

Research Article

Differences in sensitivity of reproduction to water quality in parthenogenetic European invasive lineages of the New Zealand mud snail *Potamopyrgus antipodarum* (Gray, 1843) (Caenogastropoda, Tateidae)

Carmen M. Sierra Lemus¹, Angela Schmitz Ornés¹, Martin Haase¹

¹ AG Vogelwarte, Zoological Institute and Museum, University of Greifswald, Soldmannstraße 23, D-17489 Greifswald, Germany

Corresponding author: Martin Haase (martin.haase@uni-greifswald.de)



Academic editor: Ian Duggan

Received: 12 June 2024

Accepted: 15 August 2024

Published: 10 December 2024

Citation: Sierra Lemus CM, Schmitz Ornés A, Haase M (2024) Differences in sensitivity of reproduction to water quality in parthenogenetic European invasive lineages of the New Zealand mud snail *Potamopyrgus antipodarum* (Gray, 1843) (Caenogastropoda, Tateidae). *Aquatic Invasions* 19(4): 361–371. <https://doi.org/10.3391/ai.2024.19.4.135332>

Copyright: © Carmen M. Sierra Lemus et al. This is an open access article distributed under terms of the Creative Commons Attribution License ([Attribution 4.0 International – CC BY 4.0](https://creativecommons.org/licenses/by/4.0/)).

Abstract

The dependence of freshwater snails on the availability of ions through their ambient water varies widely across species and deficiencies may limit reproduction and other physiological functions. Nevertheless, in many studies on the New Zealand mud snail (NZMS) *Potamopyrgus antipodarum*, where reactions on components dissolved in the water are not the focus, the composition of the water the snails are kept in is not specified. We tested the sensitivity of reproduction to three types of artificial fresh water in three parthenogenetic lineages invasive to Europe. The three lineages descended each from a single mother collected in the same population in northern Germany. Reproduction was measured as the number of offspring sired within 12 weeks and varied across water types, however, lineages differed in their reaction norms. This indicates 1) that reproduction of the NZMS is indeed sensitive to the composition of the water and 2) that there is fitness relevant genetic variation which probably has arisen only within the 160–180 years the NZMS is present in Europe, i.e. we witness clonal evolution. For experiments, this means that the water composition should not be neglected and that potential genetic variation even among closely related clonal lineages has to be accounted for in order to ensure comparability and reproducibility.

Key words: Clonal adaptation, clonal evolution, comparability, experimental setup, frozen niche variation, reproducibility

Introduction

Many life history traits are phenotypically plastic, hence, their expression depends on the environment. For aquatic organisms, the composition of the water is arguably the most immediate external factor influencing probably the majority of physiological processes. Interestingly, as trivial as this is, many studies investigating life history traits in the New Zealand mud snail (NZMS) *Potamopyrgus antipodarum* (Gray, 1843) do not specify the composition of the water the snails are kept in. Explicit protocols are virtually the exception, in particular in cases where dissolved

components are not the focus of the experiment (e.g., King et al. 2022; Männer et al. 2022; Levri et al. 2023). In many experiments, water quality may be regarded as a factor of minor importance as long as the conditions are equal across the experiment. This probably holds for a specific experiment, however, comparability and reproducibility are certainly limited. This is particularly evident in ecotoxicological tests, where husbandry conditions need to be standardized to achieve comparable results across labs (Duft et al. 2007; OECD 2010), but in principle also applies to all other studies, including those on life history traits.

Against this background, we investigated the sensitivity of reproduction to the composition of freshwater in the NZMS. The NZMS is native to New Zealand, where it is the most common and wide-spread freshwater snail occurring in practically all types of freshwaters including standing and running waters from springs down to estuaries, thus also tolerating brackish water (Winterbourn 1970; Verhaegen et al. 2018b). Population densities can reach hundred-thousands of individuals per m² (Geist et al. 2022). The broad ecological tolerance is paralleled by an enormous morphological variation largely correlated with the habitat. Shell size ranges from about 2.5 mm in spring populations and reaches up to 12 mm in larger rivers and lakes (Verhaegen et al. 2018b). Shape is similarly variable, and often shells bear periostracal spines (Holomuzki and Biggs 2006). A great deal of this morphological variation appears to be due to phenotypic plasticity, in size more so than in shape (Kistner and Dybdahl 2013; Verhaegen et al. 2018a, b; Männer et al. 2022). The NZMS is ovoviviparous and originally diploid as well as gonochoristic, hence outcrossing. However, diploid, triploid, and tetraploid parthenogenetic, almost all-female lines have been evolving repeatedly (Neiman et al. 2011; Paczesniak et al. 2013). The broad ecological tolerance and the reproductive characteristics are probably mainly responsible for the invasive success of those lineages that established in Europe, Australia, N-America, Japan, Chile, and lately also in N-Africa (summarized in Donne et al. 2020; Taybi et al. 2021), which are exclusively parthenogenetic. In Europe, the NZMS is Nr. 42 among the worst alien species (Nentwig et al. 2018). Apart from this notoriousness, the NZMS has become an important experimental species for the investigation of life history traits, host-parasite interactions, morphological adaptation, as well as in ecotoxicology (e.g., Dybdahl and Lively 1995a; Negovetic and Jokela 2001; Stange et al. 2012; Verhaegen et al. 2019).

In Europe, where the NZMS has been reported first in 1859 (Ponder 1988), two mitochondrial lineages, t and z, have established (Städler et al. 2005). Both show subtle genetic variation on the nuclear level, depending on the markers investigated (Weetman et al. 2002; Verhaegen et al. 2018a). Both lineages occur in fresh as well as in brackish waters, however, lineage t seems to dominate in inland waters while lineage z is mainly encountered in brackish waters. Sympatry does occur, but appears to be rare (Verhaegen et al. 2018a; Butkus et al. 2020; Männer et al. 2022).

Here we report on a common garden experiment comparing reproduction in three clonal lineages of the NZMS in artificial fresh waters of three different compositions. The three lineages were each started by a single parthenogenetic female isolated shortly after collection at the Neustädter Binnenwasser in northern Germany (54.1081°N, 10.8103°E) in September 2015 (Verhaegen et al. 2018a). Thus, at the time of the experiment, these lineages persisted already for more than 7.5 years in our aquaria. The experimental freshwaters mainly differed in the presence/absence and amount of carbonate and calcium ions (Table 1). We hypothesized that the lineages would react sensitively but that they would not differ in their reaction norms as the snails all originated from the same population/locality.

Table 1. Composition of artificial freshwaters.

Water type	Minerals contained in a total of 5 liters
ADAM	1.66 g NaCl
	10 ml of CaCl ₂ *2H ₂ O solution (stock: 135.24 g in 1 L H ₂ O)
	10 ml NaHCO ₃ solution (stock: 27.72 g in 1 L H ₂ O)
	0.5 ml SeO ₂ solution (stock: 0.07 g in 1 L H ₂ O)
OECD	1.25 g NaCl
	0.75 g NaHCO ₃
Sea salt	2.5 g NaCl

Material and methods

Common garden experiment

The snails from the Neustädter Binnenwasser belonged to the mitochondrial lineage z (Verhaegen et al. 2018a) and were maintained at a salinity of 1.3‰ [sea salt (Tropic Marin sea salt classic) added to deionized water], the salinity measured at the place of origin, at room temperature. Thus, they all had to acclimate to new conditions once transferred to freshwater. For each treatment, six adult (see Verhaegen et al. 2018a) snails from each lineage labelled Debin 20502, 20703, and 20803, respectively, were isolated in small glass jars. These jars contained 10 g of aquarium sand (JBL Sansibar, Red), a stone for shelter, and 200 ml experimental water, either “ADAM” (Aachener Daphnien Medium; Klüttgen et al. 1994), “OECD” (OECD recommended standard composition; Duft et al. 2007; OECD 2010), or “sea salt” (0.05% sea salt). The recipes/compositions are given in Table 1. The 54 jars were placed in climate cabinets at 21 °C with a regime of 14 hours light and 10 hours dark. Once a week, water was replaced, snails were fed spirulina flakes (JBL Spirulina Premium), and offspring counted. The experiment ran from March 23rd to June 15th 2023, a total of 85 days or roughly 12 weeks. At the end, each jar including the sand was thoroughly searched for young snails and eventually empty shells under a microscope in order to determine the final number of offspring. As aspects of reproduction have been shown to be size-dependent (e.g., Dybdahl and Kane 2005; McKenzie et al. 2013; Verhaegen et al. 2018a, b), shell height was measured parallel to the coiling axis from digital photographs taken with a Carl Zeiss Discovery V20 microscope equipped with an AxioCam MRc using the AxioVision LE software (Zeiss).

Statistical analyses

We analyzed two aspects of reproduction, success (offspring yes/no) and final number of offspring using descriptive statistics as well as generalized linear models. The data was analyzed using R v. 4.3.2 (R Development Core Team 2015), and packages dplyr v. 1.1.4 (Wickham et al. 2023b) for data manipulation, ggplot2 v. 3.4.4 (Wickham et al. 2023a) for graphics, ggfortify v. 0.4.16 (Tang et al. 2016; Horikoshi and Tang 2018), and lme4 v. 1.1-35.1 (Bates et al. 2015) as well as effects v. 4.2-2 (Fox 2003; Fox and Weisberg 2018) for models and graphics. For selecting the best model for each response variable, we started with the full general model including all factors (lineage, water type, their interaction, first week offspring were detected, and shell size), and then removed the non-significant ones in a step-wise fashion beginning with the one with the highest p-value. The families used for modeling were Poisson distribution for the final number of offspring and

binomial for the success rate. The model that best explained the final number of offspring included all factors except shell size. In case of the success rate, only the interaction between water type and clonal lineage tended to show an effect.

Results

Of the 54 individuals, 26 produced offspring (48%). Two snails died without offspring, one belonging to Debin 20803 in week 10, and one from Debin 20502 in week 12, both kept in ADAM. In lineage Debin 20502 ten individuals reproduced, in Debin 20703 nine, and in 20803 seven. Regarding the waters, snails in ADAM were most successful (eleven snails siring young), followed by OECD (eight) and Sea salt (seven). The GLM best explaining reproductive success included the interaction of water type and clonal lineage, however, the relationships remained statistically insignificant ($P \geq 0.0957$).

On average, each snail produced 2.94 ± 4.34 (SD) offspring. The maximum number produced by an individual was 20 (Debin 20703, ADAM). The number of offspring per water type and clonal lineage varied substantially and is presented in Fig. 1A.

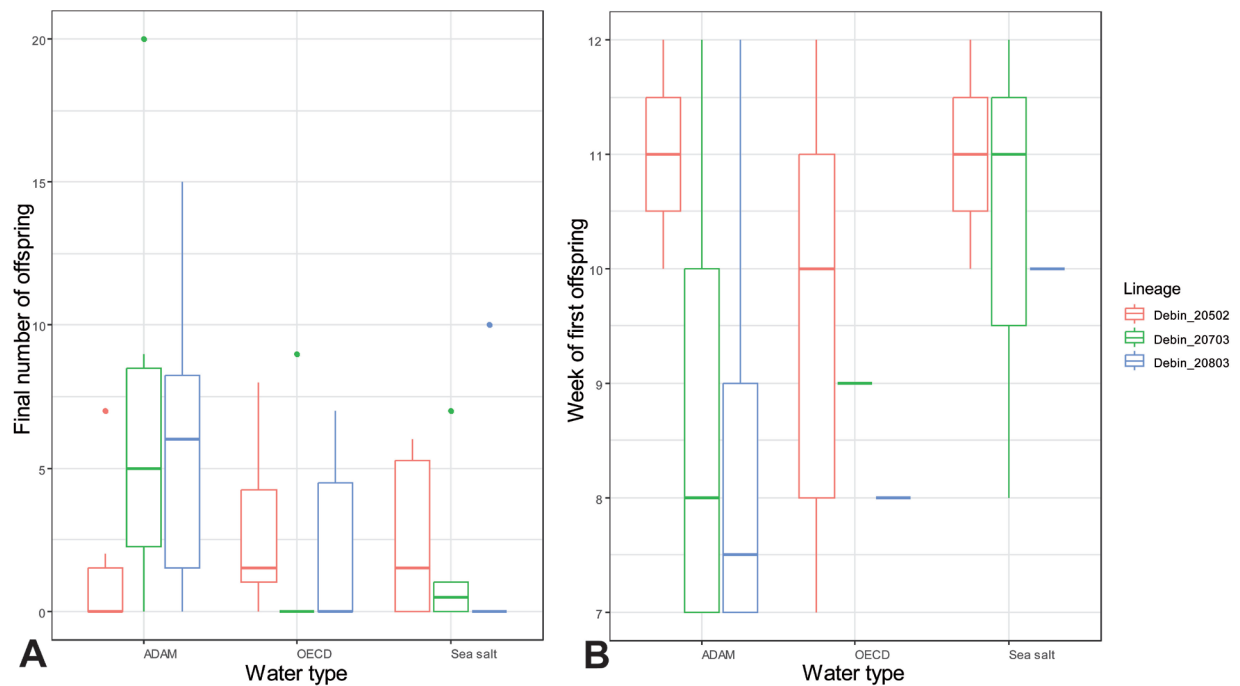


Figure 1. Final number of offspring (A) and week of first offspring (B) across water types and lineages. Box plots represent median, interquartile range (IQR), whiskers (max. 1.5 times IQR), and outliers.

The first snails to reproduce started in week 7 and the last ones in week 12 (mean 9.5 ± 1.9). The time that passed until snails started to reproduce was again very variable across water types and clonal lineages (Fig. 1B). Snails that reproduced earlier also tended to have more offspring at the end of the experiment (Fig. 2).

Thus, the final number of offspring obviously depended on water type, clonal lineage, as well as time until first reproduction, which was confirmed by a GLM including these three factors and the interaction of the former two (Table 2).

The effect plot for the interaction of lineage and water type (Fig. 3) showed that in all three lineages, the final number of offspring was intermediate in ADAM. In lineages Debin 20502 and 20803, snails in Sea salt had most offspring, in Debin 20703 those in OECD.

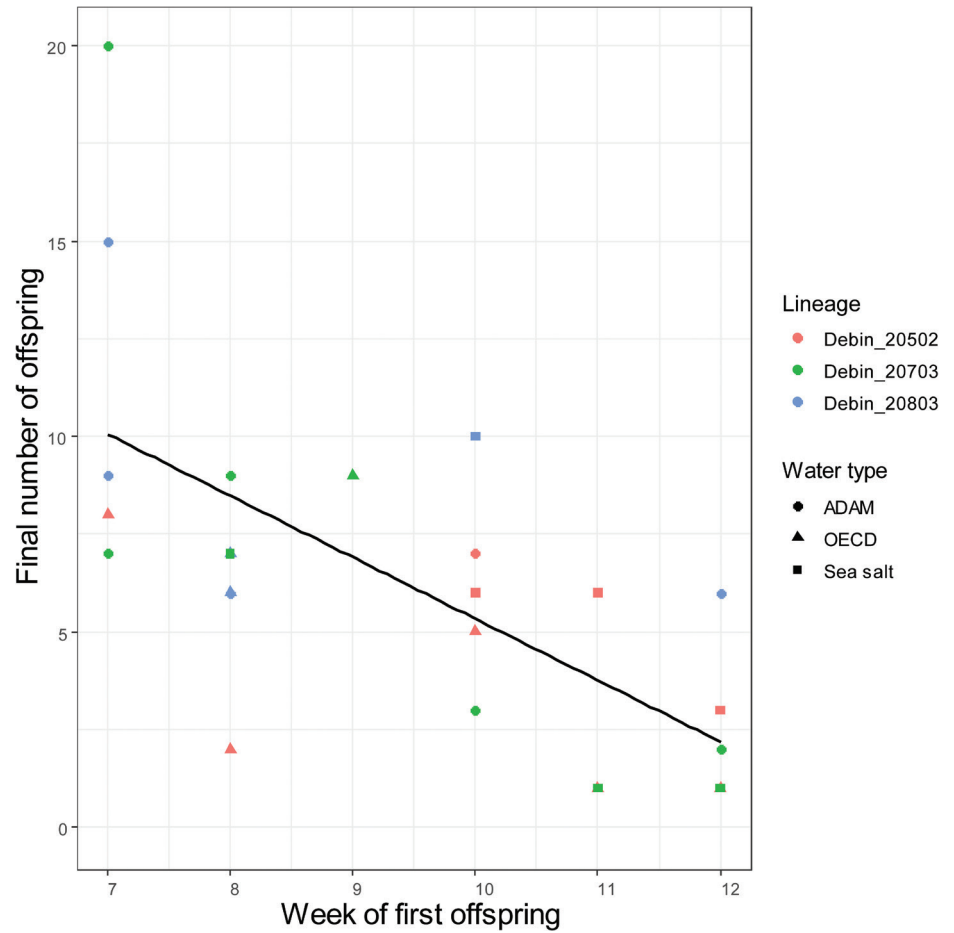


Figure 2. Best fitting linear model representing relationship between number of offspring and first week of reproduction.

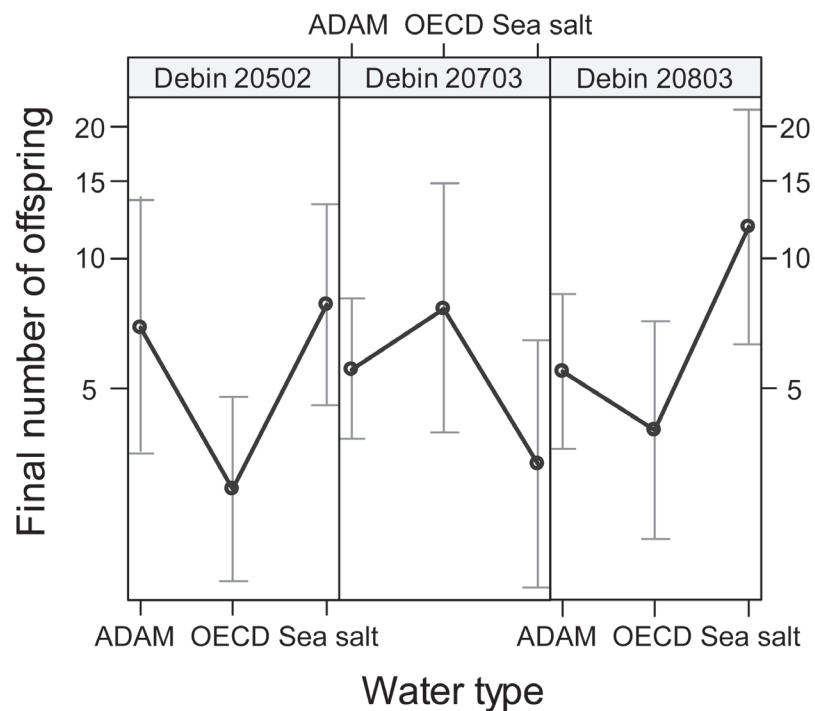


Figure 3. Effect plot from the generalized linear model for the final number of offspring depending on the interaction of genetic lineage and water type.

Table 2. Results of the generalized linear model assuming Poisson distributed data for the final number of offspring. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’.

Coefficients	Estimate	Std. Error	z value	P (> z)	Significance
ADAM	-0.12213	0.42169	-0.290	0.77211	
OECD	-0.97958	0.38375	-2.553	0.01069	*
Sea salt	5.12407	0.75242	6.810	9.75e-12	***
Debin_20703	-0.84952	0.43199	-1.967	0.04924	*
Debin_20803	0.40491	0.41150	0.984	0.32513	
ADAM:Debin_20703	0.62469	0.56704	1.102	0.27061	
OECD:Debin_20703	1.80601	0.60265	2.997	0.00273	**
ADAM:Debin_20803	-0.63763	0.57313	-1.113	0.26590	
OECD:Debin_20803	-0.09648	0.55017	-0.175	0.86079	
Week	-0.32264	0.06551	-4.925	8.42e-07	***

Discussion

This study had two main findings: 1) reproduction of the NZMS is sensitive to water quality, and 2) clonal lineages do differ in their sensitivity to water quality indicating genetic variation even within the mitochondrial lineages of the parthenogenetic invasive populations. Aquatic snails take up minerals either from the water and/or with the food (Deaton 2008; Ponder et al. 2020). It can probably be expected that snails, whose shells consist of CaCO_3 , react differently to different mineral supplies affecting both, growth and reproduction, as well as neuro-muscular activities. Across species, the dependence on calcium concentrations in the ambient water differs widely (e.g., McMahon 1983; Madsen 1987; Dillon 2000; Briers 2003; Zaluzniak et al. 2009; Dalesman and Lukowiak 2010). For the NZMS, several field studies found evidence that calcium – either measured directly or as hardness – positively influences occurrence, abundance, and life history traits (Herbst et al. 2008; McKenzie et al. 2013; Spyra and Strzelec 2014; Vazquez et al. 2016), possibly in interaction with other ions, though (Levri et al. 2020; Larson et al. 2023). Similar findings hold for conductivity in general (Herbst et al. 2008; McKenzie et al. 2013; Verhaegen et al. 2018a; Larson et al. 2020). Controlled lab experiments are scarce, though (Vazquez et al. 2016). Levri et al. (2020) suggest that for calcium concentration, conductivity and pH there may be thresholds above which any population density is supported. Also salinity affects population parameters and life history of the NZMS (summarized in Geist et al. 2022) and different lineages may vary in their reaction norms (Drown et al. 2011). Against this background it is indeed not surprising that all three lineages reacted differently across the three water types in our experiment.

The NZMS is notorious for being phenotypically plastic in various aspects, one of the main factors considered to be responsible for its invasive success (Alonso and Castro-Díez 2008; Geist et al. 2022). Plasticity has been investigated in several traits including reproductive output, growth, mortality, adult shell morphology or behavior either depending on controlled factors such as conductivity, salinity, temperature, light intensity, population density, and predator presence, or in transplant experiments and similar settings (e.g., Negovetic and Jokela 2001; Dybdahl and Kane 2005; Holomuzki and Biggs 2006; Neiman 2006; Drown et al. 2011; Liess and Lange 2011; Kistner and Dybdahl 2013; Neiman et al. 2013; Levri et al. 2014, 2017; Verhaegen et al. 2018a, b, 2021; Männer et al. 2022). However, the reactions to different water types differed among lineages in our common garden indicating genetic differentiation. This is even more surprising as

the founding mothers were all collected from the same population on a small area of maybe 3 m². Typing 3rd codon position SNPs, Verhaegen et al. (2018a) already found two nuclear genotypes among 20 snails in population Debin. As population differentiation within the two invasive mitochondrial lineages of the NZMS in Europe was subtle, Verhaegen et al. (2018a) assumed that the few, mostly locally restricted mutations detected accumulated only after the NZMS had arrived in England in the mid 19th century (see also Weetman et al. 2002). Our experiment now indicates also fitness relevant mutations, i.e. we probably witness evolution in the parthenogenetic lineages over a period of time of maybe 160–180 years. Adaptation without recombination is considered to be slow. However, once an advantageous combination of alleles has arisen through mutation accumulation, this combination may persist over generations as this genotype will not be disrupted by recombination (Roughgarden 1972; Vrijenhoek 1998). In contrast to native New Zealand populations of the NZMS, where the clonal diversity is mainly generated by the repeated evolution of parthenogenetic lineages from sexual progenitors (Dybdahl and Lively 1995b; Paczesniak et al. 2013), mutation accumulation is the only way of diversification for the exclusively asexual invasive lineages. This way, the variation of frozen niches (Vrijenhoek 1984; Jokela et al. 1997) and thus the adaptive, hence invasive potential of parthenogenetic NZMS are increasing.

Our findings show that water composition is not a factor that can be neglected in lab experiments. On the contrary, the reactions of the snails do of course depend on the minerals offered by their environment. This has been demonstrated in experiments investigating deficiencies in supply in the NZMS and other freshwater gastropods (e.g., Herbst et al. 2008; Zalizniak et al. 2009; Dalesman and Lukowiak 2010; Vazquez et al. 2016; Larson et al. 2020), but clearly has to be considered also in cases when the ions in solution are not in the focus of the experiment in order to ensure comparability and reproducibility. Regarding the study organism, we saw that even within fairly young clonal lineages, such as the mitochondrial lineages of the NZMS established in Europe, mutations do introduce genetic variation which has to be accounted for in the experimental setup.

Author Contributions

CMSL: conceptualization, methodology, formal analysis, visualization, writing – original draft; ASO: formal analysis, visualization, writing – review and editing; MH: conceptualization, funding acquisition, writing – original draft.

Funding Declaration

This study emerged from the Research Training Group 2010 RESPONSE funded by the Deutsche Forschungsgemeinschaft DFG.

Acknowledgements

We are grateful to Lilian Horn for supporting the maintenance of the snails. Comments of three anonymous reviewers improved an earlier version of the manuscript.

References

Alonso A, Castro-Diez P (2008) What explains the invading success of the aquatic mud snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca)? *Hydrobiologia* 614: 107–116. <https://doi.org/10.1007/s10750-008-9529-3>

- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Briers RA (2003) Range size and environmental calcium requirements of British freshwater gastropods. *Global Ecology and Biogeography* 12: 47–51. <https://doi.org/10.1046/j.1466-822X.2003.00316.x>
- Butkus R, Baltrūnaite L, Arbačiauskas K, Audzijonytė A (2020) Two lineages of the invasive New Zealand mudsnail *Potamopyrgus antipodarum* spreading in the Baltic and Black sea basins: low genetic diversity and different salinity preferences. *Biological Invasions* 22: 3551–3559. <https://doi.org/10.1007/s10530-020-02340-3>
- Dalesman S, Lukowiak K (2010) Effect of acute exposure to low environmental calcium on respiration and locomotion. *Journal of Experimental Biology* 213: 1471–1476. <https://doi.org/10.1242/jeb.040493>
- Deaton LE (2008) Osmotic and ionic regulation in molluscs. In: Evans DH (Ed.) Osmotic and ionic regulation. CRC Press, Boca Raton, FL, 107–133. <https://doi.org/10.1201/9780849380525-4>
- Dillon RT (2000) The ecology of freshwater molluscs. Cambridge University Press, Cambridge, 509 pp. <https://doi.org/10.1017/CBO9780511542008>
- Donne C, Neiman M, Woodell JD, Haase M, Verhaegen G (2020) A layover in Europe: Reconstructing the invasion route of asexual lineages of a New Zealand snail to North America. *Molecular Ecology* 29: 3446–3465. <https://doi.org/10.1111/mec.15569>
- Drown DM, Levri EP, Dybdahl MF (2011) Invasive genotypes are opportunistic specialists not general purpose genotypes. *Evolutionary Applications* 4: 132–143. <https://doi.org/10.1111/j.1752-4571.2010.00149.x>
- Duft M, Schmitt C, Bachmann J, Brandelik C, Schulte-Oehlmann U, Oehlmann J (2007) Proso-branch snails as test organisms for the assessment of endocrine active chemicals - an overview and a guideline proposal for a reproduction test with the freshwater mudsnail *Potamopyrgus antipodarum*. *Ecotoxicology* 16: 169–182. <https://doi.org/10.1007/s10646-006-0106-0>
- Dybdahl MF, Kane SL (2005) Adaptation vs. phenotypic plasticity in the success of a clonal invader. *Ecology* 86: 1592–1601. <https://doi.org/10.1890/04-0898>
- Dybdahl MF, Lively CM (1995a) Host-parasite interactions: infection of common clones in natural populations of a freshwater snail (*Potamopyrgus antipodarum*). *Proceedings of the Royal Society of London B* 260: 99–103. <https://doi.org/10.1098/rspb.1995.0065>
- Dybdahl MF, Lively CM (1995b) Diverse endemic and polyphyletic cones in mixed populations of the freshwater snail *Potamopyrgus antipodarum*. *Journal of Evolutionary Biology* 8: 385–398. <https://doi.org/10.1046/j.1420-9101.1995.8030385.x>
- Fox J (2003) Effect displays in R for generalised linear models. *Journal of Statistical Software* 8: 1–27. <https://doi.org/10.18637/jss.v008.i15>
- Fox J, Weisberg S (2018) An R companion to applied regression (3rd ed). Sage, Thousand Oaks CA, 608 pp. <https://doi.org/10.32614/CRAN.package.carData>
- Geist JA, Mancuso JL, Morin MM, Bommarito KP, Bovee EN, Wendell D, Burroughs B, Lutten-ton MR, Strayer DL, Tieggs SD (2022) The New Zealand mudsnail (*Potamopyrgus antipodarum*): autecology and management of a global invader. *Biological Invasions* 24: 905–938. <https://doi.org/10.1007/s10530-021-02681-7>
- Herbst DB, Bogan MT, Lusardi RA (2008) Low specific conductivity limits growth and survival of the New Zealand mud snail from the upper Owens River, California. *Western North American Naturalist* 68: 324–333. [https://doi.org/10.3398/1527-0904\(2008\)68\[324:LSCGJA\]2.0.CO;2](https://doi.org/10.3398/1527-0904(2008)68[324:LSCGJA]2.0.CO;2)
- Holomuzki JR, Biggs BJ (2006) Habitat-specific variation and performance trade-offs in shell armature of New Zealand mudsnails. *Ecology* 87: 1038–1047. [https://doi.org/10.1890/0012-9658\(2006\)87\[1038:HVAPTI\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2006)87[1038:HVAPTI]2.0.CO;2)
- Horikoshi M, Tang Y (2018) ggfortify: Data visualization tools for statistical analysis results. <https://CRAN.R-project.org/package=ggfortify>
- Jokela J, Lively CM, Fox JA, Dybdahl MF (1997) Flat reaction norms and “frozen” phenotypic variation in clonal snails (*Potamopyrgus antipodarum*). *Evolution* 51: 1120–1129. <https://doi.org/10.1111/j.1558-5646.1997.tb03959.x>

- King E, Williams C, Stillman J (2022) Hypoxia decreases thermal sensitivity and increases thermal breadth of locomotion in the invasive freshwater snail, *Potamopyrgus antipodarum*. *Physiological and Biochemical Zoology* 95: 251–264. <https://doi.org/10.1086/719899>
- Kistner EJ, Dybdahl MF (2013) Adaptive responses and invasion: The role of plasticity and evolution in snail shell morphology. *Ecology and Evolution* 3: 424–436. <https://doi.org/10.1002/ece3.471>
- Klüttgen B, Dülmer U, Engels M, Ratte HT (1994) ADaM, an artificial freshwater for the culture of zooplankton. *Water Research* 28: 743–746. [https://doi.org/10.1016/0043-1354\(94\)90157-0](https://doi.org/10.1016/0043-1354(94)90157-0)
- Larson MD, Dewey JC, Krist AC (2020) Invasive *Potamopyrgus antipodarum* (New Zealand mud snails) and native snails differ in sensitivity to specific electrical conductivity and cations. *Aquatic Ecology* 54: 103–117. <https://doi.org/10.1007/s10452-019-09729-w>
- Larson MD, Greenwood D, Flanigan K, Krist AC (2023) Field surveys reveal physicochemical conditions promoting occurrence and high abundance of an invasive freshwater snail (*Potamopyrgus antipodarum*). *Aquatic Invasions* 18: 83–102. <https://doi.org/10.3391/ai.2023.18.1.103389>
- Levri EP, Krist AC, Bilka R, Dybdahl MF (2014) Phenotypic plasticity of the introduced New Zealand Mud Snail, *Potamopyrgus antipodarum*, compared to sympatric native snails. *PLOS ONE* 9: e93985. <https://doi.org/10.1371/journal.pone.0093985>
- Levri EP, Landis S, Smith B, Colledge E (2017) Variation in predator-induced behavioral changes within introduced and native populations of the invasive New Zealand mud snail (*Potamopyrgus antipodarum*). *Aquatic Invasions* 12: 499–508. <https://doi.org/10.3391/ai.2017.12.4.07>
- Levri EP, Hutchinson S, Luft R, Berkheimer C, Wilson K (2023) Population influences desiccation tolerance in an invasive aquatic snail, *Potamopyrgus antipodarum* (Tateidae, Mollusca). *PeerJ* 11: e15732. <https://doi.org/10.7717/peerj.15732>
- Levri EP, Macelko N, Brindle B, Levri JE, Dolney TJ, Li X (2020) The invasive New Zealand mud snail *Potamopyrgus antipodarum* (J.E. Gray, 1843) in central Pennsylvania. *BioInvasions Records* 9: 109–119. <https://doi.org/10.3391/bir.2020.9.1.15>
- Liess A, Lange K (2011) The snail *Potamopyrgus antipodarum* grows faster and is more active in the shade, independent of food quality. *Oecologia* 167: 85–96. <https://doi.org/10.1007/s00442-011-1963-7>
- Madsen H (1987) Effect of calcium concentration on growth and egg laying of *Helisoma duryi*, *Biomphalaria alexandrina*, *B. camerunensis* and *Bulinus truncatus* (Gastropoda: Planorbidae). *Journal of Applied Ecology* 24: 823–836. <https://doi.org/10.2307/2403983>
- Männer L, Munding C, Haase M (2022) Stay in shape: Assessing the adaptive potential of shell morphology and its sensitivity to temperature in the invasive New Zealand mud snail *Potamopyrgus antipodarum* through phenotypic plasticity and natural selection in Europe. *Ecology and Evolution* 12: e9314. <https://doi.org/10.1002/ece3.9314>
- McKenzie VJ, Hall WE, Guralnick RP (2013) New Zealand mudsnails (*Potamopyrgus antipodarum*) in Boulder Creek, Colorado: environmental factors associated with fecundity of a parthenogenetic invader. *Canadian Journal of Zoology* 91: 30–36. <https://doi.org/10.1139/cjz-2012-0183>
- McMahon RF (1983) Physiological ecology of freshwater pulmonates. In: Russell-Hunter WD (Ed.) *The Mollusca*. Academic Press, London, 360–430. <https://doi.org/10.1016/B978-0-12-751406-2.50016-7>
- Negovetic S, Jokela J (2001) Life-history variation, phenotypic plasticity, and subpopulation structure in a freshwater snail. *Ecology* 82: 2805–2815. <https://doi.org/10.2307/2679962>
- Neiman M (2006) Embryo production in a parthenogenetic snail (*Potamopyrgus antipodarum*) is negatively affected by the presence of other parthenogenetic females. *Invertebrate Biology* 125: 45–50. <https://doi.org/10.1111/j.1744-7410.2006.00038.x>
- Neiman M, Paczesniak D, Soper DM, Baldwin AT, Hehman G (2011) Wide variation in ploidy level and genome size in a new Zealand freshwater snail with coexisting sexual and asexual lineages. *Evolution* 65: 3202–3216. <https://doi.org/10.1111/j.1558-5646.2011.01360.x>
- Neiman M, Warren D, Rasmussen B, Zhang S (2013) Complex consequences of increased density for reproductive output in an invasive freshwater snail. *Evolutionary Ecology* 27: 1117–1127. <https://doi.org/10.1007/s10682-013-9632-4>

- Nentwig W, Bacher S, Kumschick S, Pyšek P, Vilà M (2018) More than “100 worst” alien species in Europe. *Biological Invasions* 20: 1611–1621. <https://doi.org/10.1007/s10530-017-1651-6>
- OECD (2010) Detailed review paper (DRP) on molluscs life-cycle toxicity testing [Series on Testing and Assessment No. 121]. OECD Environment, Health and Safety Publications Series on Testing and Assessment 121, Paris, 182 pp.
- Paczesniak D, Jokela J, Larkin K, Neiman M (2013) Discordance between nuclear and mitochondrial genomes in sexual and asexual lineages of the freshwater snail *Potamopyrgus antipodarum*. *Molecular Ecology* 22: 4695–4710. <https://doi.org/10.1111/mec.12422>
- Ponder WF (1988) *Potamopyrgus antipodarum* - A molluscan colonizer of Europe and Australia. *Journal of Molluscan Studies* 54: 271–285. <https://doi.org/10.1093/mollus/54.3.271>
- Ponder WF, Lindberg DR, Ponder JM (2020) *Biology and Evolution of the Mollusca*. Volume 1. CRC Press, Boca Raton, FL, 900 pp. <https://doi.org/10.1201/9781351115254-1>
- R Development Core Team (2015) R. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- Roughgarden J (1972) Evolution of niche width. *American Naturalist* 106: 683–718. <https://doi.org/10.1086/282807>
- Spyra A, Strzelec M (2014) Identifying factors linked to the occurrence of alien gastropods in isolated woodland water bodies. *Naturwissenschaften* 101: 229–239. <https://doi.org/10.1007/s00114-014-1153-7>
- Städler T, Frye M, Neiman M, Lively CM (2005) Mitochondrial haplotypes and the New Zealand origin of clonal European *Potamopyrgus*, an invasive aquatic snail. *Molecular Ecology* 14: 2465–2473. <https://doi.org/10.1111/j.1365-294X.2005.02603.x>
- Stange D, Sieratowicz A, Horres R, Oehlmann J (2012) Freshwater mudsnail (*Potamopyrgus antipodarum*) estrogen receptor: Identification and expression analysis under exposure to (xeno-) hormones. *Ecotoxicology and Environmental Safety* 75: 94–101. <https://doi.org/10.1016/j.ecoenv.2011.09.003>
- Tang Y, Horikoshi M, Li W (2016) ggfortify: Unified interface to visualize statistical results of popular R packages. *The R Journal* 8: 474–485. <https://doi.org/10.32614/RJ-2016-060>
- Taybi AF, Mabrouki Y, Glöer P (2021) First record of the New Zealand mudsnail *Potamopyrgus antipodarum* (J.E. Gray, 1843) (Tateidae, Mollusca) in Africa. *Graellsia* 77: e140. <https://doi.org/10.3989/graeellsia.2021.v77.303>
- Vazquez R, Ward DM, Sepulveda A (2016) Does water chemistry limit the distribution of New Zealand mud snails in Redwood National Park? *Biological Invasions* 18: 1523–1531. <https://doi.org/10.1007/s10530-016-1098-1>
- Verhaegen G, von Jungmeister K, Haase M (2021) Life history variation in space and time: environmental and seasonal responses of a parthenogenetic invasive freshwater snail in northern Germany. *Hydrobiologia* 848: 2153–2168. <https://doi.org/10.1007/s10750-020-04333-8>
- Verhaegen G, McElroy KE, Bankers L, Neiman M, Haase M (2018a) Adaptive phenotypic plasticity in a clonal invader. *Ecology and Evolution* 8: 4465–4483. <https://doi.org/10.1002/ece3.4009>
- Verhaegen G, Neiman M, Haase M (2018b) Ecomorphology of a generalist freshwater gastropod: Complex relations of shell morphology, habitat, and fecundity. *Organisms, Diversity and Evolution* 18: 425–441. <https://doi.org/10.1007/s13127-018-0377-3>
- Verhaegen G, Herzog H, Korsch K, Kerth G, Brede M, Haase M (2019) Testing the adaptive value of gastropod shell morphology to flow: a multidisciplinary approach based on morphometrics, computational fluid dynamics and a flow tank experiment. *Zoological Letters* 5: 5. <https://doi.org/10.1186/s40851-018-0119-6>
- Vrijenhoek RC (1984) Ecological differentiation among clones: the frozen niche variation model. In: Levin SA (Ed.) *Population biology and evolution*. Springer-Verlag, Berlin, 217–231. https://doi.org/10.1007/978-3-642-69646-6_18
- Vrijenhoek RC (1998) Animal clones and diversity. Are natural clones generalists or specialists? *BioScience* 48: 617–628. <https://doi.org/10.2307/1313421>

- Weetman D, Hauser L, Carvalho GR (2002) Reconstruction of microsatellite mutation history reveals a strong and consistent deletion bias in invasive clonal snails, *Potamopyrgus antipodarum*. *Genetics* 162: 813–822. <https://doi.org/10.1093/genetics/162.2.813>
- Wickham H, Navarro DJ, Pedersen TL (2023a) *ggplot2: Elegant graphics for data analysis* (3rd ed). Springer-Verlag, New York. <https://ggplot2-book.org/>
- Wickham H, François R, Henry L, Müller K, Vaughan D (2023b) *dplyr: A grammar of data manipulation*. R package version 1.1.4. <https://dplyr.tidyverse.org>
- Winterbourn MJ (1970) The New Zealand species of *Potamopyrgus* (Gastropoda: Hydrobiidae). *Malacologia* 10: 283–321.
- Zalizniak L, Kefford BF, Nugegoda D (2009) Effects of different ionic compositions on survival and growth of *Physa acuta*. *Aquatic Ecology* 43: 145–156. <https://doi.org/10.1007/s10452-007-9144-9>