Are juveniles as tolerant to salinity stress as adults? A case study of Northern European, Ponto-Caspian and North American species

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Abstract

**Aim:** Global biodiversity and ecosystems are highly impacted by anthropogenic activities, such as climate change and introduction of non-indigenous species. As numerous species from the Ponto-Caspian region have established in the North and Baltic Seas, as well as in the Laurentian Great Lakes, there have been large number of studies examining environmental tolerance of these species to determine their future potential to spread. However, many of those studies were conducted only on adult stages, while neglecting the possibility that early life history stages might not be equally resilient.

**Location:** Northern European, Ponto-Caspian and North American regions.

**Methods:** To determine whether juveniles would demonstrate the same environmental tolerance as their parents, we examined the salinity tolerance of adults and juveniles of one Northern European (*Gammarus salinus*), one Ponto-Caspian (*Pontogammarus maeoticus*) and one North American species (*Gammarus tigrinus*). Additionally, we compared our study to that of Paiva et al. (*Global Change Biology*, 24, 2018, 2708), who tested the salinity tolerance of the same species using only adults.

**Results:** Our study determined that both adults and juveniles of all three species tolerated wide ranges of salinity, with juveniles of *G. salinus* tolerating only slightly narrower salinity range than their parents, while those of *P. maeoticus* and *G. tigrinus* much narrower range. Additionally, we determined better survival and higher growth rates of juveniles of *G. salinus* in higher salinities and better survival of *P. maeoticus* in lower salinities.

**Main conclusions:** Based on juvenile salinity tolerance, our study further supported findings of Paiva et al. (2018), where Northern European species may be adapted to marine, while Ponto-Caspian to lower saline and freshwater environments. The North American species is probably adapted to intermediate salinities. As juveniles do not tolerate the same salinity stress as adults, we emphasize the importance of testing all life history stages when predicting species resilience to environmental stressors.
1 | INTRODUCTION

Marine and freshwater ecosystems are largely affected by anthropogenic stressors like eutrophication, pollution, habitat loss, climate change, and biological invasions (Capinha, Essl, Seebens, Moser, & Pereira, 2015; Chapman, 2017; Lockwood, Hoopes, & Marchetti, 2013; Solan & Whiteley, 2016), all of which are a threat to global biodiversity. The introduction and establishment of non-indigenous species (NIS) via human-mediated transport can have strong impacts on marine biodiversity and ecosystem structure, altering communities worldwide (Simberloff, 2011; Strayer, Eviner, Jeschke, & Pace, 2006). Successful establishment of a NIS requires a sufficient number of viable and reproductively capable individuals (i.e., propagule pressure), certain species characteristics (e.g., phenotypic plasticity), and it also depends on the conditions of the recipient habitat (i.e., environmental conditions and interaction of NIS with native species; Lockwood et al., 2013; Ruiz, Carlton, Grosholz, & Hines, 1997; Simberloff, 2009). Recently, Briski et al. (2018) suggested that selection during the transport stage of the invasion process can facilitate local adaptation (e.g., survival of only pre-adapted individuals for particular environmental conditions), which may result in greater likelihood of invasion success. Likewise, several studies have suggested that certain geographical regions are major donors of NIS, in particular those with disturbed geological history and environmental fluctuations that have led to selection for flexible life history traits, phenotypic plasticity and consequently more robust species (Bij de Vaate, Jazdzewski, Ketelaars, Gollasch, & Van der Velde, 2002; Casties, Seebens, & Briski, 2016; Reid & Orlova, 2002; Ricciardi & Maclsaac, 2000).

The Ponto-Caspian region (i.e., Black, Azov and Caspian Seas) has been determined as one of the major sources of NIS to different types of water bodies, including brackish and freshwater habitats of Northern Europe and the Laurentian Great Lakes (Bij de Vaate et al., 2002; Casties et al., 2016; Mordukhay-Boltovskoy, 1964; Reid & Orlova, 2002; Ricciardi & Maclsaac, 2000). As only a small number of species from the Great Lakes invaded Northern European waters and vice versa, several studies suggested that Ponto-Caspian taxa more readily colonize habitats of diverse salinities than taxa from other regions (Leppäkoski et al., 2002; Reid & Orlova, 2002; Ricciardi & Maclsaac, 2000). The Ponto-Caspian basin is geologically old and was continuously affected by large-scale environmental fluctuations from fully marine environments, as a part of the Tethys Sea, to almost pure freshwater ecosystems as Sarmatian Sea (Reid & Orlova, 2002; Zenkevitch, 1963). Considering these hydrological changes, many Ponto-Caspian species have been selected for euryhalinity (Reid & Orlova, 2002). In addition, some studies suggested that Ponto-Caspian NIS, established in freshwater habitats, might not be of marine, but of freshwater origin due to the geological history of their native region (Casties et al., 2016; Paiva et al., 2018; Reid & Orlova, 2002). To support this hypothesis, there has been an increasing number of studies investigating the salinity tolerance of Ponto-Caspian species distributed in brackish and freshwater habitats (e.g., Dobrzycka-Krahel & Graca, 2018; Kobak et al., 2017; Paiva et al., 2018). Recently, Pauli and Briski (2018) conducted an extensive literature search on the salinity range of Ponto-Caspian NIS in their native and non-native habitats and determined that though Ponto-Caspian species occupy wide ranges of salinity, more than 67% of the species were recorded in freshwater habitats in their native region, with a tendency of a decreasing number of species as salinity increased. The similar evidence was provided by Pauli, Paiva, and Briski (2018) demonstrating that artificial selection of one Ponto-Caspian gammarid, originating from a salinity of 10 g/kg, is possible to lower salinities and freshwater conditions, but not to higher salinities. Finally, a comparative salinity assessment, using adults of 22 populations of eight gammarid species originating from the Ponto-Caspian, Northern European and Great Lakes–St. Lawrence River regions, revealed that Ponto-Caspian taxa performed better in freshwater, while Northern European taxa performed better in fully marine conditions (Paiva et al., 2018).

In terms of geographical range expansions and biological invasions, it was assumed that salinity would limit species dispersal from marine to brackish and freshwater habitats, and vice versa (Dahl, 1956). However, numerous studies have reported the establishment of marine and brackish species in freshwater habitats, with many of those species originating from the Ponto-Caspian basin (Casties et al., 2016; Lee & Bell, 1999; Pauli & Briski, 2018; Ricciardi & Maclsaac, 2000; Ruiz et al., 1997). As with most environmental stressors, salinity stress often more severely affects early life history stages, such as embryos and larvae, than adults (e.g., Anger, 2003; Kinne, 1964). The osmotic stress encountered when salinity limits are exceeded requires energetic costs that may not only compromise major physiological needs, but also have negative consequences on reproduction, development, growth and survival of stressed individuals (Anger, 2003; Neuparth, Costa, & Costa, 2002; Normant & Lamprecht, 2006). Even though adult organisms can tolerate a wide range of salinities, they may not be able to reproduce (Steele & Steele, 1991 and references therein), or when they do, the stress may have severe consequences for their offspring, such as reduced viability of embryos, decreased number of broods and reduced number of emergent juveniles (e.g., Mills & Fish, 1980; Steele & Steele, 1991; Vlasblom & Bolier, 1971). Finally, even when individuals of an introduced population are able to survive and reproduce, they can still fail to establish in a new habitat if the population growth rate is negative, which particularly may be the case when small populations are introduced (Blackburn et al., 2011).
Although salinity tolerance has been studied for different species globally (e.g., Dobrzycka-Krahel & Graca, 2018; Ellis & MacIsaac, 2009; Kobak et al., 2017; McFarland, Baker, Baker, Rybovich, & Volety, 2015; Ovčarenko, Audžijonytė, & Gasinjnaite, 2006; Paiva et al., 2018), it remains unclear how offspring would respond to those salinities. In this study, we extend the comparative salinity assessment of Paiva et al. (2018) by evaluating not only adults, but also juveniles of one Northern European (Gammarus salinus), one Ponto-Caspian (Pontogammarus maeoticus) and one North American species (Gammarus tigrinus) to determine whether adults and juveniles would reveal the same salinity pattern (Figure 1). The Northern European and Ponto-Caspian species were collected in their native range, while the North American species was collected in its invaded range, due to practicality and laboratory proximity. However, we emphasize that the aim of this study was not to compare populations from native and introduced locations of the same species, but to compare performance between adults and juveniles of species originating from different regions. To evaluate fitness of the tested species, we exposed pairs in precopula to different salinities and followed mortality of adults, and hatching success, growth rate and mortality of juveniles. We tested the hypotheses that there is no difference in (a) mortality of adults among different treatments and species; (b) mortality of juveniles among different hatching salinities and species; and (c) growth rate of juveniles among different hatching salinities and species. Additionally, we compared our results to those in Paiva et al. (2018) and tested the hypotheses that there is no difference in mortalities: (d) of adults between the two studies; and (e) of juveniles in this study and adults in Paiva et al. (2018).

2 | MATERIALS AND METHODS

2.1 | Specimen collection

Specimens of P. maeoticus were collected in October 2014 in Jafrud, Iran (37°37ʹΝ 49°07ʹΕ), of G. tigrinus in May 2016 in Travemünde, Germany (53°83ʹΝ 10°64ʹΕ), and of G. salinus in May 2017 in Falckenstein, Germany (54°40ʹΝ 10°20ʹΕ). Two species were collected in their native range (i.e., G. salinus and P. maeoticus), and one in its non-native region (i.e., G. tigrinus; Figure 1). While the perfect scenario would be to have all three species collected in its native

![Figure 1](image-url)  
**Figure 1** Geographic range and sampling locations of G. tigrinus (a), G. salinus (b) and P. maeoticus (c). Native and invaded ranges of G. tigrinus are shown in green and yellow, respectively; native range of G. salinus is shown in green; and native and invaded ranges of P. maeoticus are shown in green and by the yellow circle, respectively. Black asterisks denote sampling locations in our study.
range, G. tigrinus was collected in its invaded location due to practicality and laboratory proximity. After collection, individuals were transported in ambient water to the laboratories at GEOMAR in Kiel, Germany, where each individual was morphologically identified according to Köhn and Gosselck (1989) for G. salinus; Sars (1896), Birstein and Romanova (1968), Moiceev and Filatova (1985), Stock (1974) and Stock, Mirzajani, Vonk, Naderi, and Kiabi (1998) for P. maeoticus; and Lincoln (1979) for G. tigrinus. Before experiments started, animals were kept at their ambient salinity for at least two weeks to acclimatize to laboratory conditions; we emphasize that in the case of P. maeoticus the tested population was kept in the laboratory for 1.5 years before the experiments started.

2.2 Laboratory experiments

To evaluate fitness of each species, we exposed adult individuals to different salinities, and followed their mortality, as well as hatching success, growth rate and mortality of juveniles. The experiments were conducted from April 2016 to June 2017. The experimental design for adults consisted of three treatments: (a) control; (b) low salinity; and (c) high salinity. Each treatment consisted of five replicates (i.e., five 2-L tanks). Five pairs of mean-size individuals in precopula (i.e., male holding on to and carrying female) were placed in each 2-L tank. In the case of P. maeoticus, there were not enough couples available; therefore, in each tank three couples and four randomly chosen single adult individuals were used. Seawater filtered through a 20-µm mesh from Kiel Fjord (fluctuating from 10 g/kg to 16 g/kg) was used for the experiments, which salinity was then increased and decreased using artificial seawater (Instant Ocean®) and potable tap water, respectively to reach the desired salinity for each treatment. The salinity of the control treatments was identical to ambient water of the collection site for each species: for G. salinus, 16 g/kg; for P. maeoticus, 10 g/kg; and for G. tigrinus, 10 g/kg. The high and low salinity treatments began at the ambient salinity of the species collection site, which was then increased/decreased by 2 g/kg every two days, until reaching 40 g/kg and 0 g/kg, respectively (Delgado, Gueruo, & Ribera, 2011; Paiva et al., 2018; Pauli et al., 2018). Therefore, we emphasize here that due to the different ambient salinities of the three species, the high and low treatments did not start from the same salinities for all three species. Salinity was increased/decreased by removing half of the water in the tanks and replacing it with in advance prepared water of the required salinity; water of the required salinities was prepared seven days in advance to allow for proper dissolving of artificial salt (Instant Ocean®). Salinity was measured using a WTW Cond 3110 salinometer and a TetraCon 325 probe (Xylem Analytics Germany Sales GmbH & Co. KG, WTW, Germany). The accuracy of the desired salinities in the experiments was ±0.2 g/kg. As 1 L of water was immediately poured into the tanks, to apply the same disturbance/stress to the control treatment, water was also exchanged every two days. We emphasize that this method of changing the water could have resulted in additional stress and mortality. Mortality of adults was checked daily throughout the experiments. When 40 g/kg and 0 g/kg were reached, mortality of adults was followed for two more weeks before the experiment was terminated.

Before each water exchange (i.e., every second day), tanks were examined thoroughly for the presence of a new cohort of juveniles. If found, the juveniles were removed using a pipette, and placed in a new 2-L tank where they were kept for six weeks to allow us to follow their growth rates. The juveniles of different age cohorts were placed in separate tanks. Juveniles from two cohorts, corresponding to two salinity steps, were reared together at the intermediate salinity of two salinity steps. For example, juveniles hatched at 8 g/kg and 6 g/kg were reared together at 7 g/kg. Once juveniles were placed in the rearing tanks of a salinity that was 1 g/kg higher or lower than their hatching salinity, salinity of the tanks was not changed anymore during the six-week experiment. Following the experimental design of adults, juveniles were also reared in five replicates corresponding to the tank numbers of adults. The water in the rearing tanks was exchanged weekly, and the juveniles were fed ad libitum with fish food flakes. Hatching success was determined as the total number of juveniles per cohort, including dead individuals. Juvenile mortality and growth rate were checked every two weeks. Growth rate was determined by measuring the cephalon length using a stereomicroscope (Stemi 508, ZEISS) and the ZEN software (vs. 2.3, ZEISS), where a juvenile was placed in a droplet of water on a microscope slide and gently covered by a cover slip to restrain movements of the animal. The cephalon length was used as a proxy for total length to minimize handling and stressing the animals (Delgado et al., 2011; Lancellotti & Trucco, 1993). Dead animals were not measured.

2.3 Statistical analysis

To determine the effect of salinity on mortality of adults, we tested for differences in the onset and rate of mortality between treatments within species, between species, and between our study and Paiva et al. (2018). A mortality curve for each treatment for each species was created using all replicates, described by the equation (Briski, Ghabooli, Bailey, & MacIsaac, 2011; Briski, VanStappen, Bossier, & Sorgeloos, 2008):

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

(1)

where \( s \) is salinity change, \( Z \) is the rate of mortality, and \( Q \) is the onset of mortality. The model was then expanded to compare the rate and the onset of mortality between two curves using the equation:

\[ y = 100/[1 + e^{-(Z_1+Z_2)(s-Q_1-Q_2)}] \]  

(2)

where \( Z_1 \) and \( Z_2 \) were the rates of mortality, and \( Q_1 \) and \( Q_2 \) were the points of onset of mortality, for the first and second curves, respectively. All possible combinations of curve pairs were compared statistically by the fit non-linear model using generalized least squares. Significant levels for statistical comparisons of estimated...
parameters $Z_1$ and $Z_2$, and $Q_1$ and $Q_2$, were adjusted for multiple pairwise comparisons by Bonferroni-type correction to guard against inflating the type I error rate and the family-wise error rate of 0.05 was used. The analyses were performed using S-Plus 6.1 (S-Plus® 6.1, 2002, Insightful Corp.). Additionally, mortalities among three species at the end of the experiment were compared using three one-way ANOVAs, each for one treatment (i.e., control, low and high salinity treatments). Post hoc pairwise comparisons using Tukey’s HSD test were also performed. The assumptions of parametric tests were fulfilled.

In the case of juveniles, again for all comparisons, the assumptions of normality and homogeneity of variances were checked, and based on the obtained results, the decision on the type of test—parametric or non-parametric—was made. The effect of salinity on mortality of juveniles was tested using Kruskal–Wallis H test. Three separate Kruskal–Wallis H tests were conducted, each for one species. Additional post hoc pairwise comparisons using Wilcoxon’s rank sum test with Bonferroni’s adjustment were also performed. The effect of salinity on the cephalon length of juveniles of *G. salinus* was also conducted using Kruskal–Wallis H test, with an additional post hoc pairwise comparison using a Wilcoxon rank sum test with Bonferroni’s adjustment. To test for the effect of salinity on the cephalon length of *P. maeoticus* and *G. tigrinus*, two separate one-way ANOVAs were done, each for one species. Additional post hoc pairwise comparisons using Tukey’s HSD test were performed. The salinities at which no juveniles survived until the end of the experiment were excluded from the cephalon length analyses (i.e., 2 out of 11 salinities for *G. salinus*, 4 out of 9 salinities for *P. maeoticus* and 3 out of 9 salinities for *G. tigrinus*). The tanks were used as replicates in all statistical comparisons. Additional post hoc pairwise comparisons using Tukey’s HSD: *G. salinus*—*P. maeoticus* ($p = .0006$, *G. tigrinus*—*P. maeoticus* $p < .0001$, *G. tigrinus*—*G. salinus* $p = .0004$; Figure 2). In the low salinity treatment, adults of *G. salinus* started to die significantly later than those of *P. maeoticus* and *G. tigrinus*, though at similar salinities (Table 1; Figure 2). The mortality rate was similar among the three species (Table 1; Figure 2). At the onset of mortality of *G. salinus* was earlier than that of *P. maeoticus*, there was no difference in the mortality rate between the two species (Table 1; Figure 2). Consequently, at the end of the experiments, the mortality of *G. tigrinus* was the highest (94%), followed by that of *G salinus* (58%), and then by that of *P. maeoticus* (24%; ANOVA, $F(2, 12) = 55.7, p < .0001$; Tukey’s HSD: *G. salinus*—*P. maeoticus* $p = .0006$, *G. tigrinus*—*P. maeoticus* $p < .0001$, *G. tigrinus*—*G. salinus* $p = .0004$; Figure 2).

### 3 | RESULTS

#### 3.1 | Mortality of adults

In general, all three species demonstrated a wide range of salinity tolerance. Interestingly, the highest differences were observed in the control treatment where adults of *G. tigrinus* started to die significantly earlier and with a significantly faster mortality rate than those of *G. salinus* and *P. maeoticus* (Table 1; Figure 2). Though the onset of mortality of *G. salinus* was earlier than that of *P. maeoticus*, there was no difference in the mortality rate between the two species (Table 1; Figure 2). Consequently, at the end of the experiments, the mortality of *G. tigrinus* was the highest (94%), followed by that of *G salinus* (58%), and then by that of *P. maeoticus* (24%; ANOVA, $F(2, 12) = 55.7, p < .0001$; Tukey’s HSD: *G. salinus*—*P. maeoticus* $p = .0006$, *G. tigrinus*—*P. maeoticus* $p < .0001$, *G. tigrinus*—*G. salinus* $p = .0004$; Figure 2). In the low salinity treatment, adults of *G. salinus* started to die significantly later than those of *P. maeoticus* and *G. tigrinus*, though at similar salinities (Table 1; Figure 2). The mortality rate was similar among the three species (Table 1; Figure 2). At the end of the experiments, there was no significant difference in the mortalities among the species (70%, 54% and 62% for *G. salinus*, *P. maeoticus* and *G. tigrinus*, respectively; ANOVA, $F(2, 12) = 1.28, p = .313$; Figure 2). Finally, in the high salinity treatment, there were significant differences in the onset and rate of mortality among all three species (Table 1; Figure 2). At the end of the experiments, there was a difference in the mortalities between *G. salinus* and *P. maeoticus*, but not between *G. salinus* and *G. tigrinus*, nor between *P. maeoticus* and *G. tigrinus* (84%, 100% and 96% for *G. salinus*, *P. maeoticus* and *G. tigrinus*, respectively; ANOVA, $F(2, 12) = 6.5, p = .0122$; Tukey’s HSD: *G. salinus*—*P. maeoticus* $p = .0120$, *G. tigrinus*—*P. maeoticus* $p = .6708$, *G. tigrinus*—*G. salinus* $p = .0565$; Table 1; Figure 2). *Gammarus tigrinus* started to die significantly faster than *G. salinus*, while *P. maeoticus* started significantly later than the other two species (Table 1; Figure 2). However, the mortality rate of *P. maeoticus*

| Species compared | Experimental treatment | The onset of mortality (p-value) | The rate of mortality (p-value) |
|------------------|------------------------|---------------------------------|-----------------------------|
| *Gammarus salinus* | Control                | $.0001                          | .0873                       |
| *Pontogammarus maeoticus* | Low                   | .0133                           | .3701                       |
|                    | High                   | $.0001                          | $.0001                      |
| *Gammarus salinus* | Control                | $.0001                          | $.0001                      |
| *Gammarus tigrinus* | Low                   | .0015                           | .4050                       |
|                    | High                   | .0009                           | .0056                       |
| *Pontogammarus maeoticus* | Control              | $.0001                          | $.0001                      |
| *Gammarus tigrinus* | Low                   | .1357                           | .4402                       |
|                    | High                   | .0013                           | $.0001                      |

Note: The fit non-linear model using generalized least squares was used to test for differences between estimated parameters $Z_1$ and $Z_2$, and $Q_1$ and $Q_2$. Significant $p$-values are presented in bold. Bonferroni-type protection to guard against inflating the type I error rate and family-wise error rate of .05 were used for pairwise statistical comparisons.
was the fastest, followed by that of *G. salinus* and then by that of *G. tigrinus* (Table 1; Figure 2).

When mortalities of adults were compared among treatments, *G. tigrinus* performed the worst in the control treatment, while *P. maeoticus* was the worst in the high salinity treatment (Table 2; Figure 2). In the case of *G. salinus*, there were no extreme differences among the treatments, though some of them were significant (Table 2; Figure 2). The adults of *G. salinus* started to die significantly earlier in the high salinity treatment, with a faster mortality rate than those in the control and low salinity treatments (Table 2; Figure 2). Though

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**TABLE 2** Statistical comparisons of parameters between pairs of fitted curves for mortality of adults between treatments

| Species                  | Experimental treatment | The onset of mortality (p-value) | The rate of mortality (p-value) |
|--------------------------|------------------------|---------------------------------|--------------------------------|
| *Gammarus salinus*       | Control–High           | <.0001                          | <.0001                          |
|                          | Control–Low            | .6194                           | .0264                           |
|                          | High–Low               | <.0001                          | .0011                           |
| *Pontogammarus maeoticus*| Control–High           | <.0001                          | <.0001                          |
|                          | Control–Low            | <.0001                          | <.0001                          |
|                          | High–Low               | <.0001                          | <.0001                          |
| *Gammarus tigrinus*      | Control–High           | .3489                           | .4844                           |
|                          | Control–Low            | <.0001                          | .0077                           |
|                          | High–Low               | .0009                           | .0056                           |

Note: The fit non-linear model using generalized least squares was used to test for differences between estimated parameters $Z_1$ and $Z_2$, and $Q_1$ and $Q_2$. Significant $p$-values are presented in bold. Bonferroni-type protection to guard against inflating the type I error rate and family-wise error rate of .05 were used for pairwise statistical comparisons.
the onset of mortality in the control and low salinity treatments was similar, the mortality rate in the low salinity treatment was faster than that in the control (Table 2; Figure 2). In the case of *P. maeoticus*, the adults first started to die in the low salinity treatment, followed by the high salinity treatment and then by the control, with a faster mortality rate in the high salinity treatment compared to the low; the rate of mortality in the control treatment was the slowest (Table 2; Figure 2). Finally, the onset of mortality and mortality rate of *G. tigrinus* were significantly later and slower in the low salinity treatment than those in the control and high salinity treatments (Table 2; Figure 2).

### 3.2 | Comparison of mortality of adults in our study with that in Paiva et al. (2018)

While Paiva et al. (2018) clearly determined a high tolerance of *G. salinus* and *P. maeoticus* in the high and low salinity treatments, respectively, our study did not confirm the same tolerance (Table 3; Figure 2). Yet, a low tolerance of these two species was confirmed for the low and high salinity treatments, respectively (Table 3; Figure 2). In the case of *G. salinus*, adults in the low salinity treatment had a significantly faster mortality rate in our study than in Paiva et al. (2018), but with the same onset of mortality (Table 3; Figure 2). In the high salinity treatment, adults started to die significantly earlier in our study, with a significantly faster mortality rate (Table 3; Figure 2). While the mortality in the low salinity treatment at the end of the experiments was lower in our study than in Paiva et al. (2018) (70% and 87%, respectively), the opposite was observed in the high salinity treatment (84% and 25%, respectively; Table 3; Figure 2). In contrast to *G. salinus*, there was no difference between the two studies in the mortality rate of *P. maeoticus* in the high salinity treatment, but there were significant differences, with significantly faster onset and mortality rate in the low salinity treatment in our study compared to Paiva et al. (2018) (Table 3; Figure 2). In our study, the mortality in the low salinity treatment was almost double than that in Paiva et al. (2018) (54% and 29%, respectively; Table 3; Figure 2). In the case of *G. tigrinus*, our study revealed a significantly earlier onset and faster mortality rate in the high salinity treatment compared to those in Paiva et al. (2018) (Table 3; Figure 2). The mortalities in our study were also higher in both the low (62% and 53% in our study and Paiva et al. (2018) respectively) and high salinity treatments (96% and 77%, respectively; Table 3; Figure 2).

### 3.3 | Hatching success and mortality of juveniles

In general, all three species were able to reproduce across different salinities, but experienced mortality of juveniles throughout the six-week experimental period (Figure 3). However, juveniles of *G. salinus* hatched and survived in a broader salinity range than those of *P. maeoticus* and *G. tigrinus* (Figures 3 and 4). *Gammarus salinus* hatched at salinities from 1 to 34 g/kg, *P. maeoticus* from 0 to 22 g/kg, and *G. tigrinus* from 4 to 32 g/kg. The mean number of hatched juveniles was also the highest in the case of *G. salinus*, with 39.0, 19.2 and 15.7 juveniles in the control, low and high salinity treatments, respectively. The mean numbers of hatched juveniles of *P. maeoticus* were 5.0, 4.8 and 3.8, while those of *G. tigrinus* were 17.9, 18.3 and 13.5, respectively. In the third week of the experiment, all juveniles of *G. salinus* died at 1 and 7 g/kg, while in the case of *P. maeoticus* and *G. tigrinus*, there was no survival at 0, 1, 19 and 23 g/kg and 5, 27 and 31 g/kg, respectively (Figure 3). Consequently, until the end of the experiment juveniles of *G. salinus* survived in a slightly narrower salinity range than their parents (i.e., adults and juveniles survived in salinities from 0 to 40 g/kg and 3 to 33 g/kg, respectively; Figure 4). The mean mortalities across all salinities were 44.2%, 76.8% and 43.5% in the control, low and high salinity treatments, respectively. Statistical analyses determined a significant difference among the treatments for juveniles of *G. salinus* (Kruskal–Wallis test, \( \chi^2 = 12.189, df = 2, p\text{-value} = .0022 \)), with a significantly higher mortality in the low salinity treatment when compared to the control (Wilcoxon’s rank sum test, \( p = .0036 \)). The difference was not found between the low and high salinity treatments due to low statistical power (Wilcoxon’s rank sum test, \( p = .2322 \)). In the case of *P. maeoticus* and *G. tigrinus*, juveniles survived in much narrower salinity ranges than their parents (i.e., adults and juveniles of *P. maeoticus*

| TABLE 3 | Statistical comparisons of parameters between pairs of fitted curves for mortality of adults between our study and Paiva et al. (2018) |
| --- | --- | --- | --- |
| Species compared | Experimental treatment | The onset of mortality (p-value) | The rate of mortality (p-value) |
| Gammarus salinus | Low | .0551 | <.0001 |
| | High | <.0001 | <.0001 |
| Pontogammarus maeoticus | Low | <.0001 | <.0001 |
| | High | <.0001 | .6845 |
| Gammarus tigrinus | Low | .1357 | .4402 |
| | High | <.0001 | .0004 |

Note: The control treatments were not compared as Paiva et al. (2018) did not report controls. The fit non-linear model using generalized least squares was used to test for differences between estimated parameters \( Z_1 \) and \( Z_2 \), and \( Q_1 \) and \( Q_2 \). Significant p-values are presented in bold. Bonferroni-type protection to guard against inflating the type I error rate and family-wise error rate of .05 were used for pairwise statistical comparisons.
survived in salinities from 0 to 34 g/kg and 5 to 23 g/kg, respectively, while those of *G. tigrinus* from 0 to 40 g/kg and 9 to 23 g/kg, respectively; Figure 4). The mean mortalities of juveniles of *P. maeoticus* were 73.4%, 60.0% and 92.0% in the control, low and high salinity treatments, respectively; those of *G. tigrinus* were 52.3%, 63.3% and 72.2%, respectively. There was no significant difference in mortality of juveniles among treatments neither for *P. maeoticus* nor *G. tigrinus* (Kruskal–Wallis test, $\chi^2 = 5.459$, df = 2, $p$-value = 0.0653 and $\chi^2 = 4.042$, df = 2, $p$-value = 0.1325, respectively).

### 3.4 | Growth rates of juveniles

Growth of juveniles differed among species and treatments, with the fastest growth recorded for juveniles of *G. salinus* in the high salinity treatment at 25 g/kg, and the slowest for *P. maeoticus* in the control and high salinity treatment at 10 and 11 g/kg, respectively (Figure 5). The mean cephalon length of *G. salinus* increased from 375.8 (week 0) to 907.7 $\mu$m (week 6), with the juveniles at 3, 16 and 29 g/kg having a significantly shorter length than those at the other
salinities (Table 4; Figure 5). In the case of *P. maeoticus*, the mean cephalon length increased from 345.1 (week 0) to 503.3 µm (week 6, Figure 5). There was no significant difference in cephalon length among the different salinities (ANOVA, \( F(1, 51) = 0.503, p = .4810 \)). Finally, the mean cephalon length of juveniles of *G. tigrinus* increased from 348.2 (week 0) to 830.3 µm (week 6), with juveniles hatched at 23 g/kg having significantly shorter cephalons than those hatched at 9, 10, 11 and 15 g/kg (\( F(5, 190) = 6.075, p < .0001 \); Table 5; Figure 5).

4 | DISCUSSION

Due to an increasing number of NIS worldwide and their impacts on ecosystems and biodiversity (Seebens et al., 2018; Simberloff, 2011; Strayer et al., 2006), recently numerous studies have been testing species resilience to changes in environmental conditions, such as temperature and salinity (Castles, Clemmesen, & Briski, 2019; McFarland et al., 2015; Paiva et al., 2018). However, many of those studies were conducted on adult stages, while it still remains unclear whether those species will reproduce in and how their juveniles would respond to those changing conditions. In this study, we compared the salinity tolerance of adults and juveniles of three gammarid species originating from Northern Europe, the Ponto-Caspian region and North America to determine whether juveniles would perform equally well as adults. Additionally, we compared our study with Paiva et al. (2018). Not surprisingly, our study determined that both adults and juveniles of all three species tolerated wide ranges of salinity, with juveniles of *G. salinus* tolerating only slightly narrower salinity range than their parents, while those of *P. maeoticus* and *G. tigrinus* much narrower range. At the end of the experiments, mortalities of adults of *G. salinus* and *P. maeoticus* were significantly different in the high salinity treatment, but not in the low, with *P. maeoticus* having 100% mortality in the high salinity treatment above 34 g/kg. Importantly, our study determined better performance of juveniles of *G. salinus* in higher salinities and those of *P. maeoticus* in lower salinities. Consequently, even though the adults in our study did not reveal exactly the same pattern of salinity tolerance as determined by Paiva et al. (2018), we found similar pattern for juveniles. Based on juvenile salinity tolerance, our study supports further the finding of Paiva et al. (2018) that Northern European species perform better in higher, while Ponto-Caspian in lower salinities.

By investigating the salinity tolerance of adult euryhaline gammarids, Paiva et al. (2018) determined different patterns of tolerance among species from different regions, with Northern European taxa showing lower mortality in fully marine and Ponto-Caspian taxa in freshwater conditions. Therefore, the authors suggested that Northern European species and Ponto-Caspian species may be of marine and freshwater origin, respectively. Interestingly, even though we tested the same populations of *G. salinus* and *P. maeoticus* as did Paiva et al. (2018), we found similar pattern of salinity tolerance only for juveniles, but not for adults. In addition, we have to emphasize that even though juveniles of *P. maeoticus* in our study performed better in lower salinities, they did not survive in freshwater conditions. There may be two reasons why juveniles failed to survive in fresh water. The first and most probable reason was very low number of hatched juveniles. As mortality of juveniles in *r*-strategy species is very high (Ramírez-Llodra, 2002), the mortality in freshwater conditions in our experiments may be simply due to chance, not to environmental conditions. The second reason may be low genetic diversity of our population, as the population was started with 96 individuals, transferred to the laboratory, and kept for one and a half years before the experiments were conducted. However, a more pronounced difference between our study and that of Paiva et al. (2018) was in the survival of adults, with our study not demonstrating better survival of *G. salinus* and *P. maeoticus* in fully marine conditions.
and freshwater conditions, respectively, as Paiva et al. (2018) determined. Though, the lower salinity tolerance of adults in our study may be caused by the use of pairs in precopula rather than single individuals as in Paiva et al. (2018). Gammarid females can be fertilized only for a short period of time after moultting. Therefore, a male finds a pre-moult female and they form a precopula pair, with the male...
carrying the female (Hynes, 1955; Jormalainen, 1998; Parker, 1974). The precopula stage brings a number of costs to both sexes, such as energetic costs of moulting and prolonged mate guarding to a female and those of locomotion of the pair to a male (Elwood & Dick, 1990; Jormalainen, 1998; Sparkes, Keogh, & Pary, 1996). Finally, an additional reason for better survival of *P. maeoticus* in fresh water in Paiva et al. (2018) than in our study may be connected to water chemistry as the experiments in Paiva et al. (2018) were conducted using the ambient water of the species collection site, while our experiments were conducted using Baltic Sea water and tap water in Germany. Consequently, as our precopula pairs were exposed not only to salinity stress of our experiments, but also to reproductive stress, and in the case of *P. maeoticus* possible differences in water chemistry used in the experiments, the energetic costs of the species were probably exceeded leading to lower survival than that in Paiva et al. (2018).

Hatching success of the three species differed among species and in the case of *G. salinus* among treatments. In the control treatments, the hatching success of *G. salinus* was eightfold higher than that of *P. maeoticus* and twofold higher than that of *G. tigrinus*. Interestingly, while the numbers of hatched juveniles of *G. salinus* in the low and high salinity treatments were half of that in the control this was not the case for *P. maeoticus* and *G. tigrinus*. The differences in hatching success among species may be related to their different reproductive strategies. *Gammarus salinus* produces three to seven broods per generation with approximately 30 juveniles per brood, *P. maeoticus* only three broods with approximately nine juveniles per brood, while *G. tigrinus* produces at least ten broods with ten to 50 juveniles per brood (Nazarhaghighi, Shabanipour, Zarghami, & Etemadi-Deylami, 2013; Sutcliffe, 1993 and references therein). Furthermore, in our study juveniles of *G. salinus* and *G. tigrinus* hatched in much wider ranges of salinities than those of *P. maeoticus*. Environmental conditions often largely affect hatching success and development (Donelson, Munday, & McCormick, 2009; English, Pen, Shea, & Uller, 2015). Often, environmental stress causes parents to use their energy resources for their own survival instead of for the reproduction of offspring (Glazier, 1999). Consequently, viability of embryos and number of broods produced may be lower, and hatched juveniles smaller and weaker (e.g., Mills & Fish, 1980; Neuparth et al., 2002; Steele & Steele, 1991; Vlasblom & Bolier, 1971). Therefore, in our study, besides the different reproductive strategies among species, salinity stress resulted in the production of smaller broods of *G. salinus* in the low and high salinity treatments, and even prevented hatching of *P. maeoticus* in salinities above 23 g/kg.

Juvenile survival and growth also differed among the species and particularly among the treatments. While juveniles of *G. salinus* and *G. tigrinus* had better survival in higher salinities, *P. maeoticus* survived better in lower. Furthermore, the growth of juveniles of *G. salinus* was slower in very high and low salinities, while juveniles of *P. maeoticus* and *G. tigrinus* did not survive at all in those salinities. These results suggest that stressful environmental conditions affect the use of energy resources of juveniles, as they redirect energy from growth to survival (Anger, Spivak, & Luppi, 1998; Torres, Giménez, & Anger, 2011). In addition, smaller and weaker juveniles,

**Table 4** Pairwise comparisons of the effect of salinity on the cephalon growth of *G. salinus* in week 6

| Hatching salinity of the juvenile cohort (g/kg) | 3  | 11  | 15  | 16  | 17  | 21  | 25  | 29 |
|-----------------------------------------------|----|-----|-----|-----|-----|-----|-----|----|
| Hatching salinity of the juvenile cohort (g/kg) | 11 | 16  | 15  | 17  | 21  | 25  | 29  | 33 |
| Hatching salinity of the juvenile cohort (g/kg) | <0.0001 | 1.0000 | <0.0001 | 1.0000 | <0.0001 | <0.0001 | 1.0000 | 1.0000 |
| Hatching salinity of the juvenile cohort (g/kg) | 0.0066 | <0.0001 | 1.0000 | <0.0001 | <0.0001 | 1.0000 | 1.0000 | 1.0000 |
| Hatching salinity of the juvenile cohort (g/kg) | 0.0015 | 0.0015 | 0.0015 | 1.0000 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Hatching salinity of the juvenile cohort (g/kg) | 0.4229 | 0.4229 | 0.4229 | 0.4229 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| Hatching salinity of the juvenile cohort (g/kg) | 0.0371 | 0.0371 | 0.0371 | 0.0371 | 0.0371 | 0.0371 | 0.0371 | 0.0371 |
| Note: Wilcoxon's rank sum test with Bonferroni's adjustment was used to test the effect among different salinities. |
Tukey’s HSD test was used to test the effect among different salinities. Often produced by stressed parents, commonly will grow slower or even run out of energy due to reduced starting resources and additional environmental stress (e.g., Mills & Fish, 1980; Neuparth et al., 2002; Steele & Steele, 1991; Vlasblom & Boier, 1971). In our study, *P. maeoticus* adults were probably experiencing great stress as salinity was increasing, and consequently, though they survived until 34 g/kg, they did not produce any juveniles above 23 g/kg. In contrast, *G. salinus* experienced a gradual increase in stress as salinity went further from the species ambient salinity, with freshwater conditions being too stressful for production of juveniles. Interestingly, even though *G. tigrinus* adults experienced great stress and high mortality, they did not seem to have redirected much energy from reproduction to their own survival, and they were consequently able to produce juveniles in almost the whole range of salinities they survived in. Thus, our study supports the finding of Paiva et al. (2018), where *G. salinus* performs better in higher, while *P. maeoticus* in lower salinities.

In our study, as well as in Paiva et al. (2018) and Casties et al. (2019), the mortalities of adults of *G. tigrinus* were the highest in the control treatment, irrespective of whether salinity or temperature tolerance was examined. Interestingly, we did not observe the same pattern for juveniles. Juveniles in our study had the lowest mortality in the control treatment when compared to those in the low and high treatments, as well as to the mortality of their parents in any treatment. Both Paiva et al. (2018) and Casties et al. (2019) suggested that dark spots, regularly observed on animals, were most likely parasitic oomycetes that reduced immune function of animals (Kestrup, Thomas, van Rensburg, Ricciardi, & Duffy, 2011), and as the parasite was not able to tolerate changes in environmental conditions of the experiments, the highest mortalities were observed in the control treatments. In our study, dark spots were also observed on individuals. However, our juveniles did not demonstrate low performance in the control treatment. Therefore, either the oomycete cannot be transferred directly from parents to offspring or an additional parasite that needs an additional host might be reducing the immune system of adults, such as microphallid trematodes (MacNeil et al., 2003; Mouritsen, Tompkins, & Poulin, 2005; Prugnolle, Liu, de Meeüs, & Balloux, 2005). Consequently, when examining environmental tolerance of species, the possibility of parasitic infections and/or other diseases of the tested populations should be taken into account.

### Table 5

| Hatching salinity of the juvenile cohort (g/kg) | 9   | 10  | 11  | 15  | 19  |
|-----------------------------------------------|-----|-----|-----|-----|-----|
| Hatching salinity of the juvenile cohort (g/kg) |     |     |     |     |     |
| 10                                           | 0.8778 | –   | –   | –   | –   |
| 11                                           | 1.0000 | 1.0000 | –   | –   | –   |
| 15                                           | 0.8032 | 1.0000 | 1.0000 | –   | –   |
| 19                                           | 0.5443 | 1.0000 | 1.0000 | 1.0000 | –   |
| 23                                           | 0.0252 | <0.0001 | 0.0074 | 0.0332 | 0.1428 |

Note: Tukey’s HSD test was used to test the effect among different salinities.

### Conclusion

Global biodiversity and ecosystems are highly impacted by anthropogenic activities, such as climate change and introduction of NIS (Capinha et al., 2015; IPCC, 2014; Sala et al., 2000). Changes in ecosystems due to increasing temperature, heat waves, acidification and decreasing salinities pose additional energetic costs to native species, with some of them hardly coping with these stressors (Chapman, 2017; IPCC, 2014; Solan & Whiteley, 2016). In addition, continuously arriving NIS, which are often more resistant to multiple stressors and pre-adapted to anthropogenic impact, use these opportunities putting an additional burden on already stressed ecosystems (Holopainen et al., 2016; Hubbauer et al., 2011; IPCC, 2014). Consequently, numerous studies have been testing species resilience to environmental fluctuations, yet, rarely both adults and juveniles were tested (Casties et al., 2019; McFarland et al., 2015; Paiva et al., 2018). By comparing salinity tolerance of adults and juveniles of three gammarid species originating from Northern Europe, the Ponto-Caspian region and North America, our study demonstrated that juveniles were not able to tolerate the same stress as adults. Furthermore, geographic origin of species plays an important role in their environmental tolerance. Even though our tested species came from similar ambient salinities, our study determined significant differences in direction of salinity tolerance, with Northern European species performing better in higher, while Ponto-Caspian in lower salinities. Here, we emphasize that additional studies are needed to confirm whether these findings can be generalized. For example, *P. maeoticus*, tested here, has invading history only at two locations close to the Ponto-Caspian region (Figure 1). Therefore, it would be beneficial to determine whether *P. robustoides*, which is a widespread NIS (Bij de Vaate et al., 2002), has the same salinity tolerance or whether it is even more resistant to salinity stress than *P. maeoticus*. Likewise, populations of species in invaded areas may differ from the ones in native regions. Here, we tested the population of *G. tigrinus* from a non-indigenous region, where low genetic diversity due to the founder effect or high genetic diversity due to introgression from diverse source populations may have skewed mean fitness of our tested population. Therefore, studies testing populations from both native and non-indigenous regions would provide valuable information in determining stress tolerance of diverse taxa. Finally, we emphasize that multiple factors, such as

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**Table 5** Pairwise comparisons of the effect of salinity on the cephalon growth of *G. tigrinus* in week 6.
early life history stages, condition of the tested populations, and water chemistry and parasitism, should be taken into account in determining environmental tolerance of species and in constructing models to predict changes in species distributions, resilience of ecosystems and biodiversity change.

ACKNOWLEDGMENTS

We are grateful for financial support from the Alexander von Humboldt Sofja Kovalevskaja Award to EB. We would like to specially thank L. Kittu, M. Johnson, A. Lechtenbörger, C. Beckmann, I. Stoltenberg and F. Brink for help with sampling, salinity tests and measurements.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/ddi.13147.

DATA AVAILABILITY STATEMENT

The primary dataset containing experimental results is available at Pangaea: https://doi.org/10.1594/PANGAEA.90829, and Dryad: https://doi.org/10.5061/dryad.3n5tb2rf5.

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