GEOMAR
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# Warming, not acidification, favours survival of non-indigenous over native gammarid species

Master's Thesis

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## List of Abbreviations

| | |
|---|---|
| **NIS** | Non-indigenous species |
| **$CO_2$** | Carbon dioxide |
| **$pCO_2$** | Carbon dioxide partial pressure |

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## Abstract

Anthropogenic activities, including non-indigenous species (NIS) introduction and climate change, among other stressors, have considerably affected ecosystems and socio-economies globally. Despite the widely acknowledged individual roles of NIS and global warming in biodiversity change, predicting the connection between biological invasions and climate change still remains a fundamental challenge and requires urgent attention due to a timely importance for proper conservation management. To improve our understanding on the interaction between climate change and NIS on biological communities, laboratory experiments were conducted to test the temperature and $p\mathrm{CO}_2$ tolerance of four gammarid species: two native Baltic Sea species (*Gammarus locusta* and *Gammarus salinus*), one Ponto-Caspian NIS (*Pontogammarus maeoticus*) and one North American NIS (*Gammarus tigrinus*). The results demonstrated that an increase in $p\mathrm{CO}_2$ level was not a significant driver of mortality, neither by itself nor in combination with increased temperature, for any of the tested species. However, temperature was significant, and differentially affected the tested species. The most sensitive was the native *G. locusta* which experienced 100% mortality at 24 °C. The second native species, *G. salinus*, performed better than *G. locusta*, but was still significantly more sensitive to temperature increase than either of the NIS. In contrast, NIS performed better than native species with warming, whereby particularly the Ponto-Caspian *P. maeoticus* did not demonstrate any difference in its performance between the temperature treatments. With the predicted environmental changes in the Baltic Sea, shifts in distributions of native taxa towards colder areas may be expected, while their niches might be filled by NIS, particularly those from the Ponto-Caspian region. As the chance of impactful NIS arriving increases with the increased number of establishing NIS, the danger of the negative impact connected with the new NIS may rise as well.

# Zusamenfassung

Anthropogene Aktivitäten, darunter die Einführung nicht einheimischer Arten (NIS) und der Klimawandel, haben neben anderen Stressfaktoren die Ökosysteme und die sozioökonomischen Verhältnisse weltweit erheblich beeinträchtigt. Trotz der weithin anerkannten individuellen Rolle von NIS und globaler Erwärmung bei der Veränderung der biologischen Vielfalt stellt die Vorhersage des Zusammenhangs zwischen biologischen Invasionen und Klimawandel nach wie vor eine grundlegende Herausforderung dar und erfordert aufgrund der zeitlichen Bedeutung für ein angemessenes Naturschutzmanagement dringende Aufmerksamkeit. Um unser Verständnis der Wechselwirkung zwischen Klimawandel und NIS auf biologische Gemeinschaften zu verbessern, wurden Laborexperimente durchgeführt, um die Temperatur- und pCO2-Toleranz von vier Gammaridenarten zu testen: zwei heimische Ostsee-Arten (*Gammarus locusta* und *Gammarus salinus*), eine ponto-kaspische NIS (*Pontogammarus maeoticus*) und eine nordamerikanische NIS (*Gammarus tigrinus*). Die Ergebnisse zeigten, dass ein Anstieg des $p\mathrm{CO}_2$-Gehalts bei keiner der untersuchten Arten einen signifikanten Einfluss auf die Mortalität hatte, weder allein noch in Kombination mit einer erhöhten Temperatur. Der Einfluss der Temperatur war jedoch signifikant und wirkte sich unterschiedlich auf die untersuchten Arten aus. Die empfindlichste Art war die einheimische *G. locusta*, die bei 24 °C eine Sterblichkeit von 100 % aufwies. Die zweite einheimische Art, *G. salinus*, schnitt besser ab als *G. locusta*, reagierte aber immer noch deutlich empfindlicher auf einen Temperaturanstieg als eine der NIS. Im Gegensatz dazu schnitten die NIS bei Erwärmung besser ab als die einheimischen Arten, wobei insbesondere die pontokaspische *P. maeoticus* keinen Unterschied in ihrer Leistung zwischen den Temperaturbehandlungen aufwies. Angesichts der prognostizierten Umweltveränderungen in der Ostsee ist zu erwarten, dass sich das Verbreitungsgebiet einheimischer Taxa in kältere Gebiete verlagert, während ihre Nischen von NIS, insbesondere

aus der ponto-kaspischen Region, besetzt werden könnten. Da mit der zunehmenden Zahl der sich ansiedelnden NIS auch die Wahrscheinlichkeit der Ansiedlung von NIS steigt, kann auch die Gefahr negativer Auswirkungen durch die neuen NIS zunehmen.

# Introduction

In the last century, anthropogenic activities, such as introduction of non-indigenous species (NIS; species moved by human activity outside their native region) and climate change, among other stressors, have significantly affected numerous ecosystems worldwide, leading to reductions in biodiversity, and occasionally, to losses of ecosystem functioning and services (Olden *et al.,* 2004; Hawkins, 2012; Simberloff, 2013; IPCC, 2014; Linders *et al.,* 2019; Pyšek *et al.,* 2020). The rate at which NIS are introduced and detected is accelerating (Bailey *et al.,* 2020; Seebens *et al.,* 2021), directly mirroring increases in globalization of trade and travel, the development of new transport pathways, and the expansion of existing ones (Hulme, 2009; Galil *et al.,* 2015; Seebens *et al.,* 2018, 2021). Predicted growth in global maritime trade may reach 240 – 1209% by 2050, with an associated 3 – 20-fold increase in invasion risk (Sardain *et al.,* 2019). As the number of problematic NIS is positively correlated with the total number of established NIS (Ricciardi & Kipp, 2008), the magnitude of adverse impacts associated with invasions is expected to increase rapidly (Blackburn *et al.,* 2019; Essl *et al.,* 2020; Pyšek *et al.,* 2020; Cuthbert *et al.,* 2021; Diagne *et al.,* 2021).

The increase in gas emissions since the beginning of the eighteenth century has caused persisting climatic changes, with rising temperatures and increase in greenhouse gases, such as methane and carbon dioxide ($CO_2$), being among the most prominent ones (IPCC, 2014). Between 1880 and 2012, ocean surface temperature has risen 0.85 °C on average globally. More noticeable changes have occurred within the upper 75 m, where in the last 40 years temperature increased on average more than 0.1 °C per decade. Furthermore, the partial pressure of $CO_2$ has risen from ~316 ppm to ~408 ppm since 1960 (Tans & Keeling, 2019). This has resulted in an increased uptake of $CO_2$ by oceans and led to a lower pH value, a process commonly referred to as ocean acidification (Doney *et al.,* 2009; NOAA, 2018). Since carbonate ions are decreasing due to a higher uptake of $CO_2$ by the oceans, marine organisms may be directly affected by the change in this water chemistry parameter. In particular, calcifying organisms that need carbonate ions for growth (corals), building shells (mollusks) and exoskeletons (crustaceans) are considered to be threatened the most by falling pH values (IPCC, 2007, 2014; Whiteley, 2011; Kroeker *et al.,* 2013; Heuer & Grosell, 2014; Waldbusser *et al.,* 2014). Consequently, many studies have already observed shifts in migrations, geographic range changes and poleward movement of species due to increase in temperatures and changes to water chemistry (Chen *et al.,* 2011; Lockwood *et al.,* 2013, Poloczanska *et al.*, 2013; VanDerWal *et al.,* 2013; Bates *et al.,* 2014; Holopainen *et al.,* 2016; Smith *et al.*, 2019; Pinsky *et al.,* 2020).

Currently, the Baltic Sea is exposed to many anthropogenic stressors (IPCC, 2014; Rutgersson *et al.,* 2014; Andersson *et al.,* 2015; Reusch *et al.,* 2018). It is a geologically young, semi-enclosed brackish waterbody that was formed by glaciation (Leppäkoski *et al.,* 2002). Interestingly, the system has the highest rate of temperature change in recent history, with an increase of more than 1 °C per decade, exceeding the global average seven times over (Belkin, 2009; IPCC, 2014; Reusch *et al.,* 2018). Further increases by approximately 2 – 3 °C have been predicted for the end of the twenty-first century (IPCC, 2007, 2014; Christensen et al., 2022). Likewise, ocean acidification corresponding to $pCO_2$ levels beyond those predicted under a 2 °C warming scenario has also been observed (Melzner *et al.,* 2013; Rutgersson *et al.,* 2014; Reusch *et al.,* 2018; Christensen *et al.,* 2022), and the system has already been invaded by at least 125 NIS (Casties *et al.,* 2016; Cuthbert *et al.,* 2022). Thereby, a potential synergy in environmental impacts between NIS and rapid environmental change may be forming. Native species in the Baltic Sea have a high phenotypic plasticity, but some populations already inhabit areas characterized by conditions at the limit of their environmental tolerance (Arndt, 1989; Gräwe *et al.,* 2013). Since anthropogenic impacts are still continuing to increase, it is of paramount importance to understand their effects on individual species as well as entire communities and ecosystems, with particular interest on both native and NIS (Kroeker *et al.,* 2013; Lockwood *et al.,* 2013; IPCC, 2014; Gunderson *et al.,* 2016; Reusch *et al.,* 2018; Casties *et al.,* 2019; Ricciardi *et al.,* 2021). Anthropogenic stressors to the Baltic Sea have caused environmental changes exceeding the global averages (IPCC, 2014; Rutgersson *et al.,* 2014; Andersson *et al.,* 2015; Reusch *et al.,* 2018), thus the studies conducted there may not only provide important insight into resistance and resilience of the Baltic ecosystems to those stressors, but also the understanding of future changes globally.

To improve our understanding on the interaction between climate change and NIS on biological communities, laboratory experiments were conducted to test the temperature and $pCO_2$ tolerance of four gammarid species. Gammarid taxa were used as they are found globally as important keystone species in ecosystems, and as they are adapted to various environmental conditions (Gerhardt *et al.,* 2011; Cuthbert *et al.,* 2020), and are successful NIS and often cause declines in abundances of native gammarid species (Witt *et al.,* 1997; Pinkster *et al*., 1992; Dermott *et al.,* 1998; Grabowski *et al.,* 2006; Kotta *et al.,* 2013; Jänes *et al.,* 2015; Cuthbert *et al.,* 2020). Two Baltic (*Gammarus locusta* and *G. salinus*), one Ponto-Caspian (*Pontogammarus maeoticus*) and one North American species (*G. tigrinus*) were tested at two temperatures (i.e. 16 and 24 °C) and four $pCO_2$ levels (400, 1600, 2700, and 3500 ppm) factorially. To determine performances of the species during the experiments, mortality of individuals in different treatment conditions was tracked. The hierarchical null hypotheses tested state that there is no difference in mortality: i) among different species; ii) within and among different species in different temperature conditions; iii) within and among different species in different $pCO_2$ conditions; and iv) within and among different species in different combinations of temperature and $pCO_2$.

## Materials and Methods

### ***Organism collection and husbandry***

Four gammarid species were collected for experimentation: two native (i.e. *Gammarus locusta* and *Gammarus salinus*) and two non-native (i.e. *Gammarus tigrinus* and *Pontogammarus maeoticus*) species. The two native gammarid species were collected from the Kiel Fjord in Kiel, Germany: *G. locusta* were collected at Falckenstein Beach (54°23'36.2"N 10°11'21.4"E) in February-April 2019; and *G. salinus* were collected at Kieler Meeresfarm (54°22'55.3"N 10°09'43.6"E) in July 2020. Collection of non-native *G. tigrinus* specimens was undertaken at Dassower Lake, Lübeck, Germany (53°53'52.1"N 10°54'54.0"E) in June 2020. Non-native *P. maeoticus* were collected near Jafrud, Iran (37°29′09″N, 49°30′20″E) in October 2014. Following collection, all species were transported in source water from their respective sampling sites to a climate chamber at GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany, and maintained at 16-18 °C under a 12:12h light and dark regime. Animals were housed at their respective averaged ambient salinities (*G. locusta* = 14 g/kg; *G. salinus* = 14 g/kg; *G. tigrinus* = 10 g/kg; *P. maeoticus* = 10 g/kg) in 56 L glass aquaria (separately per species). Each aquarium contained an internal filtration system to maintain water quality, and was supplied weekly with a mixture of commercial crustacean food (Tetra Mix, Tetra Crusta, and Dr. Shrimp Healthy). The water used for housing was pumped from the Kiel Fjord, and 5 µm-filtered. Where necessary, ambient salinity was adjusted with Instant Ocean® artificial sea water or dechlorinated tap water.

### ***Laboratory experimentation***

Separate batches of trials according to species availability were performed in a climate chamber during April–May 2019 (corresponding to *G. locusta* and performed by Louisa Langrehr, who used this data for her master’s thesis submitted at Carl von Ossietzky Universitäat

Oldenburg titled : "Do anthropogenic stressors facilitate adaptation and invasion of species? Assessment of the stress resistance of two amphipod species(Gammarus locusta and Gammarus salinus) from anthropogenically impacted and protected habitats.") and July–November 2020. A crossed experimental design consisting of two temperature levels (16 and 24 °C) and four $p$CO$_2$ levels (400, 1600, 2700, and 3500 ppm) was used, yielding a total of eight treatment combinations per species (Bleich *et al*., 2008). One replicate of a given treatment consisted of a single 2 L aquarium and contained ten randomly selected conspecific adult individuals; each treatment combination was performed in triplicate, yielding a combined total of 24 replicates across the eight 'temperature × $p$CO$_2$' scenarios per species. The 2 L aquaria were placed in water baths at the desired temperature in batches of six, with $p$CO$_2$ levels being randomly assigned within each water bath. Each 2 L aquarium contained artificial habitat, and the water was continuously infused with its determined $p$CO$_2$ level using an air stone; a fastened plastic cover stabilized the $p$CO$_2$ level of each container.

Figure 1. Experimental set up showing six experimental units in a water bath. Individual fastened plastic covers, artificial habitat, and aeration input can be observed. (Image by ©Louisa Langrehr)

One day prior to the start of experimentation, the aquaria were placed in the water baths and aerated at their assigned temperature and $p$CO$_2$ combination, to ensure the desired conditions were reached before the introduction of the animals. The salinity throughout the experiments was maintained at the ambient condition for each species, i.e. 14 g/kg for *G. locusta* and *G. salinus*, and 10 g/kg for *G. tigrinus* and *P. maeoticus*.

The experimental duration was 30 days, during which each aquarium was continuously aerated at its given $pCO_2$ level and animals were fed *ad libitum* with a ground mix of crustacean food (Tetra Mix, Tetra Crusta, and Dr. Shrimp Healthy). Mortality rates (i.e. number of dead out of the original ten supplied) were assessed daily to measure survivability among species under each replicated temperature and $pCO_2$ combination. Salinity (WTW Cond 3110 with Tetracon 325 probe), pH (WTW pH 3110 with SenTix 81 pH electrode), and temperature were recorded daily, and dead individuals, molted exoskeletons, and newly emerged juveniles were removed each day. A 50% water exchange was performed at most every ten days in each replicate. Water for the exchange was prepared in advance to meet the assigned temperature and $pCO_2$ levels.

### *Statistical analyses*

Binomial generalized linear models with logit links were used to test for differences in mortality with the raw data, with two separate models used for the mid-point (i.e. day 15) and end-point (i.e. day 30) of the experiment. Each model considered mortality as a response variable (i.e. number of dead out of original ten supplied) with species (i.e. four level factor), temperature (i.e. continuous term) and $pCO_2$ (i.e. continuous term) included as factorial explanatory variables. Residuals in each model were checked for over- and under-dispersion using simulations which were compared to fitted residuals (Hartig, 2020). All possible two- and three-way interactions were included in a full model at each time point, which was reduced backward stepwise via analysis of deviance to obtain the most parsimonious structure that contained only significant terms (Crawley, 2007). Complete separation was observed in some treatment groups at the end-point (i.e. owing to complete mortality across replicates in a group), and thus bias reductions were used for model coefficient corrections (Kosmidis, 2020). Terms in the final models were computed using likelihood ratio tests *via* analysis of deviance, with Type III sums of squares incorporated in the presence of a significant interaction (Fox and Weisberg, 2019). *Post-hoc* comparisons were computed pairwise where a term yielded significance *via* Tukey tests (Lenth, 2020). Significance was always inferred considering an alpha of 0.05. Generalized linear modelling was performed in R v4.0.2 (R Core Team, 2020).

Then, tests for differences in the onset and rate of mortality between species in each treatment over time were performed. To test for differences between species, a mortality curve was constructed for each species for each treatment using data from the three replicates, described by the equation (Briski *et al.*, 2008, 2011; Paiva *et al.*, 2018):

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

where $Z$ is the slope of the mortality rate, $t$ is temperature and $Q$ is the onset of mortality. The model was then expanded to compare the slope and onset of the mortality rate between two curves using the equation:

$$y=100/[1+e^{-(Z_1+Z_2)(t-Q_1-Q_2)}] \tag{2}$$

where $Z_1$ and $Z_2$ are the slopes of the mortality rates and $Q_1$ and $Q_2$ the onsets of mortality, for the first and second curves respectively. All pairs of curves were statistically compared by the fit non-linear model using generalized least squares. Significance 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. The family-wise error rate of 0.05 was used (i.e. 0.001). All tests were performed using S-Plus 6.1 (S-

Plus ® 6.1, 2002; Insightful Corp., Seattle, WA, USA). Raw data are available as Supplementary Information.

## Results

Species, temperature, and their interactions were significant variables at both time-points of the experiment (i.e. the mid-point and end-point), while $p\mathrm{CO}_2$ and any interactions involving it were not shown to be a significant driver of mortality of the tested species (Table 1). In general, the native gammarid species (i.e. *G. locusta* and *G. salinus*) had higher mortality compared to the two non-native species (i.e. *G. tigrinus* and *P. maeoticus*) in both tested temperatures (i.e. 16 and 24 °C; Figure 1). *Gammarus salinus* had the highest mortality at 16 °C at both time-points of the experiment, whereas *G. locusta* at 24 °C for both time points (Figure 1). *Gammarus locusta* was also the only species demonstrating 100% mortality at 24 °C; there was no 100% mortality for any species at 16 °C (Figures 1, 2 and 3).

Table 1. Results of binomial generalized linear models with logit links used to test for differences in mortality rates for the mid-point (i.e. day 15) and end-point (i.e. day 30) of the experiment. Results are shown from the full model (i.e. with all terms) and refined model (i.e. following backward stepwise deletion). Likelihood-ratio (LR) Chi-square values were computed from analysis of deviance with Type III (day 15) and Type II (day 30) sums of squares and significant terms are emboldened.

| | Day 15 (Type III tests) | | | Day 30 (Type II tests) | | |
|---|---|---|---|---|---|---|
| | df | LR Chi-square | *p* | df | LR Chi-square | *p* |
| full model | | | | | | |
| species | 3 | 21.9064 | **< 0.0001** | 3 | 49.345 | **< 0.0001** |
| temperature | 1 | 18.1881 | **< 0.0001** | 1 | 53.484 | **< 0.0001** |
| $p\mathrm{CO}_2$ | 1 | 0.4688 | 0.4935 | 1 | 0.170 | 0.6799 |
| species:temperature | 3 | 29.2944 | **< 0.0001** | 3 | 79.431 | **< 0.0001** |

| | | | | | | |
|---|---|---|---|---|---|---|
| species:$p$CO$_2$ | 3 | 1.2141 | 0.7496 | 3 | 1.056 | 0.7877 |
| temperature: $p$CO$_2$ | 1 | 0.5160 | 0.4725 | 1 | 0.166 | 0.6833 |
| Species:temperature: $p$CO$_2$ | 3 | 2.0028 | 0.5718 | 3 | - 1.033 | 1.0000 |
| most parsimonious model | | | | | | |
| species | 3 | 69.912 | < **0.0001** | 3 | 62.367 | < **0.0001** |
| temperature | 1 | 64.771 | < **0.0001** | 1 | 85.372 | < **0.0001** |
| species:temperature | 3 | 90.756 | < **0.0001** | 3 | 78.470 | < **0.0001** |

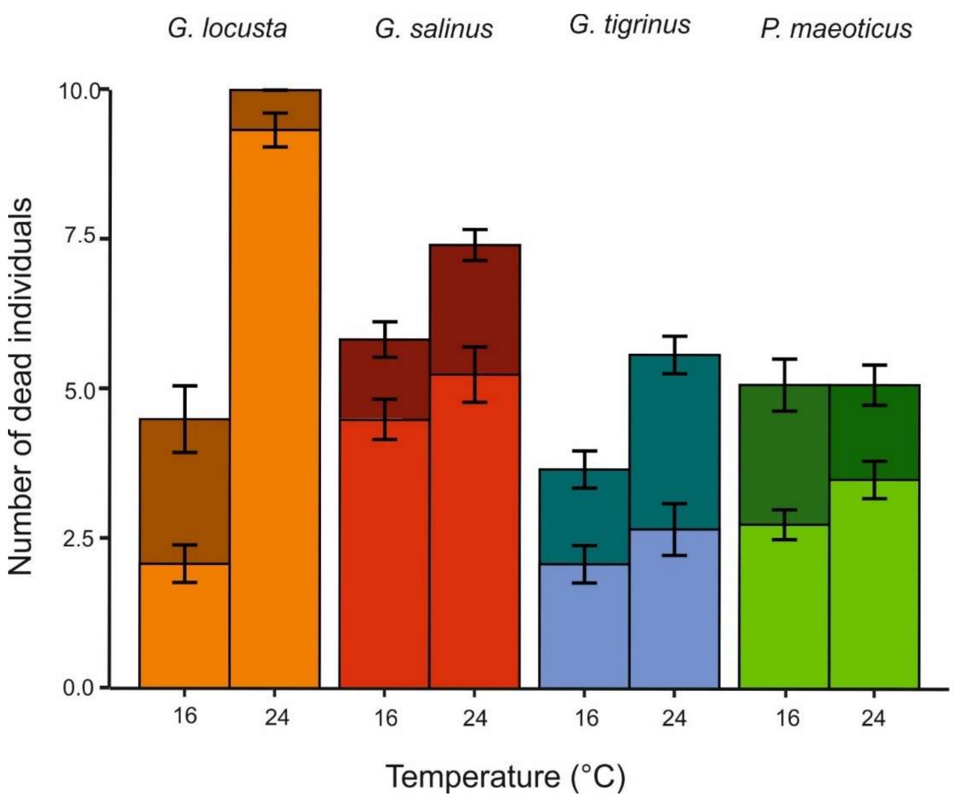

Figure 2. Means and standard errors of mortality in 16 and 24 °C treatments for four species (G. locusta, G. salinus, G. tigrinus, and P. maeoticus, in that order) at the mid-point and end-point of experiments (i.e. day 15 and day 30, respectively). Mortality in different pCO2 treatments were used to calculate means and standard errors (i.e. 400, 1600, 2700, and 3500 ppm). Lighter colors denote values at the mid-point, while darker at the end-point.

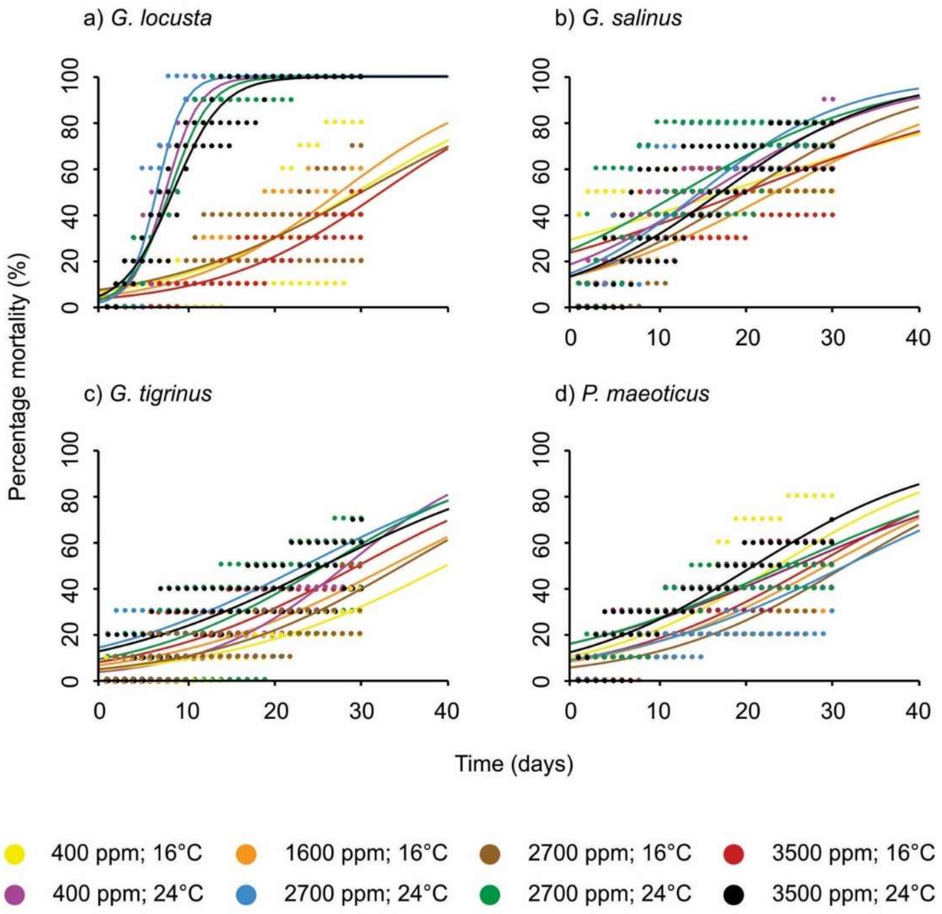

Figure 3. Mortality rates (%) of four gammarid species: *G. locusta* (a), *G. salinus* (b), *G. tigrinus* (c) and *P. maeoticus* (d) in experiments conducted in 16 and 24 °C in 400, 1600, 2700, and 3500 ppm $p\mathrm{CO}_2$ treatments. The curves were constructed using pooled data from three replicates.

The onset of mortality was significantly different among the four species in all comparisons at 24 °C and in most comparisons at 16 °C (Table 2; Figure 2). In all treatments, *G. salinus* began to die first, often followed by *P. maeoticus*. *Gammarus locusta* began to die last in most treatments. However, due to differences in mortality rate among species and treatments, our experiments resulted in *G. salinus* and *G. locusta* having the highest overall mortality at 16 and 24 °C, respectively (Tables 1 and 2; Figures 1 and 2). *Gammarus locusta* had significantly higher mortality in 24 °C than in 16 °C already at the mid-point of the experiment ($p < 0.001$; Figures 1 and 3), while for *G. salinus* and *G. tigrinus* that was the case only at the end of the experiment ($p = 0.011$ and $p = 0.003$, respectively; Figures 1 and 3). There was no difference in mortality of *P.*

*maeoticus* in either of the time-points of the experiment considering temperature treatments (*p* = 0.211 and *p* = 0.999, respectively; Figures 1 and 3).

At the end of the experiment in both temperature treatments, there was no significant difference in mortality between *P. maeoticus and G. tigrinus* (*p* = 0.131 and *p* = 0.872, respectively; Figures 1 and 3), whereas both of the species demonstrated significantly lower mortality at 24 °C than *G. locusta* and *G. salinus* (*p* < 0.001 and *p* = 0.002, respectively for *P. maeoticus*; *p* < 0.001 and *p* = 0.020, respectively for *G. tigrinus*; Figures 1 and 3). At 16 °C only *G. tigrinus* demonstrated significantly lower mortality than *G. salinus* by the end of the experiment (*p* = 0.006); there was no significant difference in mortality among other species at 16 °C at this time-point (Figure 1).

Table 2. Statistical comparisons of parameters between pairs of fitted curves for the species in experiments conducted in 16 and 24 °C and in 400, 1600, 2700, and 3500 ppm pCO2 treatments. The *t*-test incorporated in the fit nonlinear model using generalized least squares was used to test for differences between estimated parameters - rate of mortality ($Z_1$ and $Z_2$) and onset of mortality ($Q_1$ 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 0.05 were used for pairwise statistical comparisons (i.e. 0.001).

| Species compared | Temperature treatment [ °C] | *p*CO$_2$ treatment [ppm] | *p* -value of mortality onset | *p* -value of mortality rate |
|---|---|---|---|---|
| *Gammarus locusta –*<br>*Gammarus salinus* | 16 | 400 | **< 0.0001** | 0.0068 |
| | | 1600 | 0.0121 | **< 0.0001** |
| | | 2700 | **< 0.0001** | 0.0334 |
| | | 3500 | **< 0.0001** | **0.0009** |
| | 24 | 400 | **< 0.0001** | **< 0.0001** |
| | | 1600 | **< 0.0001** | **< 0.0001** |
| | | 2700 | **< 0.0001** | **< 0.0001** |

| | | | | |
|---|---|---|---|---|
| | | 3500 | **< 0.0001** | **< 0.0001** |
| *Gammarus locusta – Gammarus tigrinus* | 16 | 400 | **0.0010** | 0.4366 |
| | | 1600 | **< 0.0001** | **< 0.0001** |
| | | 2700 | 0.0023 | 0.3764 |
| | | 3500 | 0.0292 | 0.0288 |
| | 24 | 400 | **< 0.0001** | **< 0.0001** |
| | | 1600 | **< 0.0001** | **< 0.0001** |
| | | 2700 | **< 0.0001** | **< 0.0001** |
| | | 3500 | **< 0.0001** | **< 0.0001** |
| *Gammarus locusta – Pontogammarus maeoticus* | 16 | 400 | **< 0.0001** | 0.0223 |
| | | 1600 | **< 0.0001** | **< 0.0001** |
| | | 2700 | **< 0.0001** | **< 0.0001** |
| | | 3500 | **< 0.0001** | 0.0379 |
| | 24 | 400 | **< 0.0001** | **< 0.0001** |
| | | 1600 | **< 0.0001** | **< 0.0001** |
| | | 2700 | **< 0.0001** | **< 0.0001** |
| | | 3500 | **< 0.0001** | **< 0.0001** |
| *Gammarus salinus – Gammarus tigrinus* | 16 | 400 | **< 0.0001** | 0.0012 |
| | | 1600 | **< 0.0001** | 0.0200 |
| | | 2700 | **< 0.0001** | 0.0852 |
| | | 3500 | **< 0.0001** | 0.0416 |
| | 24 | 400 | **< 0.0001** | 0.0087 |

|  |  |  |  |  |
|---|---|---|---|---|
|  |  | 1600 | < **0.0001** | **0.0001** |
|  |  | 2700 | < **0.0001** | 0.6071 |
|  |  | 3500 | < **0.0001** | 0.0071 |
| *Gammarus salinus – Pontogammarus maeoticus* | 16 | 400 | < **0.0001** | < **0.0001** |
|  |  | 1600 | < **0.0001** | 0.2929 |
|  |  | 2700 | < **0.0001** | 0.1585 |
|  |  | 3500 | < **0.0001** | < **0.0001** |
|  | 24 | 400 | < **0.0001** | **0.0003** |
|  |  | 1600 | < **0.0001** | 0.008 |
|  |  | 2700 | < **0.0001** | 0.0203 |
|  |  | 3500 | < **0.0001** | 0.1736 |
| *Gammarus tigrinus – Pontogammarus maeoticus* | 16 | 400 | < **0.0001** | **0.0005** |
|  |  | 1600 | 0.0271 | 0.6994 |
|  |  | 2700 | **0.0009** | 0.0567 |
|  |  | 3500 | 0.1723 | 0.6439 |
|  | 24 | 400 | < **0.0001** | < **0.0001** |
|  |  | 1600 | < **0.0001** | 0.4850 |
|  |  | 2700 | < **0.0001** | 0.0016 |
|  |  | 3500 | < **0.0001** | 0.0032 |

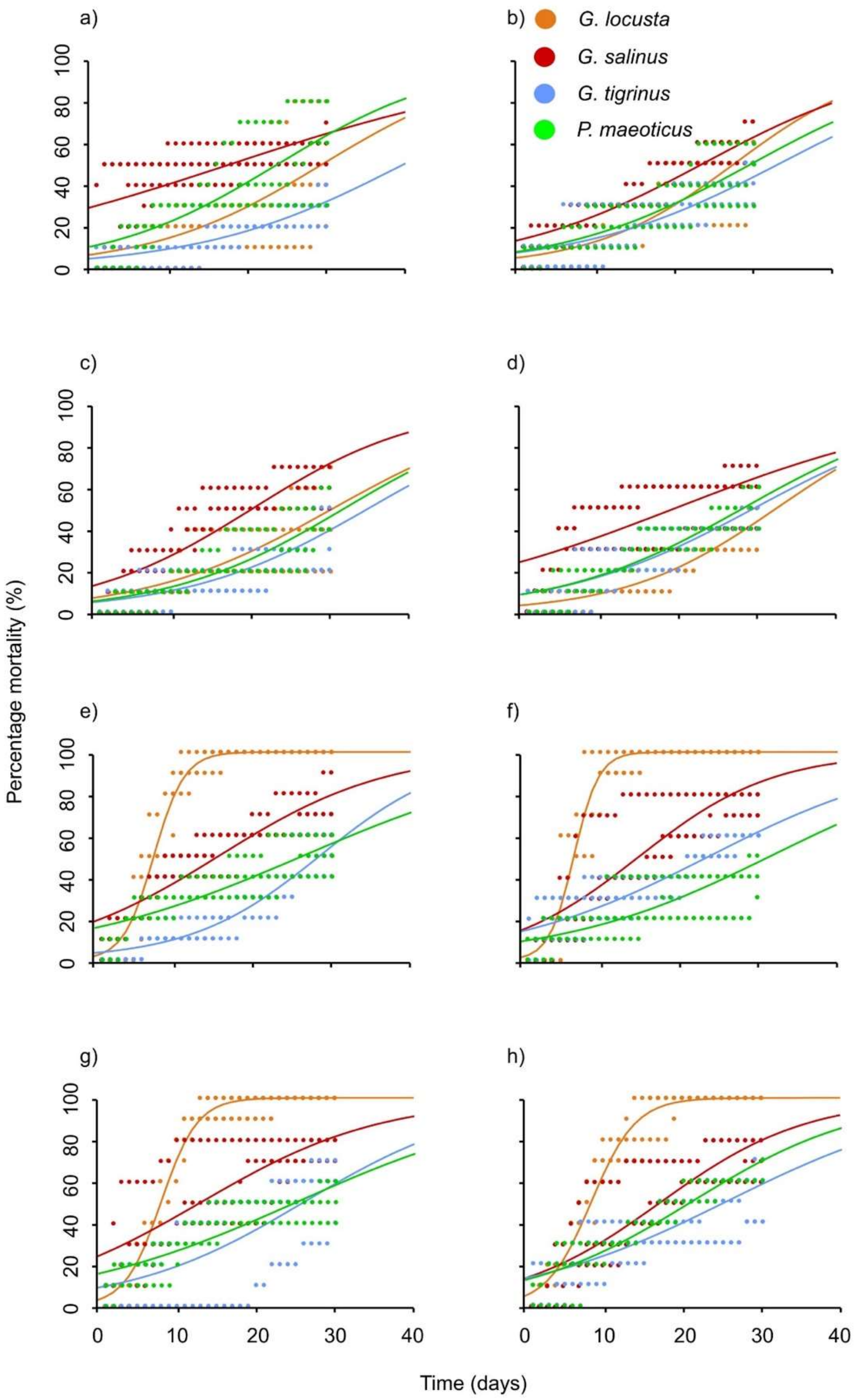

Figure 4. Mortality rates (%) of four gammarid species: *G. locusta* (orange), *G. salinus* (red), *G. tigrinus* (blue) and *P. maeoticus* (green) in experiments conducted in two temperature and four $p\mathrm{CO}_2$ treatments: 16 °C and 400 ppm (a), 16 °C and 1600 ppm (b), 16 °C and 2700 ppm (c), 16 °C and 3500 ppm (d), 24 °C and 400 ppm (e), 24 °C and 1600 ppm (f), 24 °C and 2700 ppm (g), and 24 °C and 3500 ppm (h). The curves were constructed using pooled data from three replicates.

## Discussion

Ecosystems worldwide are impacted by anthropogenic activities and globalization, with climate change and species introductions being among their greatest stressors (Olden *et al.,* 2004; Hawkins, 2012; Simberloff, 2013; IPCC, 2014; Linders *et al.,* 2019; Pyšek *et al.,* 2020). In this study, laboratory experiments were conducted to compare temperature and $p$CO2 tolerances of four gammarid species originating from three different regions - the Northern European, Ponto-Caspian and North American Atlantic regions - to detect interactions between climate change and NIS. Conversely to expectations, an increase in $p\mathrm{CO_2}$ level was not a significant driver of mortality in this study, neither by itself nor in combination with increased temperature, for any of the tested species. However, temperature was significant, and it affected the tested species to different extents, particularly favouring NIS over native species. The most sensitive was the native species *G. locusta* which experienced 100% mortality by the end-point of the experiment. The second native species, *G. salinus*, performed better than *G. locusta*, but was still more sensitive to temperature increase than any of the NIS. In contrast, *P. maeoticus*, the tested NIS from Ponto-Caspian, not only performed better than native species, but also did not demonstrate any difference in its performance between the temperature treatments. Consequently, with the predicted environmental changes in the Baltic Sea, and in particular with temperature increases, one may expect shifts in distributions among native gammarid taxa moving towards colder areas, while at the same time, their niches might be filled by NIS which exhibit better-suited environmental tolerances. In the best-case scenario, those NIS may take over roles of retreated native species and maintain ecosystem functioning. Nonetheless, as the chance that impactful NIS will arrive increases with the increased number of establishing NIS, the danger of negative impacts connected with the new NIS may rise as well (Ricciardi & Kipp, 2008; Blackburn *et al.,* 2019; Essl *et al.,* 2020; Pyšek *et al.,* 2020; Cuthbert *et al.,* 2021; Diagne *et al.,* 2021).

Earlier studies reported that marine organisms, including crustaceans, experience negative effects on growth rate, moulting frequency and survival because of acidification, particularly in combination with elevated temperature (Whiteley, 2011; Kroeker *et al.,* 2013). In contrast to those studies, a recent review and meta-analysis based on 5153 observations from 985 studies, determined that many calcifiers (e.g. echinoderms, crustaceans, and cephalopods) are tolerant to acidification levels predicted for the year 2100, and the authors pointed out the underestimated calcifiers' phenotypic plasticity (e.g. physiological, mineralogical, structural, and molecular adjustments) and adaptability (Leung *et al.,* 2022). That said, Leung *et al.* (2022) did not account for any additional stressors in their study, and emphasized crustaceans being underexplored compared to other taxa. This study did not explore physiological consequences of elevated $p\mathrm{CO}_2$, nor survival of gammarid taxa at juvenile stages, but survival of adults in this study was in concordance with Leung's *et al.* (2022) review, even when elevated temperature was considered. Interestingly, as stated above, this was not always the case, and there are many studies reporting contradictory results. For example, two Antarctic gammarid species, *Gondogeneia antarctica* and *Paradexamine fissicauda* have been significantly impacted by exposure to water with lower pH than that of their habitats (Schram *et al.,* 2016). Survival for both species declined significantly at reduced pH and co-varied with moult frequency. Schram *et al.* (2016) also specified that elevated temperature caused sub-lethal impacts on the species, while reduced pH caused significant mortality (Schram *et al.,* 2016). As their findings are contradictory to the findings of the present study and those of Leung *et al.* (2022), it is necessary to point out that differences in robustness to certain stressors among taxonomic groups and species, or even at population levels, may relate to the species/population biogeographical origin and evolutionary history (Preisser *et al.*, 2008; Kuo & Sanford, 2009; O'Neil *et al.*, 2008; Paiva *et al.,* 2018). For example, in the case of Baltic Sea taxa, many species may have already been adapted to ocean acidification due to already increased pH levels in the system (Melzner *et al.,* 2013; Rutgersson *et al.,* 2014; Reusch *et al.,* 2018; Christensen *et al.,* 2022). However, these same species may still be sensitive to increased temperature as they live at their upper thermal tolerance limits. On the other hand, the opposite may be the case for Antarctic gammarid species. Consequently, further studies are needed using different crustacean taxa, originating from environmentally diverse ecosystems, to make more general conclusions on ocean acidification impacts itself, and its combination with other stressors, on this taxonomic group.

While predicted changes in $p\mathrm{CO}_2$ concentrations and acidification turned out to be insignificant factors for the four tested species in this study, the temperature increase was especially detrimental for the native gammarid *G. locusta*, leading to mortality of all experimental individuals during the tests. Temperature was also a significant driver of mortality of the second native species tested, *G. salinus*, as well as of the North American NIS *G. tigrinus*, while it did not have a statistically clear effect on the Ponto-Caspian NIS, *P. maeoticus*. Casties *et al.* (2019) reported similar results for gammarid taxa originating from the same regions, with a Ponto-Caspian NIS being robust not only to temperature increase, but also to salinity changes. As mentioned above, the resilience to higher and/or lower temperature conditions is probably due to evolutionary history of the species (Cristescu *et al.,* 2003; Preisser *et al.*, 2008; Kuo & Sanford, 2009; O'Neil *et al.*, 2008; Paiva *et al.,* 2018). Both native species colonized the Baltic Sea from the Atlantic Ocean after the last Glacial Maximum, where they evolved under colder conditions than the Ponto-Caspian taxa (Zenkevitch, 1963; Björck, 1995; Reid & Orlova, 2002; Johannesson & André, 2006). The North American NIS, *G. tigrinus*, originates from even colder areas of the North Atlantic coast, where the cold Labrador Current flows from the Arctic Ocean southwards along the coast of North America. Therefore, with the predicted temperature increases in the Baltic Sea, as well as globally in general, taxa unable to adapt to new conditions undoubtedly will shift in their distributions towards colder areas, as has already been observed (Chen *et al*. 2011; Poloczanska *et al*. 2013; Bates *et al*. 2014; Smith *et al*. 2019; Pinsky *et al*. 2020).

Changes in ecosystems due to climate change and other anthropogenic activities may enable or hinder introduction, spread and/or impact of NIS (Hufbauer *et al.,* 2011; Holopainen *et al.,* 2016). When focusing on gammarid taxa, and in particular on *G. tigrinus* and *P. maeoticus* tested in this study, one can determine that these NIS are more resistant to predicted changes than their conspecific natives in terms of mortality. However, fecundity and other life history traits are also affected by temperature. Warmer temperature allows for more broods in amphipods due to the brooding period becoming shorter (Holopainen *et al.,* 2016). However, because of differences in the upper thermal limits among diverse species and their resilience to temperature changes, one can suspect even stronger effects of temperature on native gammarids tested than this study demonstrated. For example, life history of *G. locusta* at 20 °C, when compared to 15 °C, was characterized by at least a four-week reduction in the life-span and lower life expectancy (Neuparth *et al.,* 2002). Though, a shorter generation time, faster individual growth, and interestingly, higher population growth rate were also observed (Neuparth *et al.,* 2002). Furthermore, *G. locusta* produces two generations per year with a maximum of 65 eggs per clutch (Kolding & Fenchel, 1981) and *G. salinus* tends to have their first clutch when they have reached an age of 3.5 months (Skadsheim, 1990). In contrast, sexual maturity in *G. tigrinus* is reached at a very small size, with females of 4 mm in length and one month of age being able to bear eggs. Their brooding time decreases at increased temperatures from 20 days at 10 °C to 9 days at 21 °C. Therefore, a newly hatched female could be brooding its first clutch in little over a month after hatching (Pinkster, 1975). Ponto-Caspian gammarids have higher temperature tolerances than *G. tigrinus* and even more so than Northern European species (this study; Casties *et al.*, 2019). Moreover, *P. maeoticus* has a two-month generation time and is able to reproduce throughout a year irrespectively of seasonality (personal observations; Nazarhaghighi *et al.,* 2013). *G. tigrinus* is able to reduce and replace local gammarid taxa through a combination of high reproductive potential and predation on their juveniles (Pinkster, 1975; Jänes et al., 2015). *P. maeoticus* is not yet established in the Baltic Sea, although other gammarid species from the Ponto-Caspian region are already in the system, and some of them are voracious predators capable of causing severe changes in local communities (Dick & Platvoet, 2000; Grabowski *et al.,* 2007; Gumuliauskaitė & Arbačiauskas, 2008; Cuthbert *et al.,* 2020). Accordingly, with the predicted changes in the Baltic Sea, there are great chances that many native gammarid taxa will move north towards colder areas of the system, while their current habitats will be filled by NIS, and particularly those from the Ponto-Caspian region.

More broadly, these results exemplify how climate change and biological invasions can interact to mediate patterns of success of NIS. Whereas some effects of global change were found to be benign here even in the presence of other stressors (i.e. $p\mathrm{CO}_2$), temperature was found to be a key determining factor that distinguished NIS from native species under changing climates. These results highlight the pertinence of considering multiple interacting stressors in the context of biological invasions, both for NIS success and impact prediction as environments change.

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## Declaration Of Authorship

I, Cindy Giselle Martinez Reyes, declare that this thesis and the work presented in it are my own and has been generated as the result of my own original research. No other person's work has been used without due acknowledgement in this thesis. All references and verbatim extracts have been quoted, and all sources of information, including graphs and data sets, have been specifically acknowledged.

Signed:

Date: 13.04.2023

# Appendix: Mortality data

*Gammarus locusta*

| | 16°C and 400 ppm | | | 16°C and 1600 ppm | | | 16°C and 2700 ppm | | | 16°C and 3500 ppm | | | 24°C and 400 ppm | | | 24°C and 1600 ppm | | | 24°C and 2700 ppm | | | 24°C and 3500 ppm | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | |
| Day | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 10 | 0 | 10 | 10 | 0 | 0 |
| 3 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 10 | 0 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 20 | 0 | 10 | 20 | 10 | 10 |
| 4 | 10 | 0 | 10 | 0 | 0 | 0 | 0 | 10 | 0 | 10 | 0 | 0 | 0 | 20 | 10 | 0 | 30 | 10 | 30 | 0 | 10 | 20 | 10 | 10 |
| 5 | 10 | 0 | 10 | 10 | 0 | 0 | 0 | 10 | 0 | 10 | 10 | 0 | 0 | 40 | 20 | 0 | 60 | 10 | 30 | 10 | 20 | 30 | 20 | 20 |
| 6 | 10 | 0 | 10 | 10 | 0 | 0 | 0 | 10 | 0 | 10 | 10 | 0 | 10 | 50 | 40 | 30 | 60 | 40 | 30 | 40 | 30 | 40 | 20 | 20 |
| 7 | 20 | 0 | 10 | 10 | 0 | 0 | 10 | 10 | 0 | 10 | 10 | 0 | 40 | 70 | 50 | 60 | 70 | 50 | 40 | 40 | 40 | 40 | 50 | 20 |
| 8 | 20 | 0 | 20 | 10 | 10 | 0 | 10 | 10 | 0 | 10 | 10 | 0 | 40 | 70 | 50 | 70 | 100 | 50 | 60 | 40 | 40 | 50 | 60 | 20 |
| 9 | 20 | 0 | 20 | 10 | 10 | 0 | 10 | 10 | 10 | 10 | 10 | 0 | 50 | 80 | 50 | 70 | 100 | 50 | 70 | 50 | 50 | 70 | 70 | 40 |
| 10 | 20 | 0 | 20 | 10 | 10 | 10 | 10 | 20 | 10 | 10 | 10 | 10 | 80 | 90 | 60 | 90 | 100 | 70 | 80 | 60 | 60 | 70 | 80 | 60 |
| 11 | 20 | 0 | 30 | 20 | 10 | 10 | 10 | 30 | 20 | 10 | 10 | 10 | 90 | 100 | 80 | 90 | 100 | 90 | 90 | 80 | 70 | 70 | 80 | 70 |
| 12 | 20 | 0 | 30 | 30 | 10 | 10 | 10 | 40 | 20 | 10 | 10 | 10 | 90 | 100 | 80 | 90 | 100 | 90 | 90 | 80 | 80 | 70 | 80 | 70 |
| 13 | 20 | 0 | 40 | 30 | 10 | 10 | 20 | 40 | 20 | 10 | 10 | 10 | 90 | 100 | 90 | 90 | 100 | 100 | 90 | 100 | 90 | 70 | 80 | 90 |
| 14 | 20 | 0 | 40 | 30 | 10 | 10 | 20 | 40 | 20 | 10 | 20 | 10 | 100 | 100 | 90 | 90 | 100 | 100 | 90 | 100 | 100 | 70 | 80 | 100 |
| 15 | 20 | 10 | 40 | 30 | 20 | 10 | 20 | 40 | 20 | 10 | 20 | 10 | 100 | 100 | 90 | 90 | 100 | 100 | 90 | 100 | 100 | 70 | 80 | 100 |
| 16 | 30 | 10 | 40 | 30 | 20 | 10 | 20 | 40 | 20 | 10 | 30 | 10 | 100 | 100 | 90 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 80 | 100 |
| 17 | 30 | 10 | 40 | 30 | 30 | 20 | 20 | 40 | 40 | 10 | 30 | 10 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 80 | 100 |
| 18 | 30 | 10 | 40 | 30 | 40 | 20 | 20 | 40 | 40 | 10 | 30 | 10 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 80 | 100 |
| 19 | 30 | 10 | 50 | 40 | 50 | 20 | 20 | 40 | 40 | 20 | 30 | 10 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 90 | 100 |
| 20 | 30 | 10 | 50 | 40 | 50 | 20 | 20 | 40 | 40 | 20 | 30 | 20 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 100 | 100 |
| 21 | 30 | 10 | 60 | 40 | 50 | 20 | 20 | 40 | 40 | 20 | 30 | 20 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 100 | 100 |
| 22 | 30 | 10 | 60 | 50 | 50 | 20 | 20 | 40 | 40 | 20 | 30 | 20 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 100 | 100 |
| 23 | 30 | 10 | 70 | 50 | 50 | 20 | 20 | 40 | 50 | 30 | 30 | 30 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 24 | 30 | 10 | 70 | 60 | 50 | 20 | 20 | 40 | 50 | 30 | 30 | 40 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 25 | 40 | 10 | 70 | 60 | 50 | 20 | 20 | 40 | 60 | 30 | 30 | 40 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 26 | 40 | 10 | 80 | 60 | 50 | 20 | 20 | 40 | 60 | 30 | 30 | 40 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 27 | 40 | 10 | 80 | 60 | 50 | 20 | 20 | 40 | 60 | 30 | 40 | 40 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 28 | 40 | 10 | 80 | 60 | 60 | 20 | 20 | 40 | 60 | 30 | 40 | 40 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 29 | 40 | 20 | 80 | 60 | 60 | 20 | 20 | 40 | 70 | 30 | 40 | 50 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 30 | 40 | 20 | 80 | 60 | 60 | 30 | 20 | 40 | 70 | 30 | 40 | 50 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

*Gammarus salinus*

| 16°C and 400 ppm | | | | 16°C and 1600 ppm | | | 16°C and 2700 ppm | | | 16°C and 3500 ppm | | | 24°C and 400 ppm | | | 24°C and 1600 ppm | | | 24°C and 2700 ppm | | | 24°C and 3500 ppm | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | |
| Day | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 |
| 1 | 40 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 | 0 | 0 | 0 | 0 | 10 | 10 | 0 | 0 | 0 |
| 2 | 50 | 0 | 10 | 10 | 0 | 20 | 0 | 0 | 10 | 10 | 10 | 10 | 10 | 10 | 20 | 0 | 10 | 0 | 0 | 40 | 10 | 0 | 0 | 0 |
| 3 | 50 | 0 | 10 | 10 | 0 | 20 | 0 | 0 | 10 | 10 | 10 | 20 | 20 | 10 | 20 | 0 | 10 | 0 | 0 | 60 | 20 | 10 | 0 | 0 |
| 4 | 50 | 20 | 10 | 10 | 10 | 20 | 20 | 0 | 20 | 10 | 10 | 20 | 20 | 10 | 20 | 0 | 10 | 10 | 0 | 60 | 30 | 30 | 0 | 0 |
| 5 | 50 | 40 | 20 | 10 | 20 | 20 | 20 | 0 | 30 | 40 | 20 | 40 | 20 | 20 | 20 | 10 | 20 | 40 | 10 | 60 | 30 | 30 | 0 | 10 |
| 6 | 50 | 40 | 20 | 10 | 20 | 20 | 20 | 0 | 30 | 40 | 30 | 40 | 20 | 30 | 20 | 10 | 20 | 40 | 10 | 60 | 30 | 40 | 0 | 20 |
| 7 | 50 | 40 | 30 | 10 | 30 | 20 | 20 | 0 | 30 | 40 | 30 | 50 | 40 | 30 | 20 | 10 | 20 | 60 | 20 | 60 | 30 | 50 | 10 | 20 |
| 8 | 50 | 40 | 30 | 20 | 30 | 30 | 20 | 0 | 30 | 50 | 30 | 50 | 40 | 40 | 30 | 10 | 20 | 70 | 30 | 70 | 30 | 60 | 20 | 30 |
| 9 | 50 | 50 | 40 | 20 | 30 | 30 | 20 | 10 | 30 | 50 | 30 | 50 | 50 | 40 | 40 | 20 | 30 | 70 | 30 | 70 | 30 | 60 | 20 | 30 |
| 10 | 50 | 60 | 40 | 20 | 30 | 30 | 30 | 10 | 40 | 50 | 30 | 50 | 50 | 40 | 40 | 30 | 40 | 70 | 40 | 80 | 40 | 60 | 20 | 30 |
| 11 | 50 | 60 | 40 | 30 | 30 | 30 | 30 | 10 | 50 | 50 | 30 | 50 | 50 | 50 | 40 | 30 | 40 | 70 | 40 | 80 | 50 | 60 | 20 | 30 |
| 12 | 50 | 60 | 40 | 30 | 30 | 30 | 40 | 20 | 50 | 50 | 30 | 50 | 50 | 50 | 40 | 40 | 40 | 70 | 40 | 80 | 50 | 60 | 20 | 30 |
| 13 | 50 | 60 | 40 | 30 | 30 | 30 | 40 | 30 | 50 | 50 | 30 | 60 | 50 | 60 | 40 | 40 | 40 | 80 | 40 | 80 | 50 | 70 | 30 | 30 |
| 14 | 50 | 60 | 40 | 40 | 30 | 30 | 40 | 40 | 60 | 50 | 30 | 60 | 50 | 60 | 40 | 40 | 40 | 80 | 40 | 80 | 50 | 70 | 40 | 40 |
| 15 | 50 | 60 | 40 | 40 | 30 | 30 | 50 | 40 | 60 | 50 | 30 | 60 | 50 | 60 | 40 | 40 | 40 | 80 | 40 | 80 | 50 | 70 | 40 | 40 |
| 16 | 50 | 60 | 40 | 40 | 30 | 30 | 50 | 50 | 60 | 60 | 30 | 60 | 60 | 60 | 40 | 50 | 40 | 80 | 40 | 80 | 50 | 70 | 40 | 50 |
| 17 | 50 | 60 | 40 | 50 | 30 | 30 | 50 | 50 | 60 | 60 | 30 | 60 | 60 | 60 | 40 | 50 | 40 | 80 | 40 | 80 | 50 | 70 | 40 | 50 |
| 18 | 50 | 60 | 40 | 50 | 30 | 30 | 50 | 50 | 60 | 60 | 30 | 60 | 60 | 60 | 40 | 50 | 60 | 80 | 40 | 80 | 60 | 70 | 50 | 50 |
| 19 | 50 | 60 | 40 | 50 | 40 | 30 | 50 | 50 | 60 | 60 | 30 | 60 | 60 | 60 | 60 | 50 | 60 | 80 | 40 | 80 | 70 | 70 | 50 | 50 |
| 20 | 50 | 60 | 60 | 50 | 40 | 50 | 50 | 50 | 60 | 60 | 30 | 60 | 60 | 60 | 70 | 60 | 60 | 80 | 40 | 80 | 70 | 70 | 50 | 60 |
| 21 | 50 | 60 | 60 | 50 | 40 | 50 | 50 | 50 | 60 | 60 | 40 | 60 | 60 | 60 | 70 | 60 | 60 | 80 | 40 | 80 | 70 | 70 | 60 | 60 |
| 22 | 50 | 60 | 60 | 50 | 50 | 50 | 50 | 50 | 60 | 60 | 40 | 60 | 60 | 60 | 70 | 60 | 60 | 80 | 50 | 80 | 70 | 70 | 60 | 60 |
| 23 | 50 | 60 | 60 | 60 | 50 | 50 | 50 | 50 | 70 | 60 | 40 | 60 | 60 | 60 | 80 | 60 | 60 | 80 | 60 | 80 | 70 | 80 | 60 | 60 |
| 24 | 50 | 60 | 60 | 60 | 50 | 50 | 50 | 50 | 70 | 60 | 40 | 60 | 60 | 60 | 80 | 60 | 70 | 80 | 60 | 80 | 70 | 80 | 60 | 60 |
| 25 | 50 | 60 | 60 | 60 | 50 | 50 | 50 | 50 | 70 | 60 | 40 | 60 | 60 | 60 | 80 | 60 | 80 | 80 | 70 | 80 | 70 | 80 | 60 | 60 |
| 26 | 50 | 60 | 60 | 60 | 50 | 50 | 50 | 60 | 70 | 60 | 40 | 70 | 70 | 60 | 80 | 70 | 80 | 80 | 70 | 80 | 70 | 80 | 60 | 60 |
| 27 | 50 | 60 | 60 | 60 | 50 | 50 | 50 | 60 | 70 | 60 | 40 | 70 | 70 | 60 | 80 | 70 | 80 | 80 | 70 | 80 | 80 | 80 | 60 | 60 |
| 28 | 50 | 60 | 60 | 60 | 50 | 50 | 50 | 60 | 70 | 60 | 40 | 70 | 70 | 60 | 80 | 70 | 80 | 80 | 70 | 80 | 80 | 80 | 60 | 70 |
| 29 | 50 | 60 | 60 | 70 | 50 | 50 | 50 | 60 | 70 | 60 | 40 | 70 | 70 | 60 | 90 | 70 | 80 | 80 | 70 | 80 | 80 | 80 | 60 | 70 |
| 30 | 50 | 60 | 70 | 70 | 50 | 50 | 50 | 60 | 70 | 60 | 40 | 70 | 70 | 60 | 90 | 70 | 80 | 80 | 70 | 80 | 80 | 80 | 60 | 70 |

*Gammarus tigrinus*

| 16°C and 400 ppm | | | | 16°C and 1600 ppm | | | 16°C and 2700 ppm | | | 16°C and 3500 ppm | | | 24°C and 400 ppm | | | 24°C and 1600 ppm | | | 24°C and 2700 ppm | | | 24°C and 3500 ppm | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | |
| Day | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 |
| 1 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 10 | 0 | 20 | 0 |
| 2 | 0 | 0 | 10 | 0 | 0 | 10 | 10 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 30 | 10 | 0 | 0 | 0 | 10 | 0 | 20 | 0 |
| 3 | 0 | 0 | 10 | 0 | 0 | 10 | 10 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 30 | 10 | 20 | 0 | 0 | 20 | 0 | 20 | 0 |
| 4 | 0 | 0 | 10 | 0 | 0 | 10 | 10 | 0 | 10 | 10 | 0 | 0 | 0 | 0 | 0 | 30 | 10 | 20 | 0 | 0 | 20 | 10 | 20 | 0 |
| 5 | 0 | 0 | 10 | 0 | 0 | 10 | 10 | 0 | 10 | 10 | 0 | 0 | 0 | 0 | 0 | 30 | 10 | 20 | 0 | 0 | 20 | 10 | 20 | 0 |
| 6 | 0 | 0 | 10 | 0 | 0 | 30 | 10 | 0 | 20 | 10 | 10 | 0 | 0 | 0 | 10 | 30 | 10 | 20 | 0 | 0 | 20 | 20 | 30 | 10 |
| 7 | 0 | 0 | 10 | 0 | 10 | 30 | 10 | 0 | 20 | 10 | 30 | 0 | 10 | 10 | 10 | 30 | 10 | 30 | 30 | 0 | 20 | 20 | 40 | 10 |
| 8 | 0 | 0 | 10 | 0 | 10 | 30 | 10 | 0 | 20 | 10 | 30 | 0 | 10 | 10 | 10 | 30 | 10 | 40 | 30 | 0 | 20 | 30 | 40 | 10 |
| 9 | 0 | 0 | 10 | 0 | 10 | 30 | 10 | 0 | 20 | 10 | 30 | 0 | 10 | 10 | 20 | 30 | 20 | 40 | 30 | 0 | 30 | 30 | 40 | 10 |
| 10 | 0 | 0 | 10 | 0 | 10 | 30 | 10 | 0 | 20 | 10 | 30 | 10 | 10 | 10 | 20 | 30 | 20 | 40 | 40 | 0 | 30 | 30 | 40 | 10 |
| 11 | 0 | 0 | 10 | 0 | 10 | 30 | 10 | 10 | 20 | 10 | 30 | 10 | 10 | 10 | 20 | 30 | 20 | 40 | 40 | 0 | 40 | 30 | 40 | 20 |
| 12 | 0 | 0 | 10 | 10 | 10 | 30 | 10 | 10 | 20 | 10 | 30 | 20 | 10 | 10 | 20 | 30 | 20 | 40 | 40 | 0 | 40 | 30 | 40 | 20 |
| 13 | 0 | 0 | 10 | 10 | 10 | 30 | 10 | 10 | 20 | 20 | 30 | 20 | 10 | 10 | 20 | 30 | 20 | 40 | 40 | 0 | 40 | 30 | 40 | 20 |
| 14 | 10 | 0 | 10 | 10 | 10 | 30 | 10 | 10 | 20 | 20 | 30 | 20 | 10 | 10 | 20 | 30 | 20 | 40 | 40 | 0 | 50 | 30 | 40 | 20 |
| 15 | 10 | 20 | 20 | 10 | 20 | 30 | 10 | 10 | 20 | 20 | 40 | 40 | 10 | 10 | 20 | 30 | 30 | 40 | 40 | 0 | 50 | 30 | 40 | 20 |
| 16 | 10 | 20 | 20 | 20 | 20 | 30 | 10 | 10 | 20 | 20 | 40 | 40 | 20 | 10 | 20 | 30 | 30 | 40 | 40 | 0 | 50 | 30 | 40 | 40 |
| 17 | 10 | 20 | 20 | 30 | 20 | 30 | 10 | 10 | 20 | 20 | 40 | 40 | 20 | 10 | 20 | 30 | 30 | 40 | 50 | 0 | 50 | 30 | 50 | 40 |
| 18 | 10 | 20 | 20 | 30 | 20 | 30 | 10 | 10 | 30 | 20 | 40 | 40 | 30 | 10 | 30 | 30 | 30 | 40 | 50 | 0 | 50 | 30 | 50 | 40 |
| 19 | 10 | 20 | 20 | 30 | 20 | 30 | 10 | 10 | 30 | 20 | 40 | 40 | 30 | 20 | 30 | 30 | 30 | 40 | 50 | 0 | 50 | 30 | 50 | 40 |
| 20 | 20 | 20 | 20 | 40 | 20 | 30 | 10 | 10 | 30 | 20 | 40 | 40 | 30 | 20 | 30 | 30 | 30 | 40 | 50 | 10 | 50 | 30 | 50 | 40 |
| 21 | 20 | 20 | 20 | 40 | 20 | 30 | 10 | 10 | 30 | 30 | 40 | 40 | 30 | 20 | 30 | 40 | 50 | 50 | 50 | 10 | 50 | 30 | 50 | 40 |
| 22 | 20 | 20 | 20 | 40 | 20 | 30 | 10 | 20 | 30 | 30 | 40 | 40 | 30 | 20 | 40 | 50 | 50 | 50 | 60 | 20 | 50 | 30 | 60 | 40 |
| 23 | 20 | 20 | 20 | 40 | 20 | 30 | 20 | 20 | 30 | 40 | 40 | 40 | 30 | 20 | 40 | 50 | 50 | 60 | 60 | 20 | 50 | 30 | 60 | 50 |
| 24 | 20 | 20 | 30 | 40 | 20 | 30 | 20 | 20 | 30 | 40 | 40 | 40 | 30 | 40 | 40 | 50 | 50 | 60 | 60 | 20 | 50 | 30 | 60 | 50 |
| 25 | 20 | 20 | 30 | 40 | 30 | 30 | 20 | 20 | 30 | 40 | 40 | 40 | 30 | 40 | 60 | 50 | 50 | 60 | 60 | 20 | 50 | 30 | 60 | 50 |
| 26 | 30 | 20 | 30 | 40 | 30 | 30 | 20 | 20 | 30 | 40 | 40 | 40 | 40 | 40 | 60 | 50 | 60 | 60 | 60 | 30 | 60 | 30 | 60 | 50 |
| 27 | 30 | 20 | 30 | 40 | 30 | 40 | 20 | 20 | 30 | 40 | 40 | 40 | 40 | 40 | 60 | 50 | 60 | 60 | 60 | 30 | 70 | 30 | 60 | 50 |
| 28 | 30 | 20 | 30 | 40 | 30 | 40 | 20 | 20 | 50 | 40 | 40 | 50 | 40 | 40 | 60 | 60 | 60 | 60 | 60 | 30 | 70 | 40 | 60 | 60 |
| 29 | 30 | 20 | 40 | 50 | 30 | 40 | 20 | 20 | 50 | 40 | 40 | 50 | 50 | 40 | 60 | 60 | 60 | 60 | 60 | 30 | 70 | 40 | 70 | 60 |
| 30 | 30 | 20 | 40 | 50 | 30 | 40 | 20 | 30 | 50 | 40 | 40 | 50 | 50 | 40 | 60 | 60 | 60 | 60 | 60 | 40 | 70 | 40 | 70 | 60 |

| *Pontogammarus maeoticus* | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16°C and 400 ppm | | | | 16°C and 1600 ppm | | | 16°C and 2700 ppm | | | 16°C and 3500 ppm | | | 24°C and 400 ppm | | | 24°C and 1600 ppm | | | 24°C and 2700 ppm | | | 24°C and 3500 ppm | | |
| | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | | Mortality (%) | | |
| Day | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 | Rep1 | Rep2 | Rep3 |
| 1 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 10 | 10 | 0 | 0 | 10 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 10 | 10 | 0 | 10 | 20 | 0 | 10 | 10 | 10 | 0 |
| 3 | 10 | 0 | 0 | 0 | 10 | 10 | 10 | 0 | 0 | 0 | 10 | 0 | 10 | 0 | 10 | 20 | 0 | 10 | 20 | 10 | 10 | 20 | 20 | 0 |
| 4 | 20 | 0 | 0 | 10 | 10 | 10 | 10 | 0 | 0 | 0 | 20 | 0 | 20 | 10 | 10 | 20 | 0 | 10 | 20 | 10 | 10 | 30 | 20 | 0 |
| 5 | 20 | 0 | 0 | 10 | 10 | 10 | 10 | 0 | 10 | 0 | 20 | 0 | 30 | 20 | 20 | 20 | 10 | 10 | 20 | 10 | 10 | 30 | 20 | 0 |
| 6 | 20 | 0 | 10 | 20 | 10 | 10 | 10 | 0 | 10 | 0 | 20 | 10 | 30 | 20 | 20 | 20 | 10 | 10 | 20 | 10 | 20 | 30 | 20 | 0 |
| 7 | 20 | 10 | 10 | 20 | 10 | 10 | 10 | 0 | 10 | 10 | 20 | 20 | 30 | 30 | 20 | 20 | 10 | 10 | 30 | 10 | 20 | 30 | 20 | 0 |
| 8 | 30 | 20 | 10 | 20 | 10 | 10 | 10 | 0 | 10 | 10 | 20 | 20 | 30 | 30 | 20 | 20 | 20 | 10 | 30 | 10 | 20 | 30 | 30 | 20 |
| 9 | 30 | 20 | 20 | 30 | 10 | 10 | 10 | 10 | 10 | 10 | 20 | 20 | 30 | 30 | 20 | 20 | 20 | 10 | 30 | 10 | 30 | 30 | 30 | 20 |
| 10 | 30 | 30 | 30 | 30 | 10 | 20 | 10 | 10 | 20 | 10 | 20 | 20 | 30 | 30 | 20 | 20 | 20 | 10 | 30 | 20 | 30 | 30 | 30 | 20 |
| 11 | 30 | 30 | 30 | 30 | 10 | 20 | 10 | 10 | 20 | 20 | 20 | 20 | 30 | 40 | 30 | 20 | 40 | 10 | 40 | 30 | 30 | 30 | 30 | 30 |
| 12 | 30 | 30 | 30 | 30 | 10 | 20 | 10 | 10 | 20 | 20 | 20 | 20 | 30 | 40 | 30 | 20 | 40 | 10 | 40 | 30 | 30 | 30 | 30 | 30 |
| 13 | 30 | 30 | 30 | 30 | 10 | 20 | 20 | 20 | 20 | 20 | 30 | 20 | 30 | 40 | 30 | 20 | 40 | 10 | 40 | 40 | 30 | 30 | 40 | 30 |
| 14 | 30 | 30 | 40 | 30 | 10 | 20 | 20 | 30 | 20 | 30 | 30 | 20 | 30 | 40 | 30 | 20 | 40 | 10 | 40 | 50 | 30 | 30 | 40 | 30 |
| 15 | 30 | 30 | 40 | 30 | 10 | 20 | 20 | 30 | 20 | 40 | 30 | 30 | 30 | 40 | 40 | 20 | 40 | 10 | 40 | 50 | 30 | 40 | 40 | 40 |
| 16 | 30 | 30 | 50 | 30 | 20 | 20 | 20 | 30 | 20 | 40 | 30 | 30 | 30 | 40 | 40 | 20 | 40 | 20 | 50 | 50 | 40 | 40 | 40 | 40 |
| 17 | 30 | 30 | 60 | 30 | 20 | 30 | 20 | 40 | 20 | 40 | 30 | 30 | 30 | 40 | 50 | 20 | 40 | 20 | 50 | 50 | 40 | 40 | 50 | 40 |
| 18 | 30 | 30 | 60 | 40 | 20 | 30 | 20 | 40 | 20 | 40 | 30 | 30 | 30 | 40 | 50 | 20 | 40 | 30 | 50 | 50 | 40 | 40 | 50 | 40 |
| 19 | 30 | 40 | 70 | 40 | 20 | 30 | 20 | 40 | 20 | 40 | 30 | 30 | 30 | 40 | 50 | 20 | 40 | 30 | 50 | 50 | 40 | 40 | 50 | 40 |
| 20 | 30 | 40 | 70 | 40 | 20 | 40 | 20 | 40 | 20 | 40 | 30 | 40 | 30 | 40 | 50 | 20 | 40 | 40 | 50 | 50 | 40 | 60 | 60 | 50 |
| 21 | 30 | 40 | 70 | 40 | 20 | 40 | 20 | 40 | 20 | 40 | 30 | 40 | 30 | 40 | 50 | 20 | 40 | 40 | 50 | 50 | 40 | 60 | 60 | 50 |
| 22 | 30 | 40 | 70 | 50 | 20 | 40 | 20 | 40 | 20 | 40 | 30 | 40 | 30 | 40 | 60 | 20 | 40 | 40 | 50 | 50 | 40 | 60 | 60 | 50 |
| 23 | 30 | 40 | 70 | 60 | 30 | 40 | 20 | 40 | 20 | 40 | 30 | 40 | 30 | 40 | 60 | 20 | 40 | 40 | 50 | 50 | 40 | 60 | 60 | 50 |
| 24 | 30 | 40 | 70 | 60 | 30 | 40 | 30 | 50 | 20 | 50 | 30 | 40 | 40 | 40 | 60 | 20 | 40 | 40 | 50 | 50 | 40 | 60 | 60 | 50 |
| 25 | 30 | 40 | 80 | 60 | 30 | 40 | 30 | 50 | 20 | 50 | 40 | 40 | 40 | 40 | 60 | 20 | 40 | 40 | 50 | 50 | 40 | 60 | 60 | 60 |
| 26 | 30 | 50 | 80 | 60 | 30 | 40 | 30 | 50 | 20 | 50 | 40 | 40 | 50 | 40 | 60 | 20 | 40 | 40 | 50 | 50 | 40 | 60 | 60 | 60 |
| 27 | 30 | 50 | 80 | 60 | 30 | 40 | 30 | 50 | 20 | 50 | 40 | 40 | 50 | 40 | 60 | 20 | 40 | 40 | 50 | 50 | 40 | 60 | 60 | 60 |
| 28 | 30 | 60 | 80 | 60 | 30 | 40 | 40 | 50 | 30 | 60 | 40 | 40 | 50 | 40 | 60 | 20 | 40 | 40 | 60 | 50 | 40 | 60 | 60 | 60 |
| 29 | 30 | 60 | 80 | 60 | 30 | 40 | 40 | 60 | 40 | 60 | 40 | 50 | 50 | 40 | 60 | 20 | 40 | 50 | 60 | 50 | 40 | 60 | 60 | 60 |
| 30 | 30 | 60 | 80 | 60 | 30 | 50 | 40 | 60 | 40 | 60 | 40 | 60 | 50 | 40 | 60 | 30 | 40 | 50 | 60 | 50 | 40 | 70 | 60 | 60 |