Biol Invasions (2024) 26:2281–2293
https://doi.org/10.1007/s10530-024-03315-4

ORIGINAL PAPER

Physiological effects of interacting native and invasive bivalves under thermal stress

Veronika Hillebrand · Andreas H. Dobler · Astrid N. Schwalb · Juergen Geist

Received: 11 October 2023 / Accepted: 8 April 2024 / Published online: 4 May 2024

Abstract Across many ecosystems in North America and Europe, native freshwater bivalves (Order Unionida) are threatened by fouling and competition for food by the invasive zebra mussel *Dreissena polymorpha*. In light of climate change, knowledge on the influence of water temperature on these competitive effects is important, yet poorly understood. This study examines the physiological impact of the interaction between *D. polymorpha* and the native European unionid *Anodonta cygnea* over a 28 day—period in response to water temperatures of 12, 19, and 25 °C by comparing their glycogen, glucose, lipid and protein concentrations. The laboratory experiment comprised three treatments: (1) fouling of *A. cygnea* by *D. polymorpha*, (2) both species present but not fouling; and (3) a control in which *A. cygnea* and *D. polymorpha* were placed separately. Increased water temperatures caused physiological stress in *D. polymorpha* as evident from reduced glycogen, glucose, lipid and protein concentrations. *Dreissena polymorpha* benefited from fouling of unionids, as individuals that fouled *A. cygnea* tended to have increased glycogen, glucose, lipid and protein concentrations. Competitive effects of *D. polymorpha* over the unionid bivalve species, however, were not intensified by elevated temperatures. Glochidia release, lower infestation intensity, and physiological stress of *Dreissena* at higher temperatures were likely confounding factors. The results of this study suggest that understanding the physiological consequences of species interactions at changing temperatures can be an important tool to assess future climate change impacts on freshwater bivalves and aquatic community structures.

Keywords *Dreissena polymorpha* · Unionidae · Freshwater bivalves · Invasive species · Climate change

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10530-024-03315-4.

V. Hillebrand · A. H. Dobler · J. Geist (✉)
Aquatic Systems Biology Unit, TUM School of Life Sciences, Technical University of Munich, Muehlenweg 22, 85354 Freising, Germany
e-mail: geist@tum.de

A. N. Schwalb
Biology Department, Texas State University, San Marcos, TX 78666, USA

Introduction

Freshwater bivalves of the order Unionida are among the most endangered species in Europe and globally (e.g., Haag et al. 1993; Lopes-Lima et al. 2017, 2018). Climate change and invasive species substantially contribute to the decline of native unionid mussels in addition to other threats such as habitat loss, overexploitation, and pollution (Geist 2011; Lopes-Lima et al. 2017). In European water

systems, five invasive freshwater bivalve species, *Dreissena polymorpha*, *D. rostriformis bugensis*, *Corbicula fluminea*, *C. fluminalis* and *Sinanodonta woodiana*, are already established and continue to spread (Lopes-Lima et al. 2017). These bivalve species occupy similar ecological niches and have been shown to outcompete their native counterparts under certain conditions (Sousa et al. 2014; Geist et al. 2023).

One of the most common threats to native unionids from invasive bivalves results from competition for food since invasive species, such as *D. polymorpha*, are also filter feeders (Strayer and Malcom 2018) and their physical attachment to unionids can affect their ability to ingest food (Ożgo et al. 2020). Furthermore, competitive advantages over native bivalve species in terms of reproduction have been observed: invasive bivalves either do not require a host fish during their life cycle (*Corbicula*, *Dreissena*) or are considered generalists regarding host fish species (*Sinanodonta,* Douda et al. 2012; Huber and Geist 2019). *Sinanodonta* is also characterised by a greater larval survival at elevated temperatures compared to native bivalves (Benedict and Geist 2021), faster glochidial growth combined with higher reproductive output (Huber and Geist 2019), and appears to be less vulnerable to predation than native species (Dobler and Geist 2022). Invasive bivalves have also been found to spread diseases and parasites, and to alter sediment composition through faecal accumulation (Lopes-Lima et al. 2017).

The zebra mussel, *Dreissena polymorpha,* is considered one of the most prominent invasive species to freshwater ecosystems (Aldridge et al. 2004), which often leads to a disruption of freshwater ecosystem functioning and thus to a decline of native species, especially in Europe and North America (Ricciardi et al. 1998; Sousa et al. 2014; Strayer and Malcom 2018; Karatayev and Burlakova 2022). *Dreissena polymorpha* can reach high densities and thus constitute a great proportion of the benthic biomass, significantly altering ecosystem processes as nutrients and energy are diverted from the open water into the benthos (Strayer et al. 1999; Zhu et al. 2006). Due to their high filtering capacity, *D. polymorpha* can reduce both phytoplankton (Jack and Thorp 2000) and zooplankton communities (Pace et al. 1998). Other filter feeders, such as native unionid bivalves, are therefore affected by increased competition for food (Strayer and Malcom 2018). In addition, unionid shells can be fouled by *D. polymorpha*. Such fouling can lead to shell deformation and impede the closure and opening of the valves, the functioning of the siphons, and the movement and burrowing behaviour of native unionid bivalves (Mackie 1991). High fouling rates lead to physiological stress (Ricciardi et al. 1995) and can cause the unionids to fall over, often resulting in mortality (Ożgo et al. 2020). In contrast, *D. polymorpha* is thought to benefit from unionid fouling, resulting in improved physiological conditions in individuals attached to other mussels compared to those attached to non-living substrates (Pilotto et al. 2016). Additionally, many freshwater ecosystems are affected by excessive amounts of fine sediment deposition (Geist and Auerswald 2007; Denic and Geist 2015; Hoess and Geist 2021), sometimes leaving native unionid bivalves and other *D. polymorpha* as the only solid substrates suitable for *D. polymorpha* attachment (Mellina and Rasmussen 1994).

Unionid bivalves are especially vulnerable to increasing water temperatures because their larvae and some of their host fishes are sensitive to high temperatures (Elliott and Elliott 2010; Payton et al. 2016; Benedict and Geist 2021). This is of particular concern for metamorphosis success and developmental time of glochidia during the parasitic life stage (Taeubert et al. 2014). Compared to adult bivalves, juveniles have higher habitat quality requirements in terms of cooler temperatures and sufficient oxygen supply (Santos et al. 2015). In addition, increased water temperatures can also cause thermal stress to host fish. For *Salmo trutta*, an important host fish species for many unionid bivalves in Europe (Lopes-Lima et al. 2017), temperatures > 20 °C are problematic and > 25 °C are lethal (Elliott and Elliott 2010; Smialek et al. 2021). A decline or lack of host fish as a consequence of high temperatures can hinder the successful recruitment of unionid mussel populations (Hastie et al. 2003). Furthermore, thermal stress can affect burrowing behaviour and byssus production and may increase the mortality of juvenile bivalves (Archambault et al. 2014).

In order to quantify the impact of stress factors on the physiological condition of unionids, the energy reserves of the bivalves can be determined, typically by quantifying the storage substances

glycogen, glucose, lipid, and protein. Glycogen and glucose in particular are well-established physiological markers of energy reserves. As glycogen plays an important role in carbohydrate storage (Stetten and Stetten 1960), it has been used in several studies to assess the effect of different stressors on the energetic stores of bivalves (Fritts et al. 2015; Hornbach et al. 2021).

In many cases, multiple stressors affect unionid bivalves simultaneously, and synergistic effects of multiple stressors can amplify negative impacts (Wild et al. 2023). While several direct negative impacts of *D. polymorpha* on native bivalve species have already been characterised in detail, there remains a gap of knowledge on how elevated temperatures affect the interspecific interactions between *D. polymorpha* and native unionids, particularly in terms of empirical evidence of physiological consequences.

Whereas many studies have investigated physiological impacts of water temperature on individual bivalve species (Payton et al. 2016; Beggel et al. 2017; Said and Nassar 2022) and several studies have examined the impact of *D. polymorpha* on unionid bivalves (e.g., Hallac and Marsden 2001; Sousa et al. 2011; Beason and Schwalb 2022), to the best of our knowledge, no study has yet examined water temperature effects on the interaction between *D. polymorpha* and unionid bivalve species. As test organisms, we used a native unionid bivalve, *A. cygnea,* and the invasive *D. polymorpha* since both species have a strong niche overlap and co-occurrence (Geist et al. 2023). While populations of *D. polymorpha* are increasing in Europe and globally, there is a significant decline in native bivalves including *A. cygnea*. *Anodonta cygnea* has a maximum life span of 30 years, becomes reproductive at 1 to 4 years of age and typically releases its glochidia in late winter and early spring (Lopes-Lima et al. 2017). The goal of this study was to experimentally examine the physiological responses of these two species to fouling and interspecific presence at different water temperatures.

In particular, we hypothesized that (i) elevated water temperatures cause energy reserves and storage substances of *A. cygnea* and *D. polymorpha* to decrease, (ii) shifts in competitive effects occur at different temperatures with stronger effects of *D. polymorpha* on *A. cygnea* at higher water temperatures, and (iii) *D. polymorpha* benefits from fouling of unionids resulting in higher energy reserves and storage substances with highest benefits gained at higher water temperatures.

Methods

Test organisms and origins

Two months before the start of the experiment, 39 specimens of *A. cygnea* with a mean length, height, width, and wet weight of 138.6 ± 10.0 mm, 69.3 ± 4.5 mm, 46.1 ± 4.8 mm, and 197.9 ± 49.5 g (mean ± standard deviation), respectively were purchased from a regional breeder in north-eastern Bavaria, Germany. Specimens of *D. polymorpha* were collected from a natural lake (Lake Starnberg; 47° 55′ 19″ N, 11° 17′ 40″ E) in the southern part of Bavaria, Germany. The acclimatization of mussels followed an established protocol, which has been used in the past to successfully hold bivalves over months and years in facilities of the Aquatic Systems Biology Unit of the Technical University of Munich. The test organisms were acclimatised in a tank with a volume of 200 L filled with the same water as used during the experiment and fed every other day with 2.5 mL of an algae mixture (Shellfish Diet 1800®, Nannochloropsis 3600®, and tap water [2:1:1]) under experimental conditions.

Experimental design

To assess effects of interacting *A. cygnea* and *D. polymorpha* at different water temperatures, a fully crossed experimental design with three treatments ($T_{fouling}$, $T_{presence}$ and $T_{control}$), each with three distinct water temperatures (12.3 ± 0.1 °C, 18.7 ± 0.3 °C and 25.2 ± 0.3 °C) was used. The experiment was conducted over 28 days with five replicates for the two treatments $T_{presence}$ and $T_{fouling}$ and three replicates for $T_{control}$. The test temperatures were chosen to reflect a range of values within the temperature limits of *A. cygnea* (Geist et al. 2023). In the following, these temperature ranges are referred to as 12 °C, 19 °C, and 25 °C, respectively. In $T_{presence}$, *A. cygnea* and *D. polymorpha* were both present in the same

aquarium spatially separated by a grid so that *A. cygnea* were not fouled. In $T_{fouling}$, the same biomass of *D. polymorpha* as used in $T_{presence}$ were allowed to foul the shells of *A. cygnea* by distributing all *D. polymorpha* specimens over the shell of *A. cygnea*. Mussels that did not attach to *A. cygnea* were left in place to keep the zebra mussel biomass the same as in $T_{presence}$. In $T_{control}$, *A. cygnea* and *D. polymorpha* were placed in different tanks.

The experimental setup consisted of 39 aquaria (each 40 cm × 25 cm × 25 cm; length, width, and height, respectively), filled with 15 L of water and nine glass beakers (17 cm diameter × 22 cm height) filled with 3.5 L of water that were placed in water bath channels to maintain constant temperatures. Aquaria and beakers were filled with 5 and 7 cm, respectively, of sandy substrate of a grain size of 0.1 to 2.0 mm. Oxygen was supplied to each tank by air stones, which were driven by air pumps (RESUN, Malsch, LP-40; SuperFish, Heinsberg, Koi-Flow 60). Aquaria and beakers were filled with well water (dissolved oxygen 8.63 ± 1.08 mg L^{-1}, electric conductivity (at 20 °C) 628 ± 59 µS cm^{-1}, pH 8.65 ± 0.04). All measured values were within the tolerance limits of both bivalve species as also known from previous experiments. Water parameters were checked every other day by a handheld probe (WTW, Weilheim, Multi 350i). In addition, turbidity was measured every other day before feeding with a turbidity meter (WTW, Weilheim, TURB 350 IR). 30% of the water volume in every aquarium and beaker was exchanged once a week. For each temperature range, three water bath channels with five aquaria respectively for $T_{fouling}$ and $T_{presence}$ as well as three aquariums and three beakers for $T_{control}$ were set up. To accommodate limited space, beakers were used instead of aquaria for $T_{control}$ of *D. polymorpha*. The arrangement of aquaria and beakers in the channels followed a fully randomised scheme. Three temperature loggers (Lascar electronics, Whiteparish, EasyLog) were placed in each channel to record water temperatures.

One unionid specimen was positioned in the centre of each aquarium 1 week before the start of the experiment to allow acclimatisation to laboratory conditions. For the experiment, in the aquaria of $T_{fouling}$ and $T_{presence}$, 100 g wet biomass of *D. polymorpha* (size 1–32 mm) were added, and 23 g wet biomass of *D. polymorpha* were used in beakers of $T_{control}$ to adjust for the smaller volume. The mean wet dreissenid biomass per individual of *A. cygnea* in $T_{fouling}$ was 10.53 ± 6.77 g, with values ranging from 0.86 to 23.49 g. This resulted in a dreissenid to unionid mass ratio of 0.06 ± 0.04, which was low compared to the ratio of 0.43 ± 0.56 observed in the case of *Pseudanodonta complanata* in Lake Siecino in Poland (Ożgo et al. 2020).

Bivalves were fed daily with 11.13 µL of the aforementioned algae mixture per litre of water volume, which contained 0.05 ng protein, 0.01 ng lipid, 0.02 ng carbohydrate, and 0.01 ng ash, respectively, per µL of algal mixture.

Sample collection and preparation

After completion of the experiment, fouling rate and wet dreissenid biomass per unionid specimen for $T_{fouling}$ were determined to the nearest 0.1 mg using an analytical balance (Kern, Balingen, ADJ 100-4). From each unionid specimen, two samples of foot tissue with an approximate volume of 0.5 cm^3 were taken. In case of *D. polymorpha*, the entire body mass was used as a sample. Three individuals of *D. polymorpha* were sampled from each replicate containing *D. polymorpha*. All samples were stored in tubes and frozen at a temperature of − 20 °C.

Before the measurements, samples were dried in open tubes at a temperature of 40 °C in a drying chamber (Memmert, Schwabach) for 48 h and subsequently placed in a desiccator (Sanplatec, Osaka, C-3) for 30 min. Subsequently, dry weight of samples was measured to the nearest 0.01 mg by an electronic semimicro balance (Sartorius, Göttingen, Research R 200 D) and the samples were finely ground in the tubes with a mortar, adding 900 µL of methanol to each tube.

Analysis of energy reserves

To examine the physiological responses of both bivalve species, energy reserves were calculated from the measurement of the four storage substances glucose, glycogen, lipid, and protein using the method of De Coen and Janssen (1997). Quantification of glucose, glycogen, and lipid was based on the method of van Handel (1985a, b) with slight modifications by Götz et al. (2021). Measurement of protein was

conducted following the method by Bradford (1976) and Walker (2002).

After processing, the samples were photometrically measured with a spectrophotometer (UVIKON 923) at a wavelength of 625 nm against an anthrone reagent as blank for glycogen and glucose quantification, at a wavelength of 525 nm against a vanillin phosphorus reagent as blank for lipid measurement and at a wavelength of 595 nm against the blank for protein quantification. Each sample was analysed three times in order to calculate the mean value. Based on the dry weight of the sample and a computed calibration line, the glucose, glycogen, lipid, and protein concentration per sample was determined. Subsequently, energy reserves were calculated using the method of De Coen and Janssen (1997).

Data analyses

We first tested the effect of "biomass of attached *D. polymorpha*" (zero for $T_{control}$ and for $T_{presence}$) as a covariate in an Analysis of Covariance (ANCOVA) but no significant effect was found ($p > 0.15$). Then, a two-way Analysis of Variance (ANOVA) was used to examine the effect of temperature and treatment separately for energy reserves and each storage substance for both *A. cygnea* and *D. polymorpha.* No significant interaction effect between temperature and treatment was detected in any of the analyses (all p values > 0.18). The interaction term was therefore excluded from the analyses. The analyses were followed by a post-hoc Tukey test. To determine whether the variances of the data were homogeneous, a Levene test was applied. A t-test was used to examine whether energy reserves and glycogen concentrations differed between individuals of *A. cygnea* that had versus had not released glochidia.

A linear regression was used to examine the relationship between the wet mass of attached *D. polymorpha* and energy reserves of fouled *A. cygnea.*

Statistical analyses were conducted using R (v4.2.1; R Core Team 2022). Statistical tests for group differences were performed by means of the R packages car and multcomp. For visualisation, the R packages ggplot2, cowplot, tidyverse, stargazer, and patchwork were used. Unless described otherwise, values in this study are given as mean ± standard deviation.

Table 1 Results of two-way ANOVA and the proportion of contribution of treatment versus temperature for energy reserves and storage substance concentrations of *D. polymorpha*

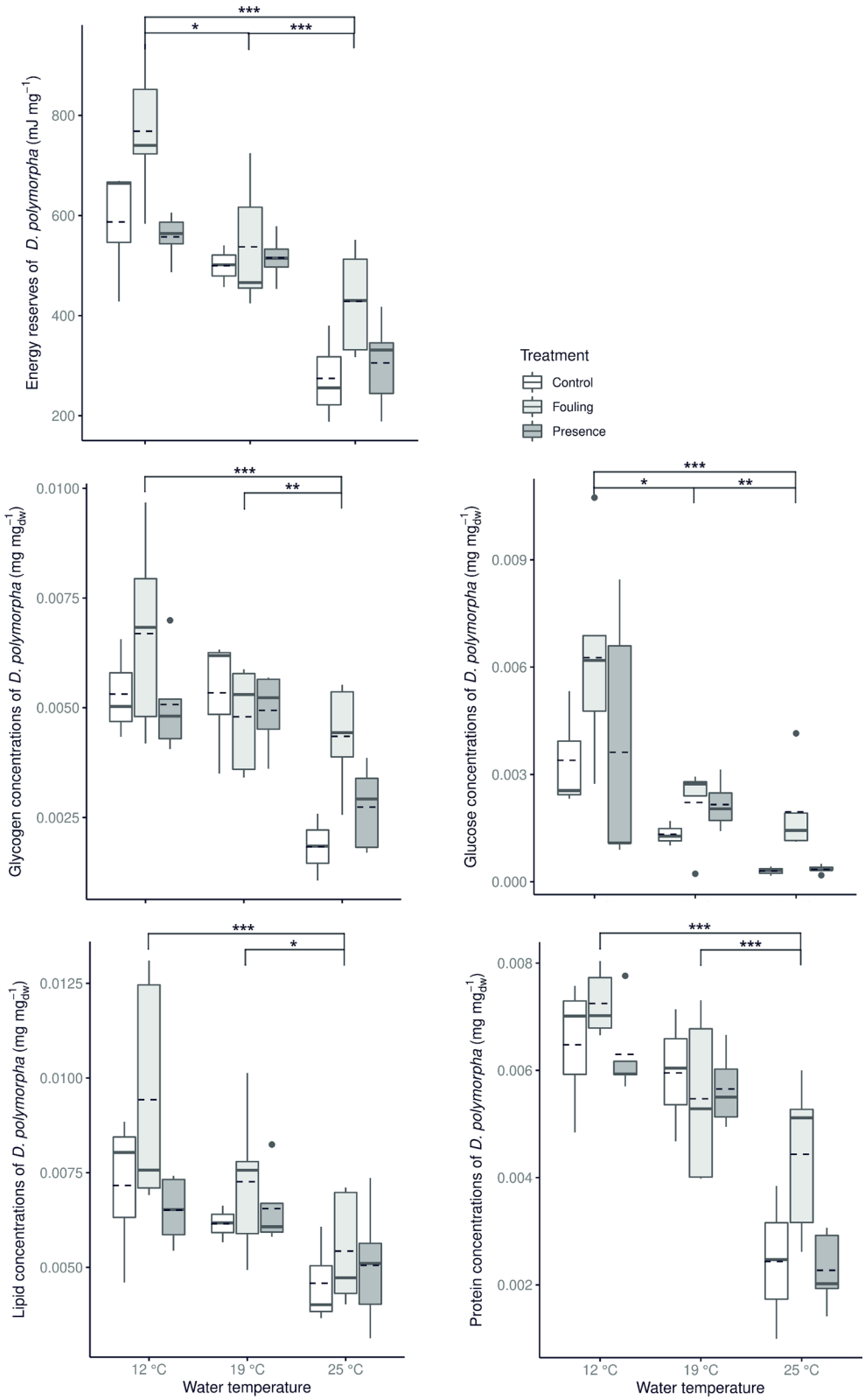
Factor	F value	*p* value	Proportion of contribution
Energy reserves			
Treatment*	6.7	< 0.01	0.13
Temperature*	28.4	< 0.001	0.55
Glycogen			
Treatment	2.8	0.07	0.07
Temperature*	12.0	< 0.001	0.38
Glucose (log glucose)			
Treatment*	5.6	< 0.01	0.14
Temperature*	17.5	< 0.001	0.44
Lipid			
Treatment	2.4	0.10	0.08
Temperature*	9.5	< 0.01	0.33
Protein			
Treatment	2.9	0.07	0.06
Temperature*	25.6	< 0.001	0.57

* indicates a significant variable with $p < 0.05$

Table 2 Results of two-way ANOVA and the proportion of contribution of treatment versus temperature for energy reserves and storage substance concentrations of *A. cygnea*

Factor	F value	*p* value	Proportion of contribution
Energy reserves			
Treatment	0.2	0.83	< 0.01
Temperature*	6.80	< 0.01	0.17
Glycogen			
Treatment*	3.4	< 0.05	0.34
Temperature*	5.0	0.01	0.50
Glucose			
Treatment	1.7	0.20	0.08
Temperature*	3.4	< 0.05	0.15
Lipid			
Treatment*	3.3	< 0.05	0.11
Temperature*	10.9	< 0.001	0.35
Protein			
Treatment	0.1	0.86	< 0.01
Temperature*	4.2	< 0.05	0.20

* indicates a significant variable with $p < 0.05$


Energy reserves of D. polymorpha (mJ mg−1)
Glycogen concentrations of D. polymorpha (mg mg−1dw)
Glucose concentrations of D. polymorpha (mg mg−1dw)
Lipid concentrations of D. polymorpha (mg mg−1dw)
Protein concentrations of D. polymorpha (mg mg−1dw)
Treatment
Control
Fouling
Presence
12 °C
19 °C
25 °C
Water temperature

◂**Fig. 1** Boxplots of **a** energy reserves, **b** glycogen, **c** glucose, **d** lipid and **e** protein concentrations of *D. polymorpha* in three different temperatures (12, 19 and 25 °C) sorted by the three treatments (fouling, presence and control) of the experiment. The horizontal dashed lines indicate the mean values, solid lines the median values, boxes the 25th to 75th percentiles and whiskers the lowest and highest values. Stars (*) indicate significant differences (* for $p<0.05$, ** for $p<0.01$ and *** for $p<0.001$). For each test scenario, five samples each were taken of $T_{fouling}$ and $T_{presence}$ and three samples of $T_{control}$

Results

Effect of temperature

In line with our first hypothesis, temperature was detected as a significant factor for energy reserves and all storage substances for both *D. polymorpha* and *A. cygnea*, explaining 33 to 57% of their variation for *D. polymorpha* and 15 to 50% for *A. cygnea* (Tables 1, 2).

In *D. polymorpha,* energy reserves and all storage substances continuously decreased from 12 °C, to 19 °C and 25 °C (Fig. 1, Table 3). This effect was pronounced and resulted in significant reductions of total energy reserves from 645 mJ mg^{-1} at 12 °C, to 521 mJ mg^{-1} at 19 °C and only 346 mJ mg^{-1} at 25 °C (Table 3). This was governed by consistent decreases in glycogen, glucose, lipid and protein at warmer temperatures, albeit being only significant for glucose across all temperatures, and for glycogen, lipid and protein for the warmest temperature against the two lower ones (Fig. 1, Table 3).

In *A. cygnea*, energy reserves were also highest at 12 °C, but the differences with the other temperatures were less pronounced than in *D. polymorpha* (Fig. 2, Table 4), overall only covering a smaller range of mean values between 319 mJ mg^{-1} at 19 °C, 429 mJ mg^{-1} at 25 °C and 432 mJ mg^{-1} at 12 °C (Table 4). In contrast to the continuous decrease of the response variables with temperature in *D. polymorpha*, energy reserves as well as glycogen, lipid and protein in *A. cygnea* were lower at 19 °C compared to both the colder and warmer temperatures. These differences were significant for most comparison with either the 25 °C (energy reserves, glycogen, protein), the 12 °C (energy reserves, lipid), or both temperatures (energy reserves) (Fig. 2, Table 4). In $T_{fouling}$, the mean wet biomass of *D. polymorpha* fouling *A. cygnea* per unionid mussel was highest at 19 °C (16.7 g ± 4.4 SD) and lowest at 25 °C (3.8 g ± 1.9 SD). At 12 °C, the mean wet dreissenid biomass was 11.1 g ± 5.6 SD.

During the experiment, we observed glochidial release in 33 of 39 *A. cygnea* (77% at 12 °C and 19 °C, and 100% at 25 °C). However, there was no significant difference (t-test: $T_{37}=0.6$, $p>0.05$ and $T_{37}=0.5$, $p>0.05$) in mean energy reserves between those individuals that released and those that did not release glochidia.

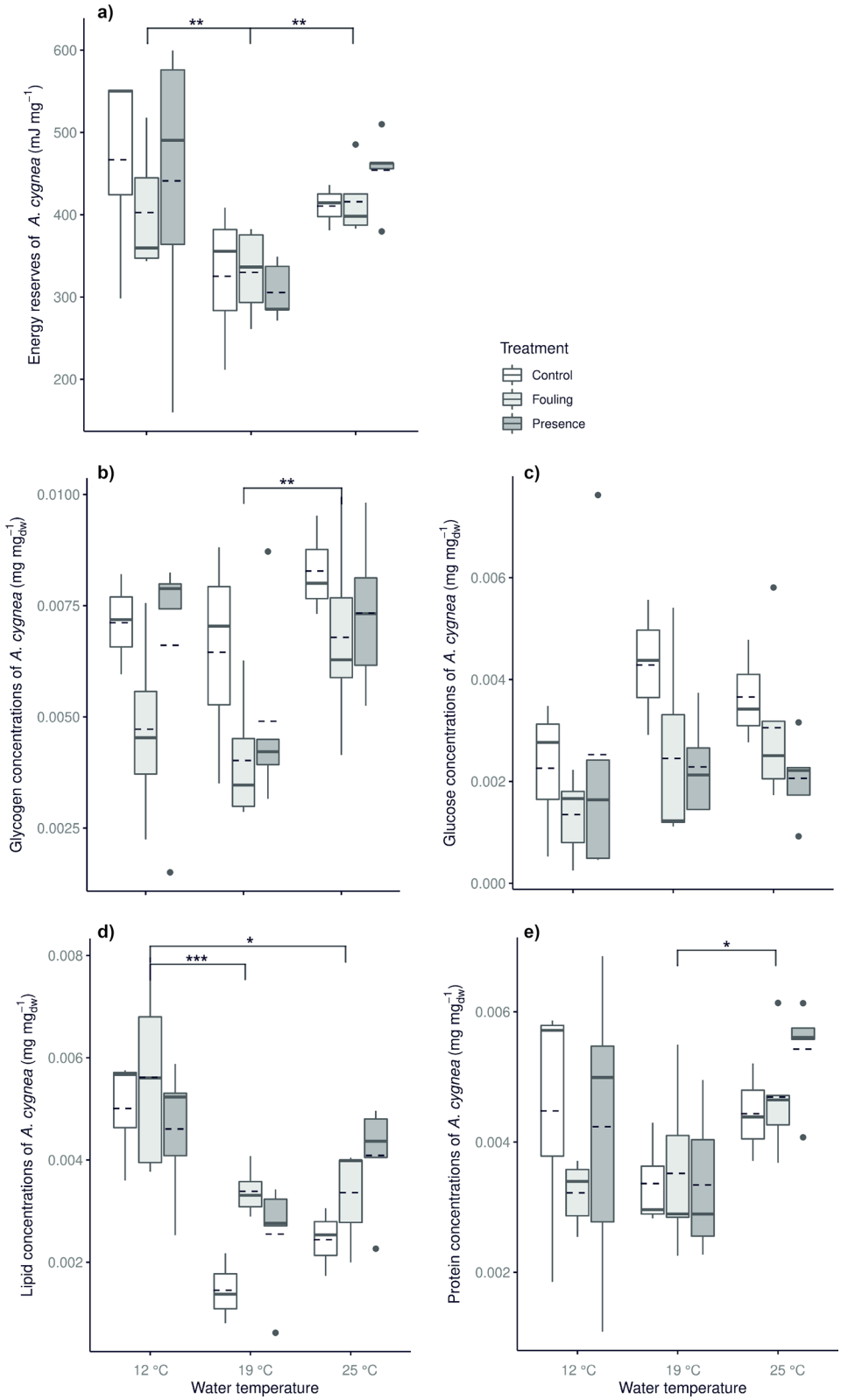
Effects of treatments

Impact of Dreissena polymorpha on Anodonta cygnea

Contrary to our second hypothesis, the physiological effects of *D. polymorpha* on *A. cygnea* were not intensified by higher water temperatures, although at the higher temperatures (19 and 25 °C) glycogen and glucose tended to be higher in $T_{control}$ compared to $T_{presence}$ and $T_{fouling}$, whereas lipid tended to be lower in $T_{control}$ (Fig. 2, Table 4). Treatment explained 34% of the variation of glycogen and 11% of lipids in *A. cygnea* and was detected as a significant factor for these variables (Table 2). The effects differed in that glycogen was lowest in $T_{fouling}$ (a 29% reduction compared to $T_{control}$), whereas lipid concentration was highest in $T_{fouling}$ (a 27% increase compared to $T_{control}$, Table 4). The differences between $T_{control}$ and $T_{fouling}$ were statistically significant for glycogen and lipids (Fig. 2 and Table 2), but not for energy reserves. However, energy reserves of fouled *A. cygnea* were lowest in $T_{fouling}$ at 12 ºC and tended to decrease with increasing wet mass of attached *D. polymorpha* ($R^2=0.18$, $p<0.05$, Fig. S1).

Impact of Anodonta cygnea on Dreissena polymorpha

In accordance with our third hypothesis, *D. polymorpha* of $T_{fouling}$ had significantly higher energy reserves and glucose compared to those of $T_{presence}$ and $T_{control}$ (Table 3), and the effect of treatment was significant for these response variables (Table 1). This effect was most pronounced at 25 °C (Fig. 1). Glycogen, protein and lipid concentration also tended to be higher in $T_{fouling}$ compared to $T_{presence}$ and $T_{control}$, but these


a)
**
**
Energy reserves of A. cygnea (mJ mg-1)
600
500
400
300
200
Treatment
Control
Fouling
Presence
b)
**
Glycogen concentrations of A. cygnea (mg mg-1dw)
0.0100
0.0075
0.0050
0.0025
c)
Glucose concentrations of A. cygnea (mg mg-1dw)
0.006
0.004
0.002
0.000
d)
*
***
Lipid concentrations of A. cygnea (mg mg-1dw)
0.008
0.006
0.004
0.002
12 °C
19 °C
25 °C
Water temperature
e)
*
Protein concentrations of A. cygnea (mg mg-1dw)
0.006
0.004
0.002
12 °C
19 °C
25 °C
Water temperature

◂**Fig. 2** Boxplots of **a** energy reserves, **b** glycogen, **c** glucose, **d** lipid and **e** protein concentrations of *A. cygnea* in three different temperatures (12, 19 and 25 °C) sorted by the three treatments (fouling, presence and control) of the experiment. The horizontal dashed lines indicate the mean values, solid lines the median values, boxes the 25th to 75th percentiles and whiskers the lowest and highest values. Stars (*) indicate significant differences (* for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$). For each test scenario, five samples each were taken of $T_{fouling}$ and $T_{presence}$ and three samples of $T_{control}$

differences were not statistically significant (Fig. 1, Table 3).

Discussion

The findings of this study provide insights into the physiological consequences of different temperatures on the interaction between the invasive *D. polymorpha* and the native European *A. cygnea*. As evident from reduced energy reserves and storage substances, elevated water temperatures caused physiological stress in *D. polymorpha*, while the temperature effect on *A. cygnea* was not definite. There were some indications of the negative impact of *D. polymorpha* on *A. cygnea,* but that effect did not intensify at higher temperatures as originally expected. One explanation for the weakness of *D. polymorpha* effects on *A. cygnea* could be the considerably lower fouling ratios at 25 °C. However, glochidia release and less severe fouling at higher temperatures may have confounded interspecific effects. *Dreissena polymorpha* benefitted consistently from fouling on *A. cygnea* and this advantage was most pronounced at 25 °C as evident from increased glycogen, glucose, lipid, and protein concentrations.

In line with our first hypothesis, elevated temperatures had pronounced negative effects on energy reserves and storage substances, especially in *D. polymorpha*. In general, invasive species are believed to benefit from increased temperatures, which is explained by both a wider ecological niche and greater adaptability (Sousa et al. 2014; Dobler et al. 2022; Geist et al. 2023). Consequently, climate change is expected to facilitate the further spread of *D. polymorpha* (Lopes-Lima et al. 2017) and species distribution models predict that its distribution range may increase significantly in the future, while native bivalves will decline and may be negatively affected by the spatial overlap with *D. polymorpha* (Gallardo and Aldridge 2013). On first glance, these expectations conflict with our observations of adverse effects of temperatures > 19 °C, which already resulted in lower energy reserves and storage substances of *D. polymorpha*. On the other hand, high temperatures have already been identified as a factor that may limit zebra mussels in subtropical climates (Schwalb et al. 2023). In contrast, in temperate climates, low water temperatures can act as a filter that currently inhibits the expansion of invasive species. Climate change can reduce the effectiveness of this filter, leading to the spread of warm-water-adapted invasive species into freshwater ecosystems with previously low water temperatures (Rahel and Olden 2008). Consequently,

Table 3 Arithmetic means (± standard deviation) of energy reserves and storage substances of *D. polymorpha* for each temperature and treatment

Factor	Energy reserves (mJ mg^{-1})	Glycogen (µg mg_{dw}^{-1})	Glucose (µg mg_{dw}^{-1})	Lipid (µg mg_{dw}^{-1})	Protein (µg mg_{dw}^{-1})
Temperature					
12 °C	645.3 ± 142.9^{A}	5.7 ± 1.7^{A}	4.6 ± 3.1^{A}	7.8 ± 2.5^{A}	6.7 ± 1.0^{A}
19 °C	520.6 ± 85.3^{B}	5.0 ± 1.1^{A}	2.0 ± 0.9^{B}	6.7 ± 1.4^{A}	5.7 ± 1.2^{A}
25 °C	345.7 ± 112.8^{C}	3.2 ± 1.4^{B}	1.0 ± 1.1^{C}	5.1 ± 1.4^{B}	3.1 ± 1.5^{B}
Treatment					
$T_{control}$	453.8 ± 164.4^{a}	4.2 ± 2.0^{a}	1.7 ± 1.6^{a}	6.0 ± 1.7^{a}	5.0 ± 2.2^{a}
$T_{presence}$	455.4 ± 132.1^{a}	4.2 ± 1.5^{a}	2.0 ± 2.5^{a}	6.0 ± 1.4^{a}	4.7 ± 2.0^{a}
$T_{fouling}$	578.1 ± 186.2^{b}	5.3 ± 1.8^{a}	3.5 ± 2.7^{b}	7.4 ± 2.7^{a}	5.7 ± 1.7^{a}

Different superscript capital letters indicate significant differences between temperature groups according to post-hoc Tukey tests. Different superscript small letters indicate significant differences between treatments according to post-hoc Tukey tests

both the limitations by low water temperatures below the 12 °C used as minimum in our experiments, and exceedance of warmer temperatures over longer periods of time (e.g. during hot summers) may limit performance and spread of *D. polymorpha*. In addition, there is evidence that reproductive and somatic energy allocation can even fluctuate within and among seasons (e.g., Jokela 1996; Jokela and Mutikainen 1995), which may indirectly depend on changing temperature regimes and also explain differences among studies.

While energy reserves and storage substances clearly decreased with temperature in *D. polymorpha*, no continuous reduction of energy reserves and storage substances with increasing temperatures was observed in *A. cygnea*. Most likely, the similarity of both energy reserves and storage substances across the studied temperatures can be explained by the greater body mass of this species (being more resilient and less affected by sudden changes) as well as by the studied temperature range matching the one to which the species is adapted as reflected in its broad occurrence in a diversity of lakes, ponds and rivers (Geist et al. 2023). Alternative explanations include that glochidia release during the experiment in this species, along with the feeding and thus energy uptake of the specimens, may have caused changes in energy reserves of *A. cygnea*. Such patterns may also strongly differ depending on species and food availability. For instance, Monroe and Newton (2001) showed that glycogen in North American *Amblema plicata* was reduced right after reproduction and that it took considerable time and adequate food quality and quantity to replenish glycogen stores. Also, thermal stress can lead to a build-up of glycogen, which is primarily used for reproduction rather than growth or performance (Guderley 2004).

Although glycogen was significantly lower for *A. cygnea* in $T_{fouling}$ compared to $T_{control}$, the effect was not more pronounced at higher temperatures as originally expected, and it was also not detected for energy reserves or the other storage substances (except for lipids), rejecting our second hypothesis. The reduction in glycogen in $T_{fouling}$ in this study (29% compared to $T_{control}$) is similar to another study on European species (Sousa et al. 2011), although a greater reduction has been found in North American species (35 to 62% reduction in glycogen, see Table 1 in Beason and Schwalb 2022). The lack of effect of zebra mussel infestation on energy reserves and storage substances (other than glycogen and lipids) is in contrast with findings by Baker and Hornbach (2000). They found lower carbohydrate and protein content in *Amblema plicata* infested with zebra mussels than individuals without infestation from the same field location, where zebra mussels were present (equivalent to our $T_{presence}$). The reasons for the discrepancy of our results with other studies could be different species, other dreissenid densities, shorter test durations, and whether glochidia discharge occurred. In addition, juvenile unionids in dreissenid-colonised habitats are often already affected by high fouling rates, which results in higher physiological stress compared to the relatively large adult unionid species

Table 4 Arithmetic mean (± standard deviation) of energy reserves and storage substances of *A. cygnea* for each temperature and treatment

Factor	Energy reserves (mJ mg^{-1})	Glycogen (µg mg_{dw}^{-1})	Glucose (µg mg_{dw}^{-1})	Lipid (µg mg_{dw}^{-1})	Protein (µg mg_{dw}^{-1})
Temperature					
12 °C	432.3 ± 132.5^{A}	6.0 ± 2.3^{AB}	2.0 ± 2.0^{A}	5.1 ± 1.5^{A}	3.9 ± 1.7^{AB}
19 °C	319.4 ± 56.4^{B}	4.9 ± 2.1^{A}	2.8 ± 1.6^{A}	2.6 ± 1.1^{B}	3.4 ± 1.0^{A}
25 °C	429.4 ± 43.3^{A}	7.3 ± 1.8^{B}	2.8 ± 1.3^{A}	3.4 ± 1.1^{B}	4.9 ± 0.9^{B}
Treatment					
$T_{control}$	400.9 ± 109.2^{a}	7.3 ± 1.8^{a}	3.4 ± 1.5^{a}	3.0 ± 1.8^{a}	4.1 ± 1.4^{a}
$T_{presence}$	400.3 ± 124.7^{a}	6.3 ± 2.4^{ab}	2.3 ± 1.7^{a}	3.7 ± 1.4^{ab}	4.3 ± 1.7^{a}
$T_{fouling}$	382.8 ± 67.1^{a}	5.2 ± 2.1^{b}	2.3 ± 1.6^{a}	4.1 ± 1.6^{b}	3.8 ± 1.1^{a}

Different superscript capital letters indicate significant differences between temperature groups according to post-hoc Tukey tests. Different superscript small letters indicate significant differences between treatments according to post-hoc Tukey tests

used in this experiment (Geist et al. 2023). The impact of *D. polymorpha* on unionid bivalves may depend on species-specific interactions, habitat quality and potential food limitations in certain habitats.

Dreissena polymorpha of $T_{fouling}$ had the highest energy reserves compared to those of $T_{presence}$ and $T_{control}$, which is in accordance with our third hypothesis, that fouling of unionids is beneficial for *D. polymorpha*. Previous studies have partly shown ambiguous results (Baker and Hornbach 2008; Pilotto et al. 2016). Whilst a study in English lowland rivers found a positive effect of fouling on body condition of *D. polymorpha* (Pilotto et al. 2016), a study in Lake Pepin, a part of the Mississippi River in Minnesota and Wisconsin, suggested the opposite effect which was explained by competition for food and burrowing behaviour of unionids (Baker and Hornbach 2008). Consequently, habitat quality and especially food availability, along with mussel densities seem to be key factors determining these interspecific effects.

Conclusion

The findings of this study highlight the advantages of including physiological endpoints in assessing performance of, and potential competition among native and invasive mussel species. Whilst our experiment was only conducted for a limited selection of species and over a limited period of time, a comprehensive assessment of the most important invasive and native species may greatly increase the mechanistic understanding of the combined impacts of climatic change and changing species interactions on freshwater mussel faunas. Such knowledge is not only important from an ecological point of view, but also highly relevant for predicting future population trends and for producing management recommendations.

Acknowledgements We would like to thank the Bavarian State Ministry of the Environment and the Bavarian Environment Agency for supporting this study via the Bavarian Mussel Coordination Office, Technical University of Munich, Germany as well as Texas State University, USA for support to AS to participate in research at TUM. We would also like to thank Dave Strayer and two anonymous reviewers whose constructive feedback contributed to the improvement of this manuscript.

Author contributions Conceptualization: VH, AD, AS, JG; methodology: VH, AD, AS, JG; data curation: VH; investigation: VH, AD, AS, JG; visualization: VH, AD; validation: AD, JG; resources: JG; supervision: JG, AS; project administration: AD, JG; funding acquisition: JG, AS; writing—original draft preparation: VH; writing—review and editing, JG, AD, AS.

Funding Open Access funding enabled and organized by Projekt DEAL. This study was partly supported by the Bavarian Mussel Coordination Office, Technical University of Munich, Germany funded by the Bavarian State Ministry of the Environment and the Bavarian Environment Agency as well as the 2021/2022 Presidential Research Award from Texas State University, USA to ANS.

Availability of data and materials Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.0vt4b8h68 (Hillebrand et al. 2024).

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no conflicts of interest.

Ethical approval N/A (Since this study only involved invertebrates (bivalves), no ethics approval was required).

References

Aldridge DC, Elliott P, Moggridge GD (2004) The recent and rapid spread of the zebra mussel (*Dreissena polymorpha*) in Great Britain. Biol Cons 119:253–261

Archambault JM, Cope WG, Kwak TJ (2014) Survival and behaviour of juvenile unionid mussels exposed to thermal stress and dewatering in the presence of a sediment temperature gradient. Freshw Biol 59:601–613

Baker SM, Hornbach DJ (2000) Physiological status and biochemical composition of a natural population of unionid mussels (*Amblema plicata*) infested by zebra mussels (*Dreissena polymorpha*). Am Midl Nat 143:443–452

Baker SM, Hornbach DJ (2008) Zebra mussels (*Dreissena polymorpha*) attached to native mussels (Unionidae) or inanimate substrates: comparison of physiological rates and biochemical composition. Am Midl Nat 160:20–28

Beason E, Schwalb AN (2022) Impact of zebra mussels on physiological conditions of unionid mussels in Texas. Aquat Sci 84:1–11. https://doi.org/10.1007/s00027-022-00853-8

Beggel S, Hinzmann M, Machado J et al (2017) Combined impact of acute exposure to ammonia and temperature stress on the freshwater mussel *Unio pictorum*. Water 9:1–16. https://doi.org/10.3390/w9070455

Benedict A, Geist J (2021) Effects of water temperature on glochidium viability of *Unio crassus* and *Sinanodonta woodiana*: implications for conservation, management and captive breeding. J Molluscan Stud 87:1–6. https://doi.org/10.1093/mollus/eyab011

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

De Coen WM, Janssen CR (1997) The use of biomarkers in *Daphnia magna* toxicity testing. IV. Cellular energy allocation: a new methodology to assess the energy budget of toxicant-stressed *Daphnia* populations. J Aquat Ecosyst Stress Recov 6:43–55

Denic M, Geist J (2015) Linking stream sediment deposition and aquatic habitat quality in pearl mussel streams: implications for conservation. River Res Appl 31:943–952. https://doi.org/10.1002/rra.2794

Dobler AH, Geist J (2022) Impacts of native and invasive crayfish on three native and one invasive freshwater mussel species. Freshw Biol 67:389–403. https://doi.org/10.1111/fwb.13849

Dobler AH, Hoos P, Geist J (2022) Distribution and potential impacts of non-native Chinese pond mussels *Sinanodonta woodiana* (Lea, 1834) in Bavaria, Germany. Biol Invasions 24:1689–1706. https://doi.org/10.1007/s10530-022-02737-2

Douda K, Vrtílek M, Slavík O et al (2012) The role of host specificity in explaining the invasion success of the freshwater mussel *Anodonta woodiana* in Europe. Biol Invasions 14:127–137. https://doi.org/10.1007/s10530-011-9989-7

Elliott JM, Elliott JA (2010) Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: predicting the effects of climate change. J Fish Biol 77:1793–1817. https://doi.org/10.1111/j.1095-8649.2010.02762.x

Fritts AK, Peterson JT, Hazelton PD et al (2015) Evaluation of methods for assessing physiological biomarkers of stress in freshwater mussels. Can J Fish Aquat Sci 72:1450–1459

Gallardo B, Aldridge DC (2013) Evaluating the combined threat of climate change and biological invasions on endangered species. Biol Cons 160:225–233. https://doi.org/10.1016/j.biocon.2013.02.001

Geist J (2011) Integrative freshwater ecology and biodiversity conservation. Ecol Ind 11:1507–1516. https://doi.org/10.1016/j.ecolind.2011.04.002

Geist J, Auerswald K (2007) Physicochemical stream bed characteristics and recruitment of the freshwater pearl mussel (*Margaritifera margaritifera*). Freshw Biol 52:2299–2316. https://doi.org/10.1111/j.1365-2427.2007.01812.x

Geist J, Benedict A, Dobler AH et al (2023) Functional interactions of non-native aquatic fauna with European freshwater bivalves: implications for management. Hydrobiologia 1:1–24. https://doi.org/10.1007/s10750-022-05121-2

Götz A, Imhof HK, Geist J et al (2021) Moving toward standardized toxicity testing procedures with particulates by dietary exposure of gammarids. Environ Toxicol Chem 40:1463–1476. https://doi.org/10.1002/etc.4990

Guderley H (2004) Locomotor performance and muscle metabolic capacities: impact of temperature and energetic status. Comp Biochem Physiol 139:371–382

Haag WR, Berg DJ, Garton DW et al (1993) Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (*Dreissena polymorpha*) in Western Lake Erie. Can J Fish Aquat Sci 50:13–19

Hallac DE, Marsden JE (2001) Comparison of conservation strategies for unionids threatened by zebra mussels (*Dreissena polymorpha*): periodic cleaning vs quarantine and translocation. J N Am Benthol Soc 20:200–210

Hastie LC, Cosgrove PJ, Ellis N et al (2003) The threat of climate change to freshwater pearl mussel populations. Ambio 32:40–46

VHillebrandAHDoblerANSchwalbJGeist2024Data from: Physiological effects of interacting native and invasive bivalves under thermal stress [Dataset]Dryad10.5061/dryad.0vt4b8h68Hillebrand, V., Dobler, AH., Schwalb, AN., & Geist, J. Data from: Physiological effects of interacting native and invasive bivalves under thermal stress [Dataset], *Dryad*, https://doi.org/10.5061/dryad.0vt4b8h68 (2024).

Hoess R, Geist J (2021) Effect of fish pond drainage on turbidity, suspended solids, fine sediment deposition and nutrient concentration in receiving pearl mussel streams. Environ Pollut 274:1–11. https://doi.org/10.1016/j.envpol.2021.116520

Hornbach DJ, Stutzman HN, Hove MC et al (2021) Influence of surrounding land-use on mussel growth and glycogen levels in the St. Croix and Minnesota River Basins. Hydrobiologia 848:3045–3063

Huber V, Geist J (2019) Reproduction success of the invasive *Sinanodonta woodiana* (Lea 1834) in relation to native bivalve species. Biol Invasions 21:3451–3465. https://doi.org/10.1007/s10530-019-02060-3

Jack JD, Thorp JH (2000) Effects of the benthic suspension feeder *Dreissena polymorpha* on zooplankton in a large river. Freshw Biol 44:569–579

Jokela J (1996) Within-season reproductive and somatic energy allocation in a freshwater clam, Anodonta piscinalis. Oecologia 105:167–174

Jokela J, Mutikainen P (1995) Phenotypic plasticity and priority rules for energy allocation in a freshwater clam: a field experiment. Oecologia 104:122–132

Karatayev AY, Burlakova LE (2022) What we know and don't know about the invasive zebra (*Dreissena polymorpha*) and quagga (*Dreissena rostriformis bugensis*)

mussels. Hydrobiologia. https://doi.org/10.1007/s10750-022-04950-5
Lopes-Lima M, Sousa R, Geist J et al (2017) Conservation status of freshwater bivalves in Europe: state of the art and future challenges. Biol Rev 92:572–607. https://doi.org/10.1111/brv.12244
Lopes-Lima M, Burlakova LE, Karatayev AY et al (2018) Conservation of freshwater bivalves at the global scale: diversity, threats and research needs. Hydrobiologia 810(1):1–14. https://doi.org/10.1007/s10750-017-3486-7
Mackie GL (1991) Biology of the exotic zebra mussel, *Dreissena polymorpha*, in relation to native bivalves and its potential impact in Lake St. Clair. Hydrobiologia 219:251–268
Mellina E, Rasmussen JB (1994) Patterns in the distribution and abundance of zebra mussel (*Dreissena polymorpha*) in rivers and lakes in relation to substrate and other physiochemical factors. Can J Fish Aquat Sci 51:1024–1036
Monroe EM, Newton TJ (2001) Seasonal variation in physiological condition of *Amblema plicata* in the Upper Mississippi River. J Shellfish Res 20:1167–1171
Ożgo M, Urbańska M, Hoos P et al (2020) Invasive zebra mussel (*Dreissena polymorpha*) threatens an exceptionally large population of the depressed river mussel (*Pseudanodonta complanata*) in a postglacial lake. Ecol Evol 10:4918–4927. https://doi.org/10.1002/ece3.6243
Pace ML, Findlay SEG, Fischer D (1998) Effects of an invasive bivalve on the zooplankton community of the Hudson River. Freshw Biol 39:103–116
Payton SL, Johnson PD, Jenny MJ (2016) Comparative physiological, biochemical and molecular thermal stress response profiles for two unionid freshwater mussel species. J Exp Biol 219:3562–3574
Pilotto F, Sousa R, Aldridge DC (2016) Is the body condition of the invasive zebra mussel (*Dreissena polymorpha*) enhanced through attachment to native freshwater mussels (Bivalvia, Unionidae)? Sci Total Environ 553:243–249
R Core Team (2022) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
Rahel FJ, Olden JD (2008) Assessing the effects of climate change on aquatic invasive species. Conserv Biol 22:521–533
Ricciardi A, Whoriskey FG, Rasmussen JB (1995) Predicting the intensity and impact of *Dreissena* infestation on native unionid bivalves from *Dreissena* field density. Can J Fish Aquat Sci 52:1449–1461
Ricciardi A, Neves RJ, Rasmussen JB (1998) Impending extinctions of North American freshwater mussels (Unionoida) following the zebra mussel (*Dreissena polymorpha*) invasion. J Anim Ecol 67:613–619
Said RM, Nassar SE (2022) Mortality, energy reserves, and oxidative stress responses of three native freshwater mussels to temperature as an indicator of potential impacts of climate change: a laboratory experimental approach. J Therm Biol 104:1–9
Santos RMB, Sanches Fernandes LF, Varandas SGP et al (2015) Impacts of climate change and land-use scenarios on *Margaritifera margaritifera*, an environmental indicator and endangered species. Sci Total Environ 511:477–488
Schwalb AN, Swearingen D, Robertson JJ et al (2023) Living on the edge: thermal limitations of zebra mussels (*Dreissena polymorpha*) in Central Texas. Biol Invasions 25:847–861. https://doi.org/10.1007/s10530-022-02950-z
Smialek N, Pander J, Geist J (2021) Environmental threats and conservation implications for Atlantic salmon and brown trout during their critical freshwater phases of spawning, egg development and juvenile emergence. Fish Manag Ecol 28:437–467. https://doi.org/10.1111/fme.12507
Sousa R, Pilotto F, Aldridge DC (2011) Fouling of European freshwater bivalves (Unionidae) by the invasive zebra mussel (*Dreissena polymorpha*). Freshw Biol 56:867–876. https://doi.org/10.1111/j.1365-2427.2010.02532.x
Sousa R, Novais A, Costa R et al (2014) Invasive bivalves in fresh waters: impacts from individuals to ecosystems and possible control strategies. Hydrobiologia 735(1):233–251. https://doi.org/10.1007/s10750-012-1409-1
Stetten JRD, Stetten MR (1960) Glycogen metabolism. Physiol Rev 40:505–537
Strayer DL, Caraco NF, Cole JJ et al (1999) Transformation of freshwater ecosystems by bivalves: a case study of zebra bivalves in the Hudson river. Bioscience 49:19–27. https://doi.org/10.1525/bisi.1999.49.1.19
Strayer DL, Malcom HM (2018) Long-term responses of native bivalves (Unionidae and Sphaeriidae) to a *Dreissena* invasion. Freshw Sci 37:697–711. https://doi.org/10.1086/700571
Taeubert J-E, El-Nobi G, Geist J (2014) Effects of water temperature on the larval parasitic stage of the thick-shelled river mussel (*Unio crassus*). Aquat Conserv Mar Freshw Ecosyst 24:231–237
Van Handel E (1985a) Rapid determination of glycogen and sugars in mosquitoes. J Am Mosq Control Assoc 1:299–301
Van Handel E (1985b) Rapid determination of total lipids in mosquitoes. J Am Mosq Control Assoc 1:302–304
Walker JM (2002) The protein protocols handbook, 2nd edn. Humana Press, Totowa
Wild R, Nagel C, Geist J (2023) Climate change effects on hatching success and embryonic development of fish: assessing multiple stressor responses in a large-scale mesocosm study. Sci Total Environ 893:1941–1954. https://doi.org/10.1016/j.scitotenv.2023.164834
Zhu B, Fitzgerald DG, Mayer CM et al (2006) Alteration of ecosystem function by zebra mussels in Oneida Lake: impacts on submerged macrophytes. Ecosystems 9:1017–1028