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M. Sc. Marine Environmental Sciences

Master thesis

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

presented by

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Oldenburg, April 23, 2020

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AIAI	Anthropogenically induced adaptation to invade
ANOVA	Analysis of variance
CO_2	Carbon dioxide
CO_3^{2-}	Carbonate ions
DIC	Dissolved inorganic carbon
H_2O	Water
H^+	Hydrogen ions
HCO_3^-	Bicarbonate ions
H_2CO_3	Carbonic acid
NNS	Non-native species
OA	Ocean acidification
pCO_2	Partial pressure of carbon dioxide
SE	Standard error
TA	Total alkalinity

Abstract

With globalization and our trade and travel the establishment of non-native species (NNS) to new habitats has steadily increased. This includes invasive species, which negatively impact their new habitats but also NNS that do not cause any harm to the existing flora and fauna. During the process of invasion, multiple stages must be conquered by a species to become a NNS. However, it is not fully understood why some species are able to establish in a new environment while others are not. For this reason, Hufbauer et al. (2012) proposed the AIAI hypothesis (´Anthropogenically induced adaptation to invade`) that states that species that originate from anthropogenically impacted habitats are pre-adapted to the environmental conditions and are therefore able to become non-native in similar-impacted habitats. Therefore, this master thesis conducted several experiments using two amphipod gammarids (*Gammarus salinus* and *Gammarus locusta*) to test the AIAI theory. The mortality of two populations, one from an anthropogenically impacted (Falckenstein beach or Kiel Fjord) and one from a protected habitat (Maasholm), of each species were determined in three increased levels of pCO_2 (partial carbon dioxide concentration) and one increased temperature level. The results showed that the populations of the anthropogenically impacted habitat performed significantly better than the populations from the protected habitat. In the case of *G. locusta*, the Falckenstein population performed better compared to the Maasholm population only when both stressors were combined. For this reason, we accept the AIAI hypothesis of Hufbauer et al. (2012) in the case of both species, but we suggest that in the case of *G. salinus* a stronger adaptation took place than in the case of *G. locusta.* Additionally, we determined that increased temperature had stronger negative impacts on the mortality of the amphipod gammarids than increased pCO_2 levels. However, when both factors were combined, all four populations showed high mortality rates suggesting that these marine species may face major problems if climate change continues at the predicted rate.

Zusammenfassung

Die Einwanderung von nicht einheimischen Arten in verschiedene Lebensräume hat mit Zunahme der menschlichen Ausbreitung stetig zugenommen. Dies betrifft nicht einheimische Arten jeglicher Art, und zwar unabhängig davon, ob die einwandernden Arten Schäden in dem neuen Habitat verursachen oder nicht. Während des Invasionsprozesses gibt es eine Vielzahl von Stufen, in denen sich entscheidet, ob die Art dazu im Stande ist, sich auszubreiten oder nicht. Kommt es in einer dieser Stufen zu einer erhöhten Sterberate der Art oder zu einem ausbleibenden Reproduktionserfolg, wird diese Art bei einer Einwanderung erfolglos bleiben. Deshalb ist es in der Wissenschaft eine häufig diskutierte Frage, welche Prozesse es nicht einheimischen Arten ermöglichen in fremde Lebensräume vorzudringen, während eine Vielzahl von Arten daran scheitert. Hufbauer et al. (2012) haben daher die Hypothese der „Anthropogenically Induced Adaptation to Invade“ kurz AIAI, aufgestellt. Diese besagt, dass Arten, die in einem vom Menschen beeinflussten Ökosystem beheimatet sind, sich bereits vor dem Invasionsprozess an die dortigen Umwelteinflüsse anpassen und daher in der Lage sind in ähnlich vom Menschen beeinflusste Lebensräume einzuwandern. Diese Theorie wurde bereits bei Pflanzen und teilweise auch bei Tieren bewiesen, jedoch weniger bei marinen Arten. Aus diesem Grund war das Ziel der vorliegenden Masterarbeit, diese Theorie anhand von zwei Arten von Gammariden (Flohkrebse) zu überprüfen. Dafür wurden jeweils zwei Populationen von *Gammarus salinus* und *Gammarus locusta* aus einem geschützten Lebensraum und einem anthropogen beeinflussten Gebiet gesammelt und auf deren Stresstoleranz durch die Überwachung der Mortalität getestet. Als geschützter Lebensraum wurde das Vogel- und Naturschutzgebiet Schleimünde in Maasholm ausgewählt, während die Kieler Förde und der stark von Touristen besuchte Falckensteiner Strand als anthropogen beeinflusste Gebiete gewählt wurden. Als anthropogene Stressfaktoren wurden in dieser Arbeit drei erhöhte CO_2 Partialdrücke und eine erhöhte Temperatur gewählt. Die Ergebnisse dieser Studie haben gezeigt, dass die Populationen von *G. locusta* sensitiver auf den Stress reagiert haben als die Populationen von *G. salinus*. Zudem ist deutlich geworden, dass die Population von *G. salinus* aus dem anthropogen beeinflussten Gebiet toleranter gegenüber dem Stress war als die Population aus dem geschützten Habitat. Die Population aus dem anthropogen beeinflussten Gebiet von *G. locusta* war erst toleranter gegenüber dem Stress als beide Stressfaktoren

zusammen getestet wurden, jedoch waren die Ergebnisse weniger deutlich. Daher wurde die AIAI Hypothese von Hufbauer et al. (2012) für beide Arten akzeptiert, es wird jedoch davon ausgegangen, dass bei *G. salinus* eine stärkere Adaption stattgefunden hat als bei *G. locusta*. Weitere Ergebnisse haben zudem gezeigt, dass insbesondere die erhöhte Temperatur ein Grund hoher Mortalitätsraten war, der erhöhte CO_2 Partialdruck allein jedoch nicht so starke negative Auswirkungen auf die Populationen hatte. Dennoch haben beide Umweltfaktoren zusammen in allen vier Populationen zu erhöhten Mortalitätsraten geführt, weshalb davon ausgegangen werden kann, dass sich der Klimawandel für diese Gammaridenarten als problematisch erweisen könnte.

1. Introduction

In the last few decades, the invasions of diverse habitats by non-native species (NNS) has been one of the major topics of research and discussions in various fields of science and conservation. However, in the past, it seemed to be less relevant whether species were introduced by accident (e.g. ships, in clothes of travelers or their luggage) or on purpose (e.g. due to biocontrol or for sport purposes). This changed when scientists have begun to realize some major problems arising from the introduction of NNS, such as changes in community structures due to suppressing of native species (Davis 2009). During that time, literature on consequences of NNS for diversified ecosystems and habitats have also become more prominent (Simberloff et al. 2012). With this, the view on justifications of deliberate introductions changed, and it became clearer that humans have played a major role in this process. Consequently, biological invasions are a result of anthropogenic activities together with urbanization, agriculture and climate change, where invasion ecology research tries to answer various questions, like why some species are successful in establishing in new habitats, while others are not (Davis et al. 2011).

A species must overcome several obstacles to establish in a new habitat and become a NNS. As Lockwood et al. (2013) pointed out, there are several main stages in the invasion process: transport, establishment, and later on, possible spread and impact (Figure 1). First, individuals must be transported and introduced into a new environment; as it has been stated earlier, the way this happens is not fundamental. Second, the individuals must be able to tolerate environmental conditions of the new habitat and integrate into a biological community to establish self-sustaining populations there. Only when the introduced population has increased its abundance, the species starts to spread. However, to make an impact on the recipient community, usually the NNS abundance must increase significantly (Lockwood et al. 2013).

1.1 Anthropogenically induced adaptation to invade (AIAI)

The fundamental hypothesis behind this thesis is based on a principle developed by Hufbauer et al. (2012) known as ‘Anthropogenically induced adaptation to invade’. In invasion ecology, a big focus of research has always been on evolutionary responses occurring only after a population was introduced to a new habitat. However, Hufbauer et al. (2012) proposed a new invasion scenario, where

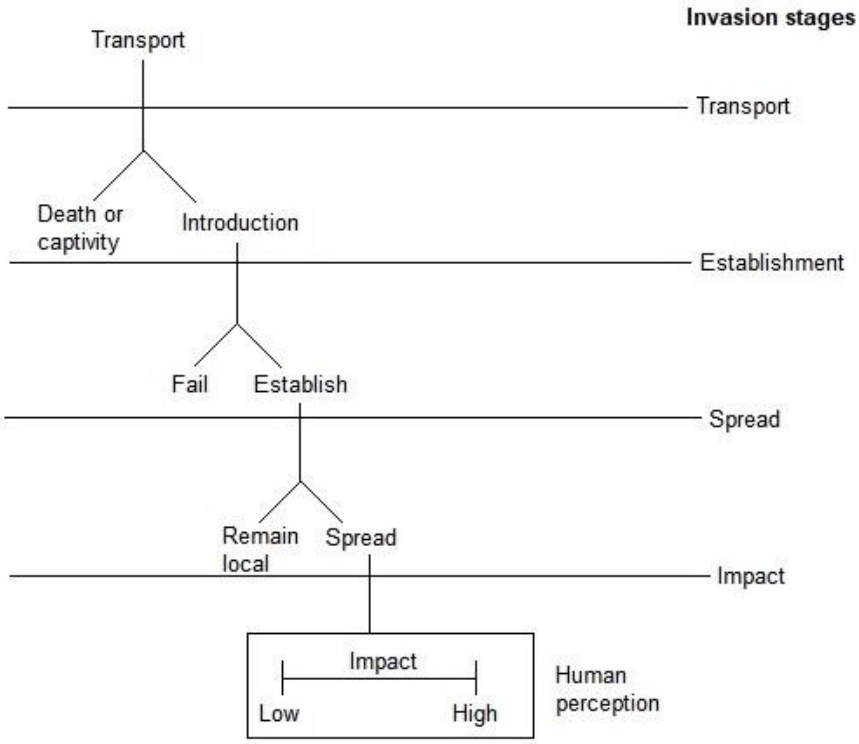


Figure 1 Stages of the invasion process by Lockwood et al. (2013).

evolution is already happening in a species' native habitat, facilitating the invasion process into another environment. As the name of the hypothesis already explains, the factor of anthropogenic influence plays a crucial role in this scenario. According to the AIAI hypothesis, native species which are exposed to anthropogenic activities and human-altered habitats become adapted to those habitats and human impacts. After transportation to a new environment, equally altered by humans, the establishment of the species is facilitated due to the pre-adaptation of the species. There is already some evidence of this hypothesis; for example, Foucaud et al. (2013) showed that the populations of the invasive fire ant *Wasmannia auropunctata* that live in human-altered habitats tolerated temperature and humidity stress better than the ones living in natural habitats. Moreover, similar patterns were seen in the Colorado potato beetle (Hufbauer et al. 2012) and a fungal pathogen of wheat (Stukenbrock et al. 2007).

1.2 Climate change and environmental stressors

As already mentioned above anthropogenic influences on ecosystems can be defined in various ways, but frequently those impacts are strongly linked to climate change. Thus, many scientific studies nowadays, including this thesis, focus on environmental factors that are closely linked to these environmental changes. Those

aspects involve an increase of greenhouse gases (e.g., methane, CO_2 etc.), rising temperatures, extreme weather events, changes in sea level and ocean currents to only name a few. Since oceans have an important role as CO_2 sinks and therefore have a direct effect on our climate, especially marine organisms are strongly affected by climate change (Duinker and Wefer 1994).

Effects of a changing climate can already be seen in the environment. Scientists predicted multiple future changes including changes in the marine carbonate chemistry (IPCC 2014). In particular, the partial pressure of carbon dioxide has increased from ~316 ppm to ~408 ppm from 1960 to 2018 (Tans and Keeling 2019). This results in an increased uptake of CO_2 by oceans and leads to a lower pH value, a process commonly referred to as ocean acidification (OA) (NOAA 2018). According to Caldeira and Wickett (2005) the global surface sea water pH is going to decrease by 0.3 to 0.5 pH units until the year 2100. Normally, the pH of the Baltic Sea fluctuates between 8.5 in spring/summer and 7.9 in autumn/winter (HELCOM 2013). However, it has been estimated that the pH of the Baltic Sea could decrease by 0.26 to 0.4 units (HELCOM 2013). To understand the problems of OA, it is necessary to understand the carbonate chemistry composition of sea water. When CO_2 reacts with water, carbonic acid is formed (H_2CO_3). The carbonic acid then disassociates into carbonate ions (CO_3^{2-}) and bicarbonate ions (HCO_3^-) by losing hydrogen ions (H^+; Doney et al. 2009). Consequently, the seawater carbonate chemistry reactions are in a near equilibrium:

$$CO_{2\,(atmo)} \Leftrightarrow CO_{2(aq)} + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \Leftrightarrow 2\,H^+ + CO_3^{2-} \quad (1)$$

As the oceans take up CO_2 from the atmosphere, H^+ ions and HCO_3^- increase, whereas the concentration of carbonate ions decreases. Consequently, the increase of H^+ ions is responsible for the decrease in the pH value (Doney et al. 2009). Since carbonate ions are decreasing due to a higher uptake of CO_2 by the oceans, marine organisms are directly affected by the change of this water chemistry parameter. Consequently, calcifying organisms that need carbonate ions to grow (corals), build shells and skeletons (mollusks) or exoskeletons (crabs) are threatened the most by a changing pH value (IPCC 2007, 2014). The rise of acidity requires more energy for these marine species to maintain shells and skeletons (United States Environmental Protection Agency 2015).

Scientists predicted a decrease in growth and survival of shallow marine calcifying species when exposed to OA on small time scales (Pörtner et al. 2004). However, they also proposed that most species, in fact, might be able to tolerate those changes if they already live in habitats with fluctuating environmental conditions. Similar results were obtained by Pansch et al. (2014), who showed that barnacles originating from habitats with high surrounding fluctuations were actually more resistant to high pCO_2 concentrations than those from more stable habitats. Still, the authors stated in the same publication that a combination of environmental stressors might have stronger effects and are equally important to be considered. In contrast, a study on the pacific krill (*Euphausia pacifica*) demonstrated negative consequences for the species, where growth of the pacific krill was negatively affected by high pCO_2 concentrations with decreasing survival at pH of 6.96 (Cooper et al. 2017).

In addition to changes in pCO_2 levels and pH, scientists forecasted an increase in mean surface water temperatures of 2 to 5°C until the late 21st century in the Baltic Sea (BACC Author Team 2008; HELCOM 2013). For a long time, it has been known that animals´ activity generally increases with a rise in temperature. However, if the thermal optimum of a species is reached, the activity decreases, leaving the animal with a reduced rate of metabolism and thus stress (Cossins and Bowler 1987). To some extent, animals can avoid this stress by moving to other habitats or adapting to the changing temperatures (Peck 2005). Still, because of climate change effects this might not be possible for every species, and therefore, the survival of those species may become problematic and questionable. For example, Carney Almroth et al. (2015) showed that Antarctic fish are negatively affected by a rise in temperature, and temperature stress of 27°C, which resultead in higher mortalities of clams and oysters compared to a temperature of 22°C (Matoo et al. 2013).

1.3 Study organisms

Gammarid species belong to the order Amphipoda and family Gammaridae (WoRMS Editorial Board). They are present in many benthic communities where they play a major role in ecosystems of shallow coastal waters. They are often used as test organisms because of their short life and reproduction cycles, successful cultivation in laboratory conditions and their role as suitable indicators to environmental changes (Conlan 1994). In this thesis, two gammarid species were used for the experiments; both species are native to the Baltic Sea (Kotta et al. 2011).

Gammarus locusta (Linnaeus, 1758) can be found in almost all regions of the Baltic Sea. The species is distributed from the low intertidal zone down to a depth of 30 m (Zettler and Zettler 2017). *Gammarus locusta* females grow to a size of 20 mm, while males can be up to 33 mm in length (Figure 2). The diet of *G. locusta* consists of macroalgae like *Ulva* spp. and detritus; also, cannibalism has been observed in laboratory cultures (Costa and Costa 2000).

Gammarus salinus (Spooner, 1947) is commonly found in algae, under rocks or in mussels (Zettler and Zettler 2017). It is very common in the Baltic Sea and can be found down to a depth of 10 m (Budd 2002). Individuals are slightly smaller than individuals of *G. locusta* with females growing to 18 mm and males growing to a maximum of 24 mm (Figure 3; Zettler and Zettler 2017). This species is omnivorous consuming plant material, fish and meat (Fenchel and Kolding 1979). Moreover, *G. salinus* can tolerate high fluctuations in salinity; however, it is not found in purely marine or freshwater habitats. Furch (1972) and Bulnheim (1979) already demonstrated that this species is more tolerant to changing temperatures than other gammarid species including *G. locusta*.

Figure 2 *Gammarus locusta* (Image by ©Louisa Langrehr)

Figure 3 *Gammarus salinus* (Image by ©Filipa Paiva)

1.4 Thesis objectives

In this thesis, we conducted a comparative assessment of stress tolerance of two *G. salinus* and two G. *locusta* populations. For each species, one population was collected from an anthropogenically impacted and one from a protected habitat, allowing us to test whether the population from the anthropogenically impacted habitat would better tolerate induced stresses than the population from a protected habitat. Two types of stressors were applied: increased temperature and three increased pCO_2

levels. If the populations from the anthropogenically impacted habitats will perform better than those from the protected habitat, we will accept the AIAI hypothesis of Hufbauer et al. (2012) that populations from anthropogenically impacted habitats are pre-adapted to anthropogenic stressors and therefore to become NNS. Consequently, the main question of this thesis is: Do anthropogenic alterations in Falckenstein beach and Kiel Fjord cause *G. salinus* and/or *G. locusta* populations to pre-adapt and therefore to become non-native?

Therefore, four hypotheses were tested in this thesis:

(1) There is a difference in the stress tolerance between the populations of *G. salinus* originating from protected and anthropogenically impacted habitats;

(2) There is a difference in the stress tolerance between the populations of *G. locusta* originating from protected and anthropogenically impacted habitats;

(3) There is a difference in the stress tolerance among different treatments within each population;

(4) There is a difference in the stress tolerance between *G. salinus* and *G. locusta*.

2. Material and Methods

2.1 Sampling locations

Two populations of two gammarid species (i.e., *Gammarus locusta* and *Gammarus salinus*) were collected for the experiments. The first population of each species was collected at anthropogenically impacted habitats, while the second at protected habitats, where anthropogenic pressures are assumed to be low. The sampled anthropogenically impacted habitat of *G. locusta* was Falckenstein beach (54°23'36." N 10°11'21.4"E), but due to insufficient number of individuals of *G. salinus* at Falckenstein beach, this species was sampled in front of the GEOMAR institute (54°19'45.7"N 10°08'55.7"E) in the Kiel Fjord (Figure 4). The protected sampling location of both species was Maasholm (54°40'33.0"N 10°01'48.0"E).

Falckenstein beach is the longest beach in Kiel, Germany, and it is consequently visited by lots of tourists. It is located at the mouth of the Kiel Fjord leading into the Baltic Sea (Figure 4). The Kiel Fjord is impacted by city infrastructure, ship traffic, ports as well as military harbors. Also, the coastal waters are contaminated with metals e.g., cadmium, zinc and lead (Landesamt für Natur und Umwelt des Landes Schleswig-Holstein 2001). Both Falckenstein beach and Kiel Fjord are assumed to be exposed to the same anthropogenic impacts.

Figure 4 Collection sites of specimens at Falckenstein beach (54°23'36.2"N 10°11'21.4"E), in front of the GEOMAR institute (54°19'47.5"N 10°08'58.1"E) and on the peninsula Schleimünde (54°40'33.0"N 10°01'48.0"E) ; image from www.maps.google.de, accessed on 16th July 2019.

On the other hand, Maasholm is part of a major nature reserve (Figure 4). The samples were collected on a small peninsula called Schleimünde, located next to Maasholm. Schleimünde separates the river Schlei and the Baltic Sea, thus there is a high inflow of fresh water in this area. Since 1927, the area is a conservation area for birds and therefore it is considered as a habitat without major anthropogenic influence (Verein Jordsand zum Schutze der Seevögel und der Natur e.V.).

2.2 Collection of specimens

Specimens were collected from March to July 2019. Using a sampling net, brown algae, rocks and chunks of mussel beds were transferred to buckets. Animals were collected directly from the algae or mussels by tweezers, placed in small buckets filled with ambient water, and transferred to GEOMAR for further processing. At the institute, the animals were relocated to bigger buckets, providing artificial shelter, air supply and food (TetraMin®). The water in the buckets was stepwise lowered or raised to the desired salinity of 14.2 PSU. All animals were kept in 50 L aquaria at 16°C until the start of the experiments.

2.3 Identification of specimens

Specimens were identified under a stereo microscope (ZEISS Stemi 305). Using a net and tweezers, an animal was transferred to a petri dish. If an animal was moving too actively, it was stabilized with a piece of net (Figure 5). Species identification was determined following (Zettler and Zettler 2017). Species characteristics used for identification are shown in Table 1.

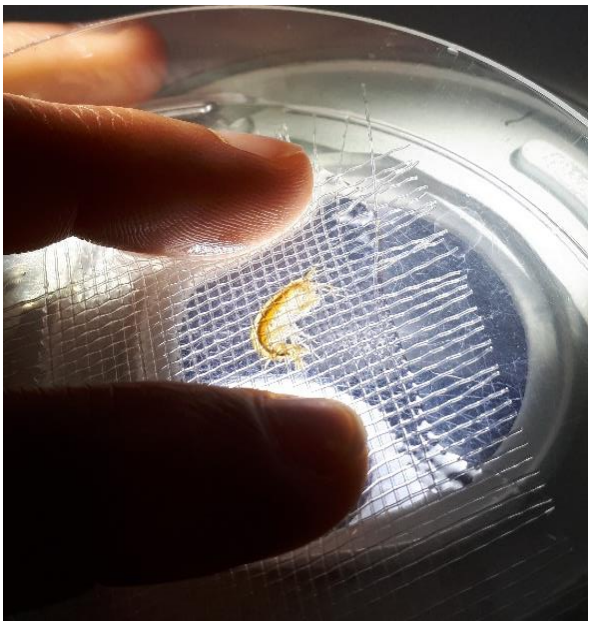
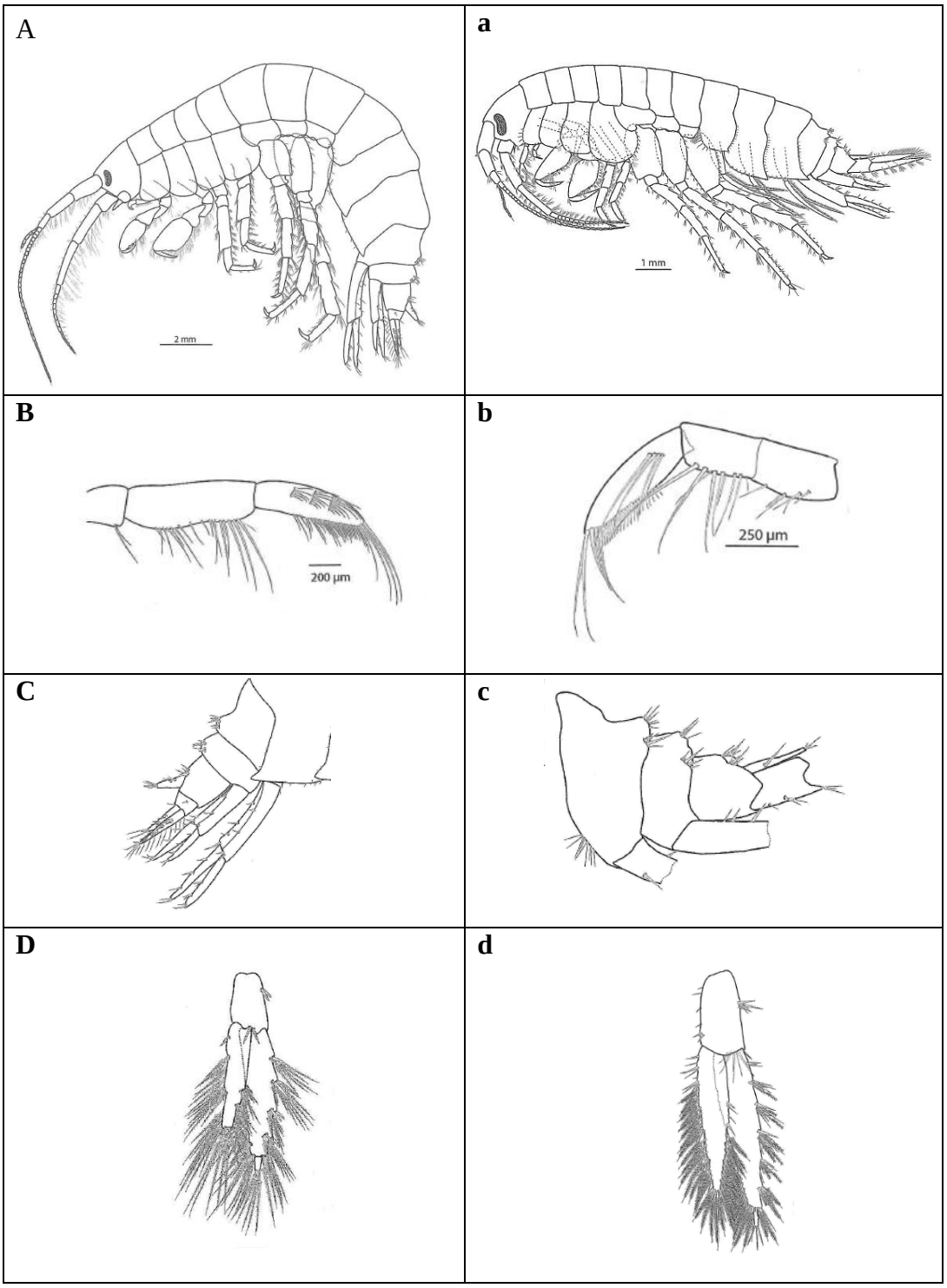


Figure 5 Identification of specimens with the help of a net (Image by ©Louisa Langrehr)

Table 1 Morphological characteristics of *G. salinus* (A-D) and *G. locusta* (a-d) used for identification of specimens. Morphology of full body of *G. salinus* (A) and *G. locusta* (a), mandible palpus (B and b), ursosome segments (C and c), uropod 3 with rami (D and d). Illustrations from Zettler and Zettler (2017)



2.4 Stress experiments with increased pCO_2 and temperature levels

2.4.1 Experimental setup

In this study, two types of stressors were applied: increased temperature and increased pCO_2. The experimental design consisted of one control and seven treatments (Table 2). Each treatment was conducted in triplicates, containing 10 randomly selected individuals. The control group with ambient temperature (16°C) and ambient pCO_2 level (400 ppm) refers to natural conditions as it was no subject to any stressor. Treatments T1, T2, and T3 had only pCO_2 stress applied, starting with lower stress level of 1600 ppm, followed by 2700 ppm and then by 3500 ppm (Table 2). Treatment T4 had only temperature stress, where temperature was increased to 24°C, while treatments T5, T6, and T7 had both temperature and pCO_2 increased (Table 2). The same experimental set up was used for each of the four populations tested.

Table 2 Experimental treatments. Used pCO_2 concentrations - 400 ppm (control and Treatment 4) – 1600 ppm (Treatment 1 and 5) – 2700 ppm (Treatment 2 and 6) – 3500 ppm (Treatment 3 and 7). Each pCO_2 concentration was tested at 16 and 24°C. T denotes treatment.

Treatment	Control	T1	T2	T3	T4	T5	T6	T7
pCO_2 [ppm]	400	1600	2700	3500	400	1600	2700	3500
Temperature [°C]	16.0	16.0	16.0	16.0	24.0	24.0	24.0	24.0

In total, 24 plastic aquaria (2 L) were used during each stress experiment. All aquaria were filled with water from the Kiel Fjord in front of the GEOMAR institute and filtered through Sediment Filters (1 µm and 5 µm). Each aquarium was closed with a plastic lid and secured with two rubber bands. The lid had an opening at one side for the pH electrodes. Every tank was provided with artificial shelter and an air stone for the infusion of pCO_2. All aquaria were put into water baths for temperature regulation (Figure 6).

After the aquaria were prepared for the experiments, ten animals of the respective species and from the respective location were put in each aquarium, and mortality was observed for 30 days. Mortality was checked every day by counting all

living individuals in every tank. An animal was considered dead when there was no reaction to touching by tweezers.

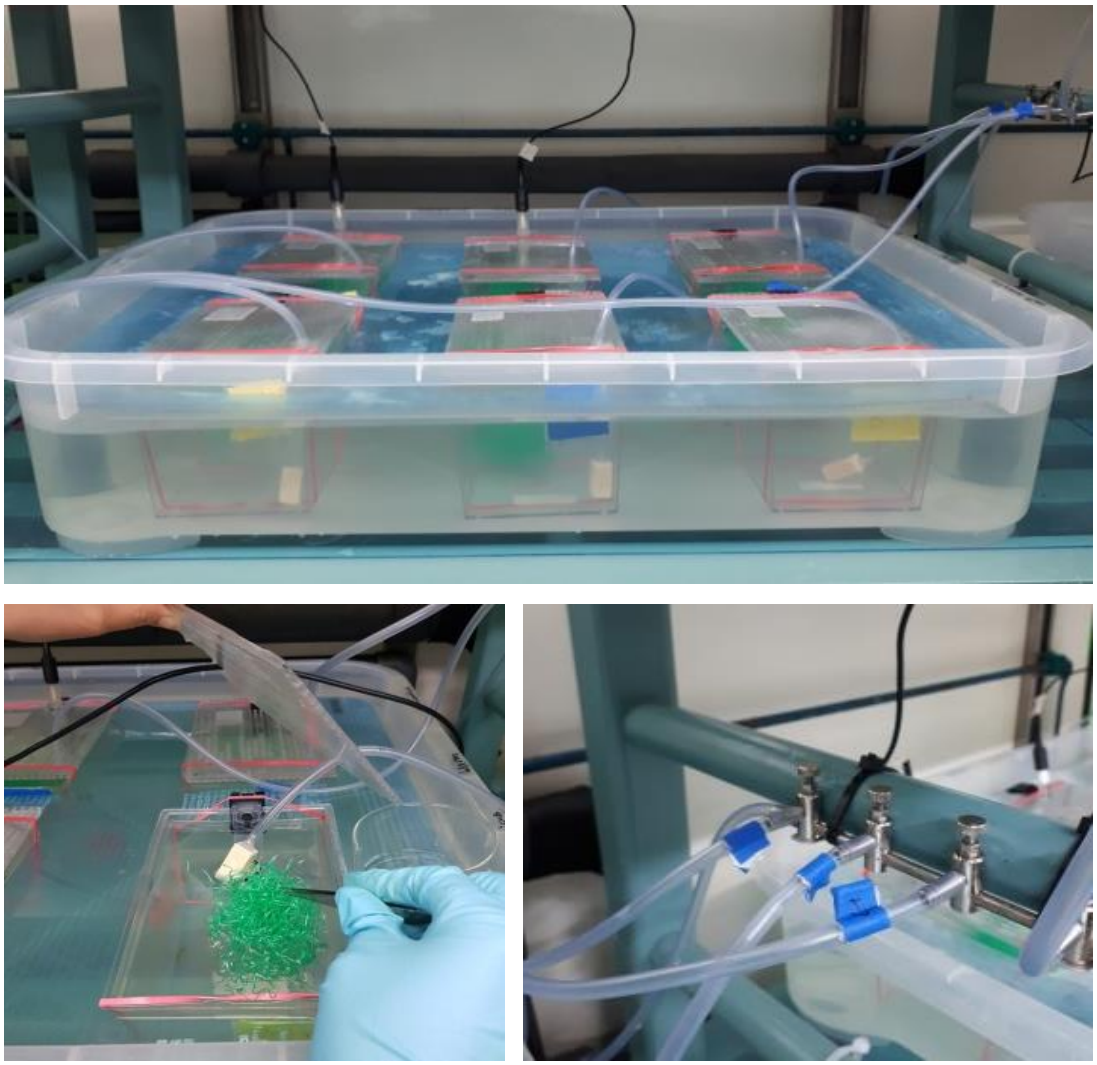


Figure 6 Experimental setup. Experimental tanks in water bath showing pH electrodes and pCO_2 supply (top). Process of counting animals; artificial shelter and bubble stone are visible here (bottom left). The pCO_2 supply of one treatment with three replicates (bottom right).

2.4.2 Monitoring of the carbonate system

During the experiment, pH_{NBS} and temperature were measured daily with a pH meter (WTW pH 3110) and a pH electrode (SenTix® 81). Salinity was measured every second day by a conductometer (Cond 3310 with TetraCon® 325). When evaporation raised salinity above 14.4 PSU, distilled water was added to the tanks to return it to 14.2 PSU. Every five days, water samples (250 mL) were taken from all aquaria and aquaria were refilled with fresh sea water. To ensure clean water conditions, 50% of the water was exchanged every ten days. Water samples for total alkalinity (TA) and

dissolved inorganic carbon (DIC) were filled into brown glass bottles with plastic screwing caps (250 mL bottles). All sample bottles were poisoned with mercury chloride solution following the guidelines *Guide to Best Practices for Ocean CO_2 Measurements* by Dickson et al. (2007). The samples were stored in the dark at room temperature until processing. Total alkalinity samples were titrated at room temperature with 0.05 M HCl-solution in an automated titration device (TitroLine® 7000). The sample volume was 25 mL and a dual measurement was performed for each sample. Batch160 (bottled 9th September 2016) was used as the reference material with an initial TA of 2212.44 ± 0.67 µmol/kg. The corresponding values for DIC were calculated with the software CO2Sys developed by Pierrot et al. (2011) using dissociation constants from Mehrbach et al. (1973), and refit by Dickson and Millero (1987).

The pH sensors were calibrated once per week with WTW™ Technical Buffer Solution pH 4.01 and 7.00. The pH meter was calibrated every day with the same technical buffer solution and with an additional buffer of pH 10.00 before measurements were taken.

2.5 Data analysis

To determine differences in mortality among different treatments, several statistical tests were performed using SPSS (SPSS 26.0 IBM Corp.). To test for differences among treatments, eight Kruskal-Wallis tests were conducted each for one of the four populations (i.e., two populations of each species) on the experimental day 15 and 30. Additionally, to test for effects of the two stressors (i.e., temperature and pCO_2) on mortality between two populations of the same species, four two-factorial analysis of variance (two-factorial ANOVA) were conducted each for one species on the experimental day 15 and 30. Also, a Mann-Whitney U test was conducted to determine differences in mortality between the two populations of each species. Therefore, four tests (i.e., two populations of each species) were performed on the experimental day 15 and 30.

Finally, to get an insight in mortality throughout the experiments, we have tested for difference in onset of mortality and mortality rate for each treatment between two populations for each species separately. For this purpose, mortality curves have been constructed for each treatment for each population and for each species, using data from all three replicates, according to the following equation:

$$y = 100/[1+e\text{-}Z(s\text{-}Q)] \quad (2)$$

with *t* being time, *Z* the mortality rate and *Q* representing the onset of mortality. With an expanded model, both the rate and the onset of mortality were compared between two curves using the equation:

$$y = 100/[1+e\text{-}(Z_1+Z_2)\ (s\text{-}Q_1\text{-}Q_2)] \quad (3)$$

with Z_1 and Z_2 representing the mortality rate and Q_1 and Q_2 being the points of the onset of mortality for both curves, respectively. The comparison of the curves was done statistically by the Fit Nonlinear Model applying Generalized Least Squares (S - Plus® 6.1, 2002, Insightful Corp., Seattle, Washington, USA).

3. Results

3.1 Response of *G. locusta* to temperature and pCO_2 stress

The mortality of two populations of *G. locusta* exposed to temperature and pCO_2 stress was observed for 30 days. In case of the Falckenstein population, mortality on day 15 ranged from 13% to 97%, with a mean of 57.1%. In the case of the population from Maasholm, mortality values were higher, and ranged from 40% to 100%, with a mean of 71.6%. On day 30, the mortality of the Falckenstein population ranged from 40% to 100%, with a mean of 72.5%. Mortality of the Maasholm population was from 73% to 100% with a mean of 95% (Table 3). The mean mortality of the Maasholm population was higher than that of the Falckenstein population on both days, respectively. Statistical analysis determined a significantly higher mortality of the Maasholm population on day 30, when compared to the Falckenstein population (Mann-Whitney U test, $p = 0.023$), with a medium effect size ($r > 0.329$). Moreover, the mortality differed among treatments of the Falckenstein population (Kruskal-Wallis test, $p = 0.008$ (day 15) and $p = 0.006$ (day 30)), and also of the Maasholm population (Kruskal-Wallis test, $p = 0.008$ (day 15) and $p = 0.014$ (day 30)). Mortalities were always higher in T4, T5, T6, T7 and T8, where temperature was 24°C. Indeed, a two-factorial ANOVA showed a significant effect of temperature on the mortality of both populations of *G. locusta* on both days (Two-factorial ANOVA, $p < 0.001$). However, a significant effect of pCO_2 or both stressors combined was not confirmed (Two-factorial ANOVA, $p > 0.05$).

Table 3 Mortality of *G. locusta* populations from Falckenstein and Maasholm. Values are mean mortality values in % on day 15 and day 30 of the experiment.

	Mean mortality on day 15		**Mean mortality on day 30**	
Treatment	Falckenstein	Maasholm	Falckenstein	Maasholm
Control	23	40	47	73
Treatment 1	20	50	50	93
Treatment 2	27	57	43	100
Treatment 3	13	47	40	93
Treatment 4	97	93	100	100
Treatment 5	97	93	100	100
Treatment 6	97	100	100	100
Treatment 7	83	93	100	100
Total Mean	**57.1**	**71.6**	**72.5**	**94.9**

Consequently, the Falckenstein population performed significantly better than the Maasholm population in all pCO_2 treatments, where the temperature has not been increased (for details see below).

Additionally, the onset of mortality (*Q*) and the rate of mortality (*Z*) were compared between the same treatments of the two populations (Table 4). Mortality rates differed significantly in all treatments, except between the control, T4 and T5 treatments (Table 4; Figure 7). The onsets of mortality were significantly different in all treatments except in T4 (Table 4).

In the case of the control treatment, mortality rates were not significantly different between the two populations (Table 4; Figure 7a). However, individuals from Maasholm started to die earlier than individuals from Falckenstein (Table 4; Figure 7a). As the onset of mortality of the Maasholm population was earlier than that of the Falckenstein population, the mortality on day 30 was also higher than that of the Falckenstein population. Already on day 15, the mortality of the Maasholm population reached 40%, while that of the Falckenstein population was 23% (Table 3; Figure 7a). On day 30, the mortality values have increased to 73% and 47% for the Maasholm and Falckenstein populations, respectively (Table 3; Figure 7a).

T1 showed a similar pattern to the control, although in this case, both the mortality rates and the onset of mortality were significantly different between the two populations (Table 4). Again, the onset of mortality was earlier for the Maasholm population, with a higher mortality on day 30 (Table 4; Figure 7b). The mortality of the Falckenstein population on day 15 reached 20%, while that of the Maasholm population was 50% (Table 3; Figure 7b). On day 30, the mortality of the Maasholm population was 93%, while that of the Falckenstein population was 50% (Table 3; Figure 7a).

In the case of T2, both the mortality rate and the onset of mortality were significantly different between the populations (Table 4). Maasholm individuals again started to die earlier than those of the Falckenstein population. However, this time, the Maasholm individuals were dying much faster than those of the Falckenstein population (Table 3; Figure 7c). Consequently, in the middle of the experiment, mortality of the Maasholm population reached 57%, while that of the Falckenstein population was only 27% (Table 3Figure 7c). On day 30, the mortality reached 100% and 43% in the Maasholm and Falckenstein population, respectively (Table 3; Figure 7c).

Table 4 Mortality rates (Z) and onset of mortality (Q) of compared treatments of *G. locusta* populations from Maasholm and Falckenstein. Shown values are p-values. Significant values are highlighted bold.

Compared treatments	Mortality rates (Z)	Onset of mortality (Q)
Control Maasholm – Control Falckenstein	0.2001	< **0.0001**
T1 Maasholm – T1 Falckenstein	**0.0056**	< **0.0001**
T2 Maasholm – T2 Falckenstein	< **0.0001**	< **0.0001**
T3 Maasholm – T3 Falckenstein	**0.0023**	< **0.0001**
T4 Maasholm – T4 Falckenstein	0.0872	0.1320
T5 Maasholm – T5 Falckenstein	0.5787	< **0.0001**
T6 Maasholm – T6 Falckenstein	< **0.0001**	< **0.0001**
T7 Maasholm – T7 Falckenstein	< **0.0001**	< **0.0001**

In T3, individuals from Maasholm again started to die earlier than individuals from Falckenstein (Table 4). The mortality rates between the two populations were significantly different, with the Maasholm population reaching a higher mortality during the experiment (Table 3; Figure 7d). On day 15, the mortality of the Maasholm population was 47%, while that of the Falckenstein population was 13% (Table 3; Figure 7d). Towards the end of the experiment, the mortality of the Maasholm population increased to 93% and that of the Falckenstein population to 40% (Table 3; Figure 7d).

In the case of T4, where the temperature was 24°C, there was no difference neither in the onset of mortality nor in the mortality rates between the two populations (Table 4; Figure 7e). However, the mortality in general was much higher than in the previous treatments. In the case of the Maasholm population, the mortality on day 15 was already 93%, while that of the Falckenstein population reached 97% (Table 3; Figure 7e). On day 30, the mortality of both populations rose to 100% (Table 3; Figure 7e).

In T5, similar trends as in the T4 were observed (Table 3 and 4; Figure 7f). The mortality in general was elevated and there was no significant difference in the mortality rates between the two populations (Table 4; Figure 7f). However, the Maasholm population had an earlier onset of mortality than the Falckenstein population (Table 4; Figure 7f). Mortality values on day 15 and day 30 were similar to the previous treatment, with the Maasholm population reaching 93% and 100%, and the Falckenstein population 97% and 100%, respectively (Table 3; Figure 7f).

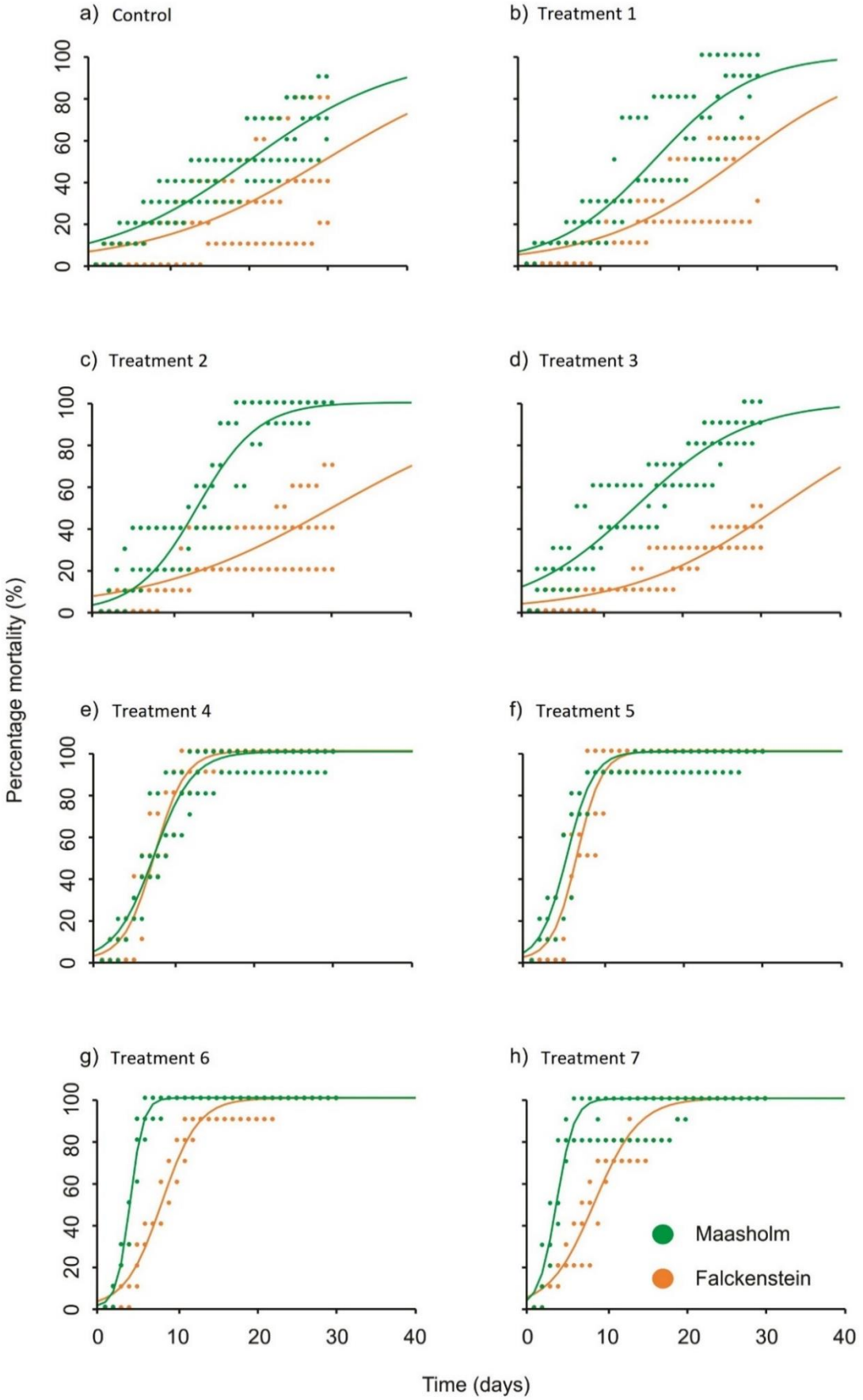


Figure 7 Mortality rates of two populations of *G. locusta* shown separately for each treatment (a – Control (400 ppm, 16°C); b – T1 (1600 ppm, 16°C) ; c – T2 (2700 ppm, 16°C); d – T3 (3500 ppm, 16°C); e – T4 (400 ppm, 24°C); f – T5 (1600 ppm, 24°C); g – T6 (2700 ppm, 24°C); h – T7 (3500 ppm, 24°C)).

In T6, the Maasholm population started to die earlier than the Falckenstein population (Table 4; Figure 7g). The mortality rates were significantly different from each other (Table 4; Figure 7g). On day 15, the mortality of the Maasholm population already reached 100%, while that of the Falckenstein population was 97% (Table 3; Figure 7g). However, until day 30, all individuals of both populations were gone (Table 3; Figure 7g).

T7 was similar to T6 (Table 3 and 4; Figure 7h). The mortality of the Maasholm population started earlier than that of the Falckenstein population, with significantly faster mortality rates (Table 4; Figure 7h). The Maasholm population reached 93% on day 15 and 100% on day 30; the Falckenstein population had a mortality of 83% and 100%, respectively (Table 3; Figure 7h).

3.2 Response of *G. salinus* to temperature and pCO_2 stress

Apart from the two populations of *G. locusta*, the mortality under temperature and pCO_2 stress was also monitored for two populations of *G. salinus.* On day 15, the mortality of the Kiel population ranged from 3% to 17%, with a mean of 9.4%. The mortality on day 30 of the Kiel population was generally higher with a minimum of 13%, a maximum of 37%, and a mean of 24.8%. In case of the Maasholm population, the mortality on day 15 ranged from 23% to 77%, with a mean of 53.3%. On day 30, the mortality of the Maasholm population was from 63% to 100%, with a mean of 77.9% (Table 5). The mean values differed greatly between the two populations. On days 15 and 30, the mean mortalities of the Maasholm population were significantly higher than that of the Kiel population (Mann-Whitney U test, $p = < 0.001$ (day 15) and $p < 0.001$ (day 30)). Furthermore, the mortality of the Kiel population did not differ between treatments on both days (Kruskal-Wallis test, $p = 0.547$ (day 15) and $p = 0.546$ (day 30)). This appeared to be different for the Maasholm population, as the mortality was significantly higher on day 15 in the warmer treatments, but not on day 30 (Kruskal-Wallis test, $p = 0.023$ (day 15) and $p = 0.104$ (day 30)). In the case of the Kiel population, there were no major effects of any of the tested factors (Two-way ANOVA, $p > 0.121$ for both stressors on both days). In contrast, a temperature effect was observed for the Maasholm population on both days (Two-way ANOVA, $p < 0.001$ (day 15) and $p = 0.001$ (day 30)). In the case of the pCO_2 as a stressor, and both stressors combined, no effect was confirmed (Two-factorial ANOVA, $p > 0.05$).

Table 5 Mortality of *G. salinus* populations from Kiel and Maasholm. Values are mean mortality values in % on day 15 and day 30 of the experiment.

	Mean mortality on day 15		**Mean mortality on day 30**	
Treatment	Kiel	Maasholm	Kiel	Maasholm
Control	10	43	27	63
Treatment 1	7	23	17	67
Treatment 2	17	30	20	73
Treatment 3	3	43	13	63
Treatment 4	7	67	20	80
Treatment 5	17	70	27	90
Treatment 6	7	77	37	100
Treatment 7	7	73	37	87
Total mean	**9.4**	**53.3**	**24.8**	**77.9**

Consequently, the Kiel population performed significantly better than the Maasholm population in all treatments (for details see below).

In addition, differences in the mortality rate (*Z*) and the onset of mortality (*Q*) were compared between the same treatments of the two populations (Table 6). The onset of mortality of the two populations was significantly different between all treatments (Table 6). Treatment 1, T2, T5, T6 and T7 showed significant differences in the mortality rates, while there was no difference between the control treatments and T3 (Table 6).

The mortality rates in the control treatments of the two populations showed no significant differences (Table 6; Figure 8a). However, individuals of the Maasholm population started to die earlier than those of the Kiel population (Table 6; Figure 8a). On both days, day 15 and 30, the Maasholm population had a slightly higher mortality (43% and 63%, respectively), than the Kiel population (10% and 43%, respectively; Table 5; Figure 8a).

In the case of T1, the mortality values were a little bit lower compared to the control treatments (Table 5; Figure 8b). However, the mortality rates were significantly different between the two populations (Table 6; Figure 8b). Again, the mortality of the Maasholm population was higher, also because of the earlier onset of mortality of the Maasholm population (Table 6; Figure 8b). On day 15 and day 30, the Maasholm population had a mortality of 23% and 67%, while that of the Kiel population was 7% and 17%, respectively (Table 5; Figure 8b).

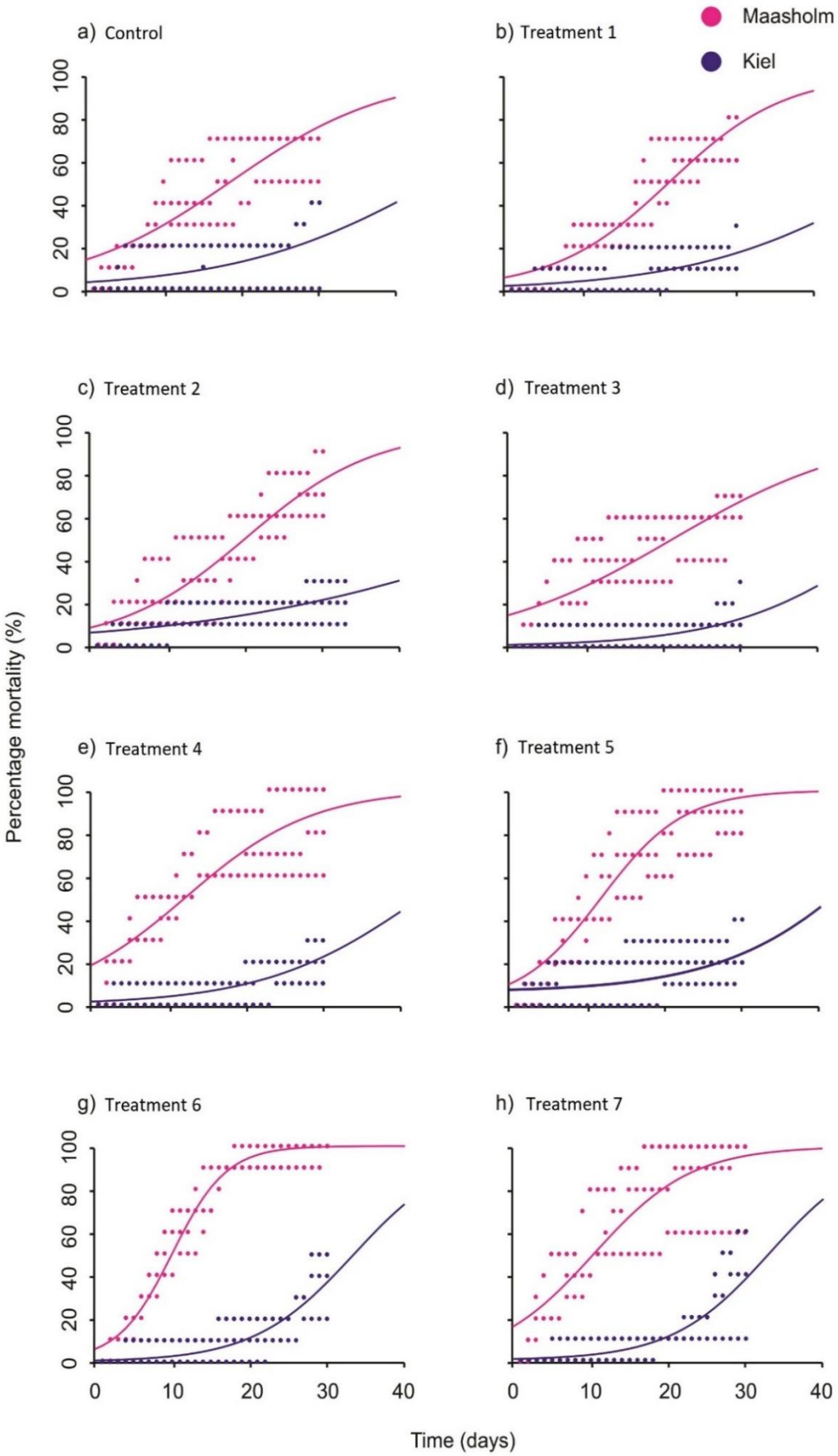


Figure 8 Mortality rates of two populations of *G. salinus* shown separately for each treatment (a – Control (400 ppm, 16°C); b – T1 (1600 ppm, 16°C) ; c – T2 (2700 ppm, 16°C); d – T3 (3500 ppm, 16°C); e – T4 (400 ppm, 24°C); f – T5 (1600 ppm, 24°C); g – T6 (2700 ppm, 24°C); h – T7 (3500 ppm, 24°C)).

Table 6 Mortality rates (Z) and onset of mortality (Q) of compared treatments of *G. salinus* populations from Maasholm and Kiel. Shown values are p-values.

Compared treatments	Mortality rates (Z)	Onset of mortality (Q)
Control Maasholm – Control Kiel	0.2929	< **0.0001**
T1 Maasholm – T1 Kiel	**0.0001**	< **0.0001**
T2 Maasholm – T2 Kiel	< **0.0001**	< **0.0001**
T3 Maasholm – T3 Kiel	0.6216	< **0.0001**
T4 Maasholm – T4 Kiel	0.1016	< **0.0001**
T5 Maasholm – T5 Kiel	**0.0016**	< **0.0001**
T6 Maasholm – T6 Kiel	< **0.0001**	< **0.0001**
T7 Maasholm – T7 Kiel	**0.017**	< **0.0001**

The mortality rates in T2 were significantly different between the populations, as the Maasholm population had a higher and faster mortality than the Kiel population (Table 6; Figure 8c). Also, the Maasholm population started to die earlier than the Kiel population (Table 6; Figure 8c). On day 15, the Maasholm population had a mortality of 30%, while that of the Kiel population was 17% (Table 6; Figure 8c). On day 30, the mortalities of both populations increased to 73% and 20%, respectively (Table **6**; Figure 8c).

In the case of T3, mortality rates were not different between the two populations, although mortality of the Maasholm population was higher than that of the Kiel population, due to an earlier onset of mortality (Table 6; Figure 8d). On day 15, the mortalities of the Maasholm and Kiel populations were 43% and 3%, respectively (Table 6; Figure 8d). On day 30, mortalities were 63% and 13% (Table **6**; Figure 8d).

Similar results as in T3 were observed in T4 (Table 6; Figure 8e). However, the mortality in general was slightly more elevated than in the previous treatments (Table 6; Figure 8e). There was no significant difference in mortality rates between the two populations (Table 6; Figure 8e). Still, the onset of mortality of the Maasholm population was earlier than that of the Kiel population (Table 6; Figure 8e). Therefore, on day 15 and 30, the Maasholm population had a mortality of 67% and 80%, while that of the Kiel population was 7% and 20%, respectively (Table 6; Figure 8e).

In the case of T5, the mortality rate of the Maasholm population was significantly faster than that of the Kiel population, resulting in notably higher mortality of the Maasholm population (Table 6; Figure 8f). Also, the Maasholm

population started to die much earlier than the Kiel population (Table 6; Figure 8f). Consequently, the Maasholm population had a mortality of 70% and 90% on day 15 and 30, while that of the Kiel population was 17% and 27%, respectively (Table 6; Figure 8f).

In the case of T6, the results were very similar to those of T5, with significant differences in the mortality rates between the two populations (Table 6; Figure 8g). Again, the mortality and the onset of mortality of the Maasholm population were higher and earlier than that of the Kiel population (Table 6; Figure 8g). The mortalities of the Maasholm population on day 15 and day 30 were 77% and 100%, while those of the Kiel population were 7% and 37%, respectively (Table 6; Figure 8f).

In the case of T7, the Maasholm population had a significantly faster mortality rate than the Kiel population, which resulted in higher mortality of the Maasholm population (Table 6; Figure 8h). Additionally, the Maasholm population had an earlier onset of mortality, when compared to the Kiel population (Table 6; Figure 8h). Finally, the mortality of the Maasholm population on day 15 and 30 population were 73% and 87%, while those of the Kiel population were 7% and 37%, respectively (Table 6; Figure 8h).

3.3 Water chemistry and parameters

During the experiment, water chemistry parameters (i.e., pH_{NBS}, DIC and TA) were continuously monitored or calculated for all populations. Additionally, temperature and salinity were measured frequently to guarantee constant conditions during the experiments. Salinity was kept constant during the experiments with fluctuations between 0 and 0.7 PSU; so, this parameter will be not further presented in the following chapter.

3.3.1 Water parameters of the G. locusta populations

Temperature was measured daily for both populations of *G. locusta* (Appendix: Figure 9 and 10). The ambient temperature treatments of the Falckenstein population had a total mean of 15.6°C with the lowest temperature being 15.1°C and the highest reaching 16°C (Appendix: Figure 9). In the case of the warm treatments, the total mean temperature was 24.4°C, with the minimum temperature of 24.2°C and maximum of 24.9°C (Appendix: Figure 9).

In the case of the Maasholm population, the ambient temperature treatments had a total mean of 15.7°C with a minimum of 15.4°C and maximum of 16°C (Appendix: Figure 10). In the warm treatments, temperature ranged from 23.4°C to 23.8°C with a mean of 23.7°C (Appendix: Figure 10).

In the case of the Falckenstein population, pH values ranged from 8.0 in the control treatment to 7.3 in the highest pCO_2 concentration treatment (Appendix: Figure 11). There were also some minor differences in the pH values between the warmer and ambient treatments; the warmer treatments showed slightly higher pH values than the ambient temperature treatments (Appendix: Figure 11). Data points were missing towards the end of the experiment, as some experimental aquaria already experienced 100% mortality and thus water parameter measurements were stopped (Appendix: Figure 11).

In the case of the population from Maasholm, pH values were fluctuating more throughout the experiment than those of the Falckenstein population (Appendix: Figure 12). The pH values ranged from 8.1 (control treatments) to 7.3 (highest pCO_2 concentration treatments), with similar oscillations visible in all treatments (Appendix: Figure 12).

Over the course of the experiment, TA values for the Falckenstein population ranged between 1700 µmol/kg and 2400 µmol/kg (Appendix: Figure 13). In the case of the control treatment and T1, T2 and T3, TA values were rather constant. However, higher oscillations were observed in the T4, T5, T6, and T7 (Appendix: Figure 13).

In the case of the Maasholm population, TA values looked different from those of the Falckenstein population; this may be, because more samples were taken during the Maasholm experiments (Appendix: Figure 14). Here, TA values showed higher oscillations with measured values from 1400 µmol/kg to 2000 µmol/kg (Appendix: Figure 14). Also, in all treatments, the values decreased towards the end of the experiment (Appendix: Figure 14).

Dissolved Inorganic Carbon trends followed those of the TA measurements as the DIC values were calculated using the TA values. In the case of the Falckenstein population, DIC values ranged from 1700 µmol/kg to 2500 µmol/kg (Appendix: Figure 15). The values in the warmer treatments were a little bit lower than those in the ambient temperature treatments (Appendix: Figure 15).

Dissolved Inorganic Carbon values of the Maasholm population ranged from 1300 µmol/kg to 2100 µmol/kg (Appendix: Figure 16). The values in all treatments

decreased as the experiment progressed, with T4, T5, T6 and T7 remaining on the same level and the control treatment, T1, T2 and T3 steadily decreasing further towards the end of the experiment (Appendix: Figure 16).

3.3.2 Water parameters of the G. salinus populations

Temperature of the Kiel population minimally varied in all treatments during the 30 days experiment (Appendix: Figure 17). In the case of the ambient temperature treatments, temperature varied between 15.6°C to 15.9°C, with a mean of 15.8°C (Appendix: Figure 17). The warmer treatments´ temperature oscillated between 23.2°C and 23.8°C with a mean temperature of 23.4°C (Appendix: Figure 17).

The temperature of the Maasholm population ranged between 15.1°C and 15.8°C, with a mean of 15.6°C (Appendix: Figure 18). In the warmer treatments, temperature ranged between 23.2°C and 23.8°C, with a mean of 23.5°C (Appendix: Figure 18). In all treatments, the temperature stayed constant for 30 days, with only a small fluctuation around the mean temperature (Appendix: Figure 18).

In the ambient temperature treatments, pH values of the Kiel population varied between 7.2 and 8.2 (Appendix: Figure 19). Values were relatively stable, however, with slight decrease towards the end of the experiment (Appendix: Figure 19). The warmer treatments had slightly higher pH values than the ambient temperature treatments, with pH values from 7.2 to 8.2 (Appendix: Figure 19).

In the case of the Maasholm population, pH values were more stable and only showed minor oscillations (Appendix: Figure 20). In the case of the ambient temperature treatments, minimum and maximum pH values were 7.2 and 8.1, respectively (Appendix: Figure 20). Again, the warmer treatments had a slightly higher pH values with a minimum of 7.3 and maximum of 8.2 (Appendix: Figure 20).

Total Alkalinity values showed a declining trend in the case of the Kiel population (Appendix: Figure 21). In all treatments, values ranged from 1400 µmol/kg to 2000 µmol/kg (Appendix: Figure 21). The warmer treatments showed slightly lower values than the ambient temperature treatments (Appendix: Figure 21).

In the case of the Maasholm population, TA values showed different pattern (Appendix: Figure 22). In general, the values were more constant in the control treatment and T1, T2 and T3, with TA between 1600 µmol/kg and 2000 µmol/kg (Appendix: Figure 22). In the case of the warmer treatments, TA values varied between 1500 µmol/kg and 1900 µmol/kg (Appendix: Figure 22).

Dissolved Inorganic Carbon values followed TA values for both populations (Appendix: Figure 23, Figure 24). In the case of the Kiel population, DIC of the ambient temperature treatments varied between 1400 µmol/kg and 2100 µmol/kg (Appendix: Figure 23). In the warmer treatments, values ranged from slightly under 1400 µmol/kg to merely 1800 µmol/kg. Thus, DIC values were lower in T4, T5, T6 and T7 (Appendix: Figure 23).

The population from Maasholm had more constant DIC values (Appendix: Figure 24). The values ranged from 1600 µmol/kg to 2100 µmol/kg in the ambient temperature treatments (Appendix: Figure 24). In the warmer treatments, values were between 1400 µmol/kg and 2000 µmol/kg (Appendix: Figure 24). However, in all treatments, a strong decline of DIC values was visible as the experiment progressed (Appendix: Figure 24).

4. Discussion

This master thesis tested the stress tolerance of two populations of two gammarid species to high pCO_2 and temperature to determine whether the conditions in anthropogenically impacted habitats caused the populations to pre-adapt to become potential NNS. Therefore, the mortality of tested species in several treatments of increased pCO_2 levels and one increased temperature level was monitored. Our results revealed that the Kiel and Falckenstein populations of *G. salinus* and *G. locusta*, respectively, the populations from anthropogenically impacted habitats, performed significantly better than the populations from Maasholm. Though, in the case of *G. locusta*, the population from an anthropogenically impacted habitat, the Falckenstein population, was performing better than the Maasholm population only when increased pCO_2 stress was applied. In the increased temperature condition, there was no difference between populations. Therefore, our results suggested that adaptation to anthropogenic impacts has been occurring for both species. Though, it looks like the adaptation is stronger in the case of *G. salinus* than in the case of *G. locusta*. Consequently, we accepted the AIAI hypothesis.

Several studies have been conducted and various examples were found that supported the AIAI hypothesis. For instance, Foucaud et al. (2013) tested the thermotolerance of native and non-native populations of the invasive fire ant *Wasmania auropunctata*. They discovered a better tolerance towards dry and hot conditions of the populations originating from human-altered habitat when compared to those from the natural habitat. In their study, they concluded that populations of *W. auropunctata* were pre-adapted to the conditions of the anthropogenically impacted habitats supporting a successful invasion of this species (Foucaud et al. 2013). Similar results of the same invasive ant species in Israel were obtained by Rey et al. (2012) and the authors confirmed pre-adaptation in *W. auropunctata*. Moreover, pests can also be taken as an example to confirm the Hufbauer et al. (2012) hypothesis. The Colorado potato beetle *Leptinotarsa decemlineata* is a pest of potato (*Solanum tuberosum*) and has already invaded several continents including Europe (Margus 2018). In the dissertation, Margus (2018) shows that anthropogenic stress (i.e., herbicide and insecticide exposure) increased the stress tolerance of this species which could facilitate future invasion processes to other anthropogenically impacted habitats. There are also examples from marine environments. Huhn et al. (2016) tested the

performance of populations of the Asian green mussel *Perna viridis* from heavily impacted Jakarta Bay and two natural sites. Several response variables under salinity stress and oxygen depletion were measured. The results obtained by Huhn et al. (2016) showed that the mussels from the impacted habitat performed better under hypoxia than those from the two natural habitats. The authors suggested that the mussels are pre-adapted to the stressors in Jakarta Bay and can therefore survive better under anthropogenic stress.

Considering some of the evidence of the AIAI hypothesis explained in the previous paragraph, amphipod gammarids might also become potential NNS due to a pre-adaptation in the anthropogenically impacted habitat. As a matter of fact, it has been shown that there are non-native gammarid species including the species *G. tigrinus* (Spooner, 1947; Herkül et al. 2009). This gammarid amphipod is an invader from the North American coast of the Atlantic Ocean and it has successfully established in the Baltic Sea, where it occurs in high numbers next to *G. locusta* and *G. salinus*. This nonindigenous species has extensively reduced the number and diversity of native gammarid species in the Baltic Sea since its successful establishment (Grabowski et al. 2006). This example shows very well that amphipod gammarids might be suitable organisms to become NNS.

Though, one tested population of both *G. salinus* and *G. locusta* were pre-adapted to anthropogenic impacts, this work revealed different strengths of adaptation between the two species, with *G. salinus* being able to tolerate higher stress than *G. locusta*. One reason for this might be due to the different sampling locations of the two species. As already mentioned, due to the low number of individuals of *G. salinus* at Falckenstein beach during the experimental period in 2019, this population was collected from the Kiel Fjord. Although both locations are very close to each other and are expected to have the same anthropogenic impact, it cannot be proven that this is truly the case. Both sampling locations are areas with a lot of ship traffic and low water exchange rates. Especially, as the Kiel Fjord is a closed system with a lot of ship traffic through the canal and harboring ships, it might have a higher anthropogenic impact on organisms than previously anticipated (Nikulina et al. 2008). In fact, the Kiel Fjord is declared to be an important local hot spot of cadmium, lead, copper, and zinc contamination (Landesamt für Natur und Umwelt des Landes Schleswig-Holstein 2002). Additionally, a study by Haarich et al. (2003) showed that concentrations of these pollutants were highest in the inner Fjord and decreased towards the outer Fjord

areas. Also, a pCO_2 monitoring in the Kiel Fjord revealed that values can already reach 3500 ppm in the late summer months (Hiebenthal et al. 2016). For instance, Pane and Barry (2007) support the idea that tolerance to pCO_2 stress differs according to the species´ habitat. Therefore, those reasons might have slightly impacted the different mortalities of the populations from the supposedly same habitat.

Moreover, without inducing further stress, the populations of *G. locusta* already showed a high mortality during the acclimatization period at the institute. Consequently, as the animals were apparently stressed during acclimation, it might have induced a higher mortality during the experimental trials. Furthermore, this showed that *G. locusta* already exhibits signs of stress without any additional environmental factors, simply due to laboratory conditions. Also, as cannibalism was reported in laboratory cultures of *G. locusta* by Costa and Costa (2000), it cannot be ruled out that individuals were eating each other during the experiments, resulting in a higher mortality. Yet, we did not observe any eaten animal, and animals were fed ad libitum.

In addition, our results showed no effect of increased pCO_2 concentrations on the mortality of the two species. However, a negative effect of temperature was visible. The effects of increased temperature were lower in the populations of *G. salinus* than in those of *G. locusta*, but in all populations, mortality rapidly increased when both stressors were combined.

Temperature itself can already have a negative effect on crustaceans; for example, it can decrease the number of offspring (Maranhão and Marques 2003) and lower the survival rate (Gülzow 2015). Therefore, it is not surprising that in the present study, temperature had a very strong effect on the mortality of the test organisms. Indeed, it can be noticed that the populations of *G. salinus* showed lower mortality rates under temperature stress than the populations of *G. locusta*. As Furch (1972) and Bulnheim (1979) already reported, *G. salinus* has quite a high resistance to temperature stress, compared to other amphipod species. However, it does not become clear why *G. locusta* seems to be more temperature sensitive than *G. salinus*. Crustaceans are poikilothermic animals, which means that their body temperature is linked to the temperature of their environment (Lagerspetz and Vainio 2006). Thus, to avoid high temperatures, they must either escape the high temperatures through locomotion or have a high thermal acclimation capacity (Lagerspetz and Vainio 2006). As both

G. locusta and *G. salinus* were not able to escape from the temperature stress during the experiment, the thermal acclimatization capacity might differ between the species. Due to the different environmental conditions, that the species originated from, it could be reasoned that *G. salinus* has a higher capacity to balance temperature changes and consequently is more resistant to high temperatures. It was also observed that animals were molting frequently during the experimental trial. As Cossins and Bowler (1987) point out, high temperature enhances growth and thus molting in crustaceans increases. However, this process costs energy and it was suggested by Halcrow and Boyd (1967) that animals are more likely to be stressed during the molting period. Nevertheless, as amphipods molt frequently in their life cycle and the age of the gammarids was not known, this fact probably is not the major reason for a high mortality under temperature stress. It must also be pointed out, that this experiment only tested constant temperature stress and did not include changing temperatures during day or night periods and thus the results regarding this objective must be seen critically.

Both species do not seem to be affected differently by changing pCO_2 concentrations, which was a rather unexpected result. Due to the fact, that temperature was affecting the amphipods´ mortality negatively, it was anticipated that high pCO_2 concentrations were also a major stress factor for both species. In comparison to recent publications (Borges et al. 2018; Lopes et al. 2019), the results presented in this study did not fully correspond to previous findings. A similar result as in this study was obtained by Hauton et al. (2009), who also did not see any influence of pCO_2 stress on the mortality of gammarids. On the contrary, Lopes et al. (2019) and Borges et al. (2018) demonstrated a decrease in survival at pCO_2 stress of 800 ppm. Yet, in the present study, a pCO_2 stress of 3500 ppm was used and a clear effect on the mortality rate was not confirmed.

Synergistic effects of both factors were visible in the mortality rates of all populations of *G. locusta* and the Maasholm populations of *G. salinus*. When both stressors were combined in the treatments, the mortality increased. However, the statistical analysis showed insignificant results when comparing the mortality of the combined stressors. In comparison, the results did not follow those of other authors. For instance, Dissanayake and Ishimatsu (2011) did find a synergistic physiological effect of pCO_2 and temperature in a shallow-water decapod. Yet, in their study higher pCO_2 concentrations (i.e., pH of < 6.9) than in the present study were used which might explain the different results. It is also possible that the species they tested is

simply more sensitive towards high pCO_2 concentrations than the gammarid species used in the present study. As it was evident in the results that mortality did increase with combined stressors, but the statistical analysis did not confirm the results, another reason for this could be the statistical test itself. For the analysis of differences in mortality between the treatments, a Kruskal-Wallis test was conducted, and all treatments were compared pairwise. However, the more pairs are compared to each other, the lower the threshold value of the analysis is. Thus, there might have been a statistical difference between the treatments, but the test was not strong enough to show this significance.

During the experiment, some fluctuations of water chemistry parameters were observed in all experimental trials. However, those fluctuations were minimal and can be ignored in most cases. At one point, during the experiments with the Kiel population of *G. salinus*, a sudden drop in pH was visible in the control, T1, T2 and T3 (see Appendix: Figure 19). At that time, a problem with the aeration occurred and the tanks were not sufficiently provided with CO_2. As food was provided constantly and biological processes of bacteria were taking place, a lot of oxygen was consumed, and CO_2 was produced which probably led to the sudden decrease in pH (Arnosti et al. 1998). Yet, the aeration was repaired within a couple of hours after the drop and thus we do not anticipate that this incident influenced the results. This was also the reason for the frequent change of water during the experiment. It must be mentioned that a flow-through system might have been the better method as this would have guaranteed a constant water exchange and would have prevented a strong impact of biological processes on the water chemistry. Moreover, the results showed that pH was always slightly higher in all warmer treatments, probably because CO_2 is less soluble when water temperature increases (Enick and Klara 1990).

Furthermore, TA and DIC values were fluctuating a lot during all experimental trials for all populations. This is surprising as TA and therefore DIC values are usually staying constant although CO_2 is added (Wolf-Gladrow et al. 2007). In other publications regarding OA experiments, TA was rather constant and did not show high fluctuations (e.g., Garzke et al. (2016)). In the present study, the reason for this could be the distilled water that was added to the aquaria, when salinity was rising above 14.2 PSU. Although only small amounts were added, this could have been the reason for the change in TA, which was not considered before the experiments started.

5. Conclusion

This study clearly showed that populations from Maasholm had higher mortalities than populations from the anthropogenically impacted habitats, suggesting that the AIAI hypothesis is true. Considering the results and the previous discussion, *G. salinus* could be more and *G. locusta* less pre-adapted to become NNS. *Gammarus salinus* performed quite well under anthropogenic stress and seems to be a very robust species as already demonstrated by other publications. Concerning the populations of *G. locusta,* it is more doubtful that this species can become an introduced species as its performance during the experiment was rather poor. Especially, regarding the invasion stages that must be overcome by entering individuals. For instance, already during the stage of transport, individuals of *G. locusta* might not survive the meanwhile harsh conditions (e.g., in ship ballast waters) while those of *G. salinus* might have better chances of survival in this process.

The results of the present study demonstrated that there was a significant effect of increased temperature on the gammarids´ survival. Especially for the Maasholm populations and both populations of *G. locusta* temperature stress seemed to be a major reason for the high mortality. The anthropogenic stressor pCO_2 itself did not show a strong effect on the mortality of any population. However, both stressors combined could pose a threat to *G. locusta*, but probably not to *G. salinus*. Consequently, as climate change progresses, it might earlier negatively impact *G. locusta* than *G. salinus*. It is expected that high pCO_2 concentrations do not show an early effect, but might increase the overall stress with increasing climate change when more stressors are increasing simultaneously. Consequently, the previous hypothesis that one of the species might perform differently under anthropogenic stress has to be accepted as *G. salinus* performed better regarding the mortality than *G. locusta*. Also, the increasing pCO_2 concentrations combined with temperature of the treatments showed an effect on the mortality of some of the populations; thus, this hypothesis will partly be accepted.

Additional preferentially long-term or transgenerational studies, testing not only mortality, but also other parameters like growth, reproduction, and stress hormones, are needed to further confirm the AIAI hypothesis and effect of anthropogenic impact on different species and communities.

Acknowledgments

I want to especially thank PD Dr. habil. Elizabeta Briski for agreeing to be my supervisor and helping me to conduct my master thesis at the GEOMAR institute. Also, I want to thank her for being a very patient and reliable supervisor who would always answer all my questions and help me with the problems that occurred during my work.
Also, I want to thank Prof. Dr. Peter Schupp for being my second supervisor and Dr. Sven Rohde for agreeing to referee my thesis.

A special thanks goes to Gregor Steffen for helping me with my experimental setup and the collection of samples and supporting me during the experimental trial.
Additionally, a big thanks goes to Filipa Paiva for teaching me how to work with amphipod gammarids and giving me a lot of advice for conducting my experiments.
Also, I want to thank all employees of the GEOMAR institute who helped me with the analysis of my samples and providing me with material and advice whenever needed.
At last, I want to thank the `Verein Jordsand zum Schutz der Seevögel und der Natur e.V.` for allowing me to collect my samples in the nature reserve Schleimünde.

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Appendix

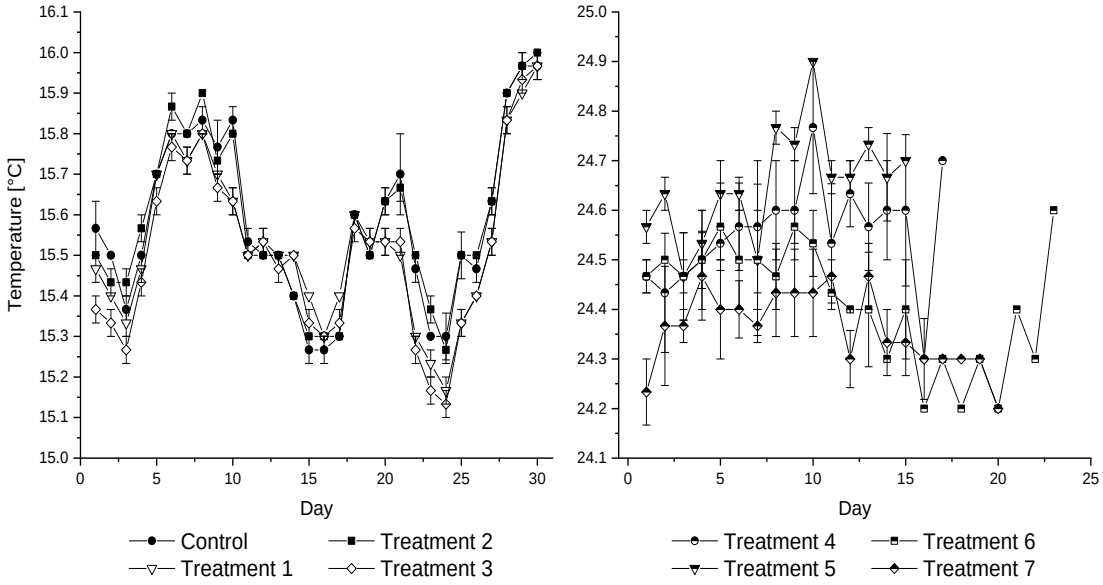


Figure 9 Temperature trend of *G. locusta* (Falckenstein). Control (400 ppm, 16°C); Treatment 1 (1600 ppm, 16°C) ; Treatment 2 (2700 ppm, 16°C); Treatment 3 (3500 ppm, 16°C); Treatment 4 (400 ppm, 24°C); Treatment 5 (1600 ppm, 24°C); Treatment 6 (2700 ppm, 24°C); Treatment 7 (3500 ppm, 24°C)).Values are mean ± SE.

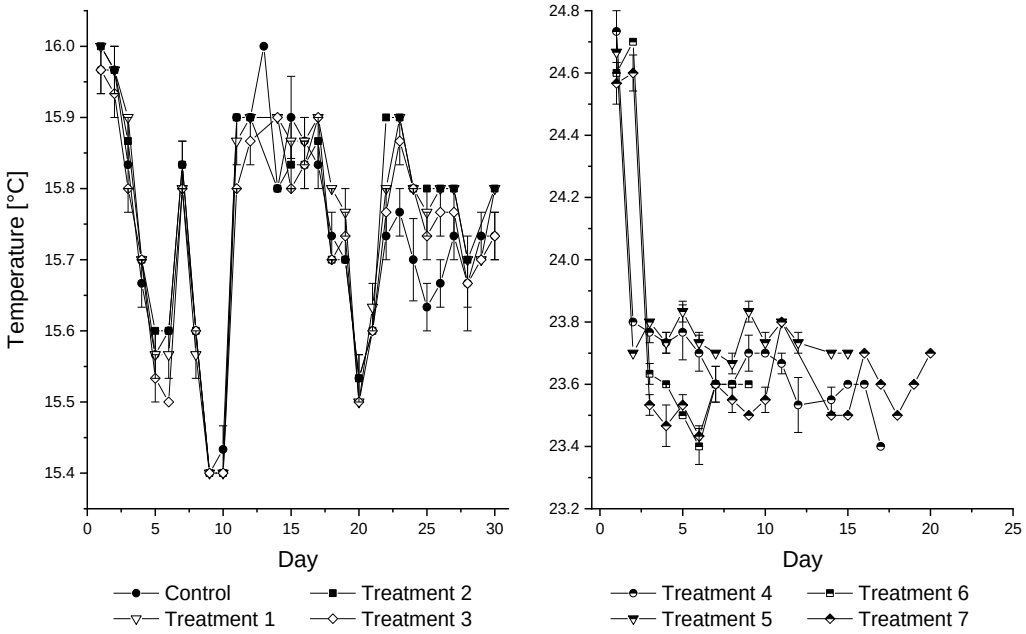


Figure 10 Temperature trend of *G. locusta* (Maasholm). Control (400 ppm, 16°C); Treatment 1 (1600 ppm, 16°C) ; Treatment 2 (2700 ppm, 16°C); Treatment 3 (3500 ppm, 16°C); Treatment 4 (400 ppm, 24°C); Treatment 5 (1600 ppm, 24°C); Treatment 6 (2700 ppm, 24°C); Treatment 7 (3500 ppm, 24°C)).Values are mean ± SE.

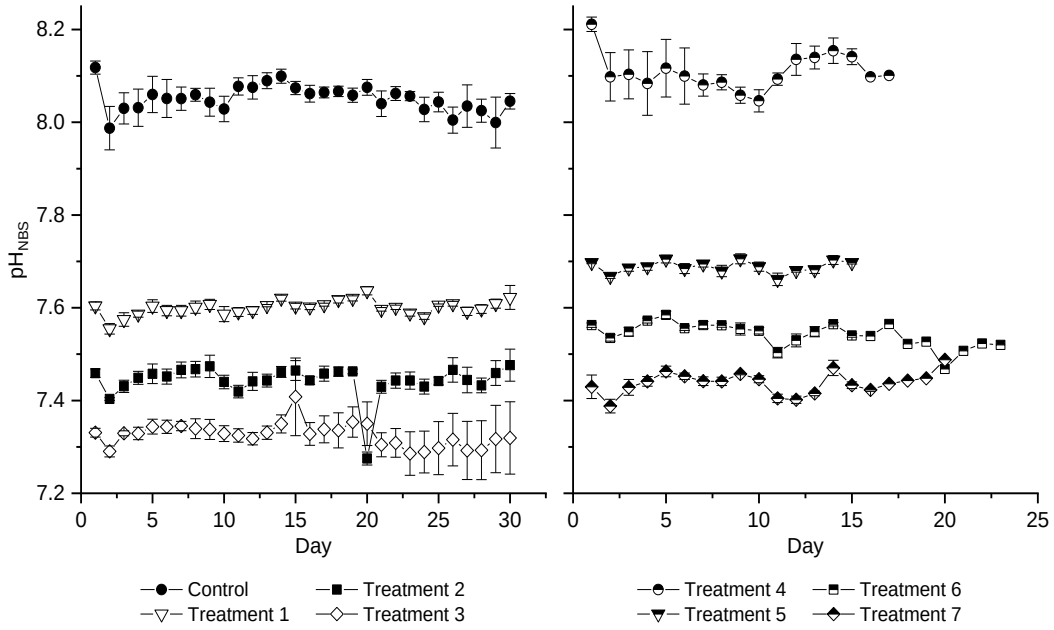


Figure 11 pH_{NBS} trend of *G. locusta* (Falckenstein). Control (400 ppm, 16°C); Treatment 1 (1600 ppm, 16°C) ; Treatment 2 (2700 ppm, 16°C); Treatment 3 (3500 ppm, 16°C); Treatment 4 (400 ppm, 24°C); Treatment 5 (1600 ppm, 24°C); Treatment 6 (2700 ppm, 24°C); Treatment 7 (3500 ppm, 24°C)).Values are mean ± SE.

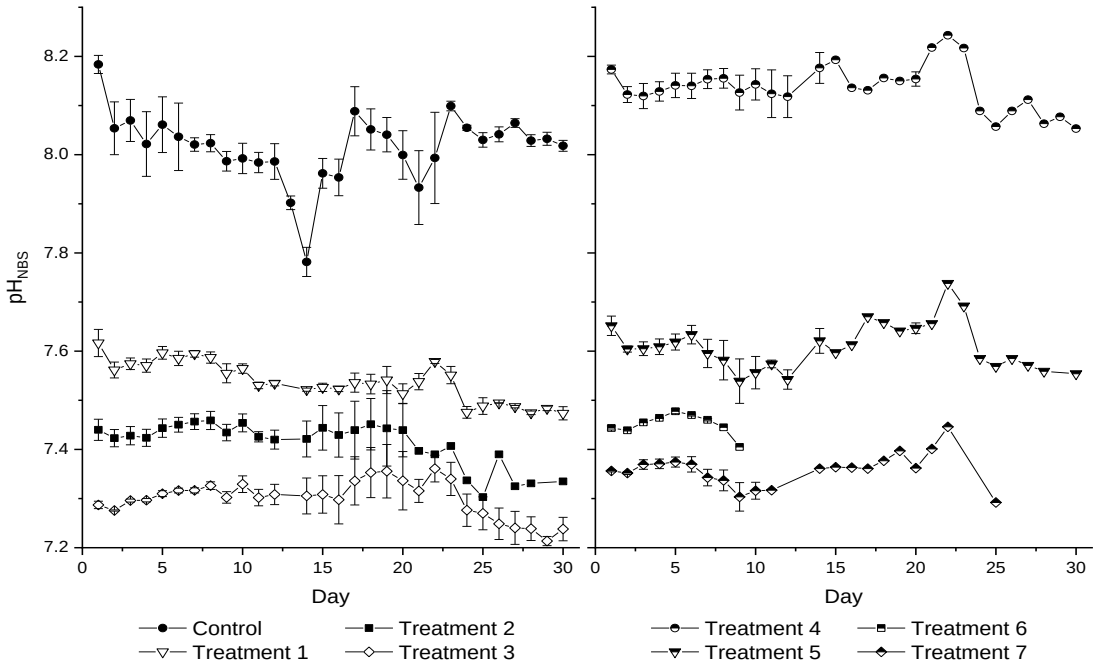


Figure 12 pH_{NBS} trend of *G. locusta* (Maasholm). Control (400 ppm, 16°C); Treatment 1 (1600 ppm, 16°C) ; Treatment 2 (2700 ppm, 16°C); Treatment 3 (3500 ppm, 16°C); Treatment 4 (400 ppm, 24°C); Treatment 5 (1600 ppm, 24°C); Treatment 6 (2700 ppm, 24°C); Treatment 7 (3500 ppm, 24°C)).Values are mean ± SE.

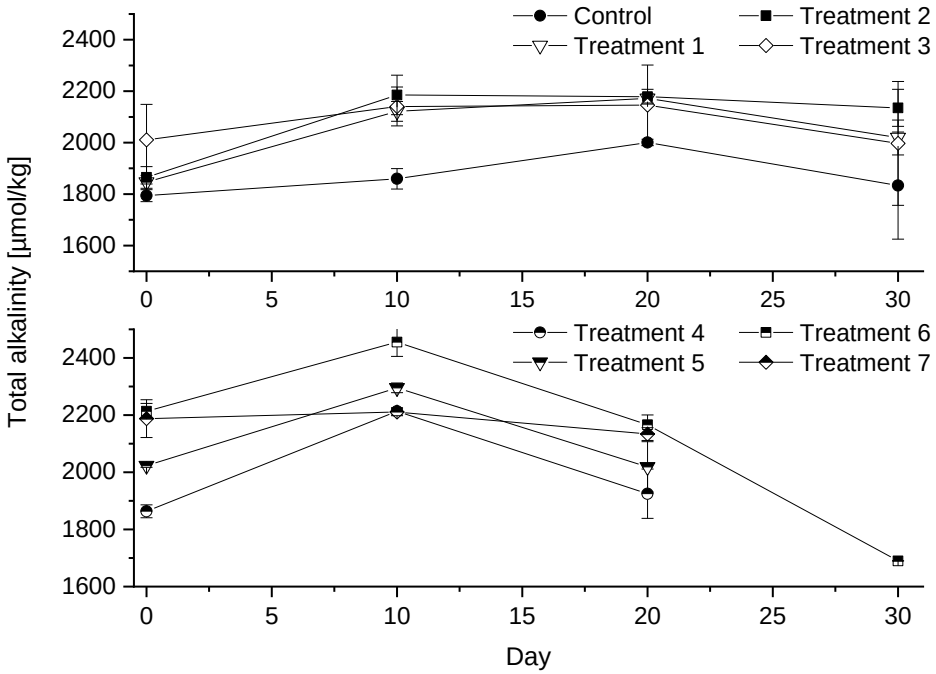


Figure 13 Total alkalinity trend of *G. locusta* (Falckenstein). Control (400 ppm, 16°C); Treatment 1 (1600 ppm, 16°C) ; Treatment 2 (2700 ppm, 16°C); Treatment 3 (3500 ppm, 16°C); Treatment 4 (400 ppm, 24°C); Treatment 5 (1600 ppm, 24°C); Treatment 6 (2700 ppm, 24°C); Treatment 7 (3500 ppm, 24°C)).Values are mean ± SE.

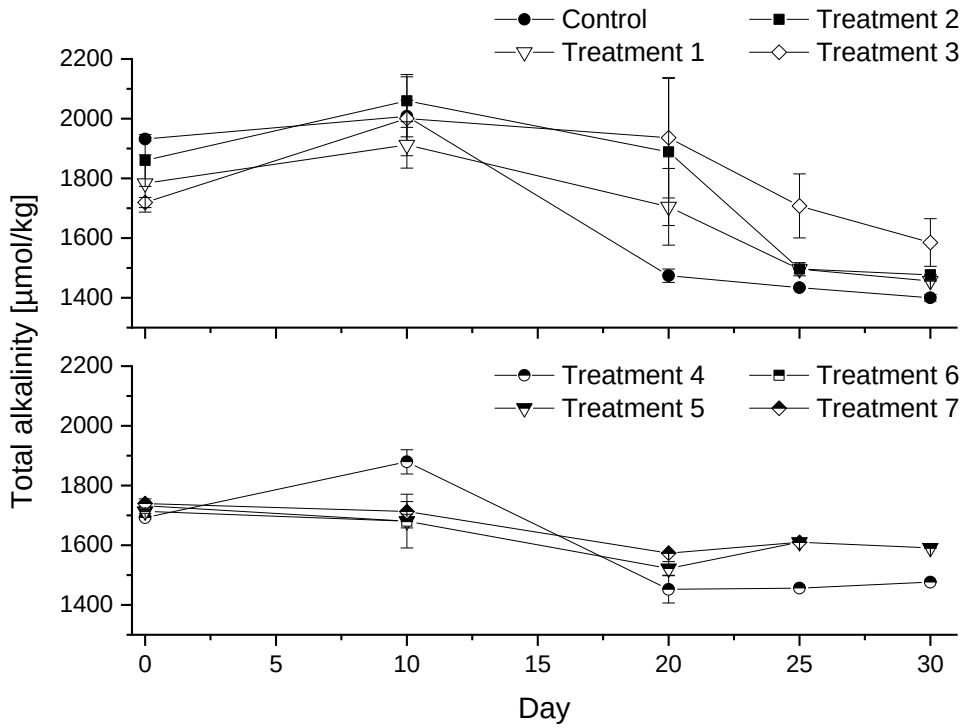


Figure 14 Total alkalinity trend of *G. locusta* (Maasholm). Control (400 ppm, 16°C); Treatment 1 (1600 ppm, 16°C); Treatment 2 (2700 ppm, 16°C); Treatment 3 (3500 ppm, 16°C); Treatment 4 (400 ppm, 24°C); Treatment 5 (1600 ppm, 24°C); Treatment 6 (2700 ppm, 24°C); Treatment 7 (3500 ppm, 24°C)).Values are mean ± SE.

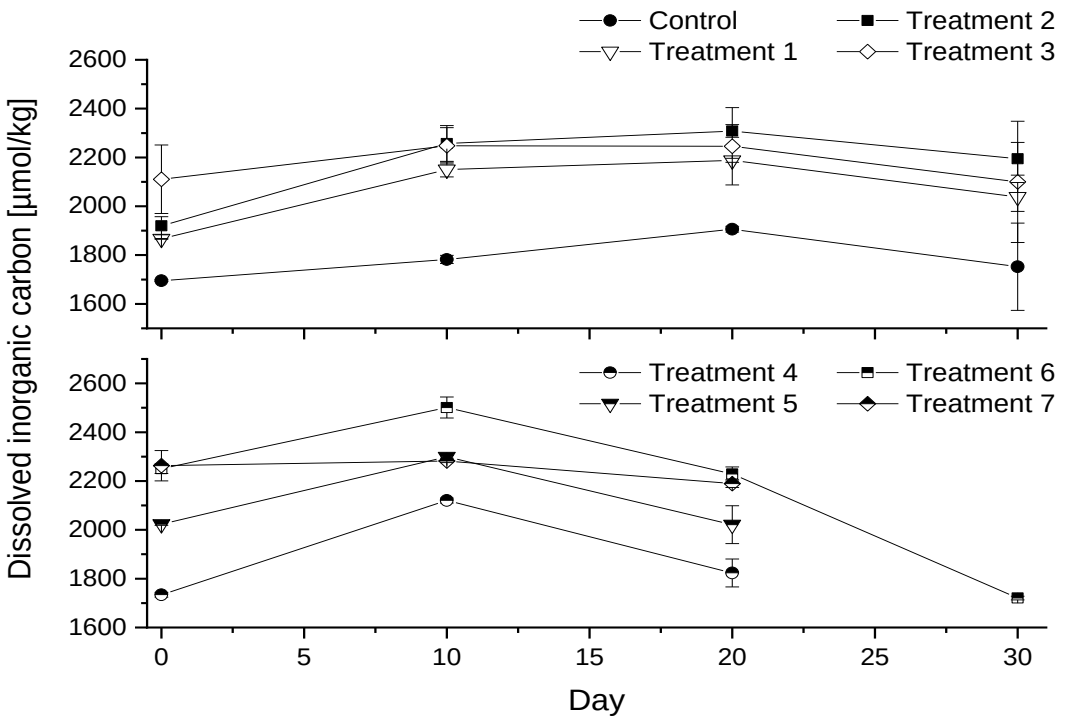


Figure 15 Dissolved inorganic carbon trend of *G. locusta* (Falckenstein). Control (400 ppm, 16°C); Treatment 1 (1600 ppm, 16°C) ; Treatment 2 (2700 ppm, 16°C); Treatment 3 (3500 ppm, 16°C); Treatment 4 (400 ppm, 24°C); Treatment 5 (1600 ppm, 24°C); Treatment 6 (2700 ppm, 24°C); Treatment 7 (3500 ppm, 24°C)).Values are mean ± SE.

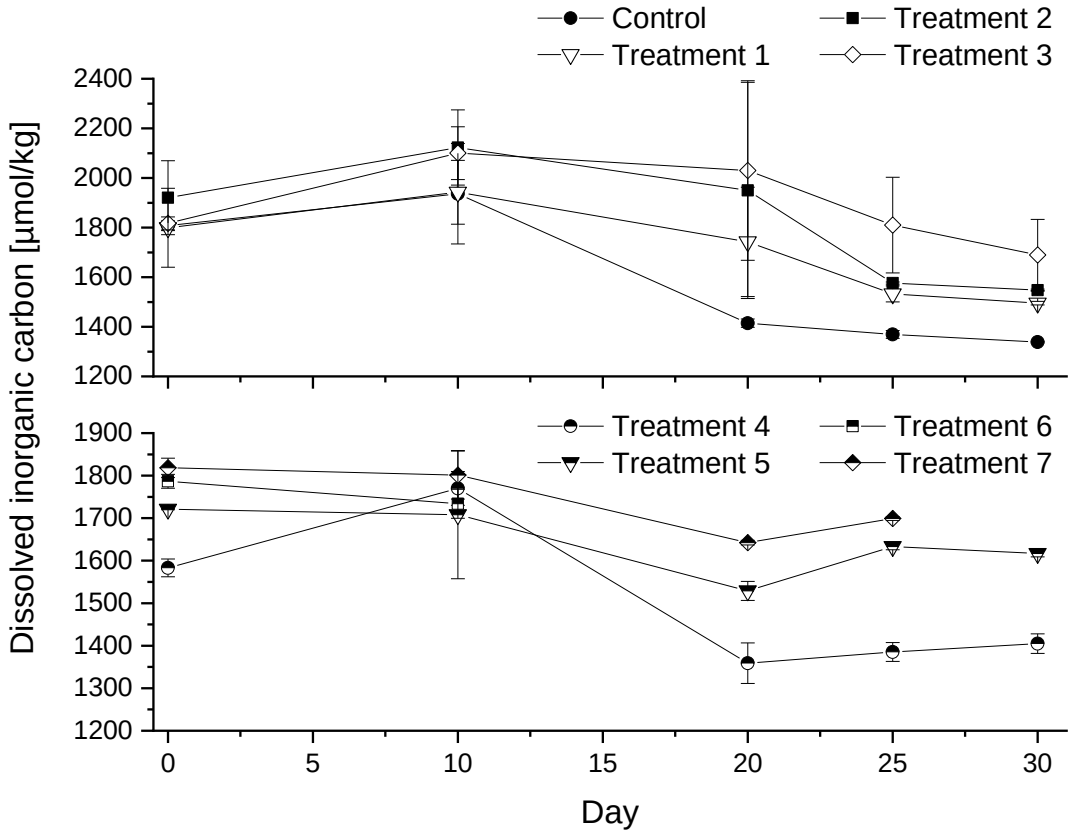


Figure 16 Dissolved inorganic carbon trend of *G. locusta* (Maasholm). Control (400 ppm, 16°C); Treatment 1 (1600 ppm, 16°C) ; Treatment 2 (2700 ppm, 16°C); Treatment 3 (3500 ppm, 16°C); Treatment 4 (400 ppm, 24°C); Treatment 5 (1600 ppm, 24°C); Treatment 6 (2700 ppm, 24°C); Treatment 7 (3500 ppm, 24°C)).Values are mean ± SE.

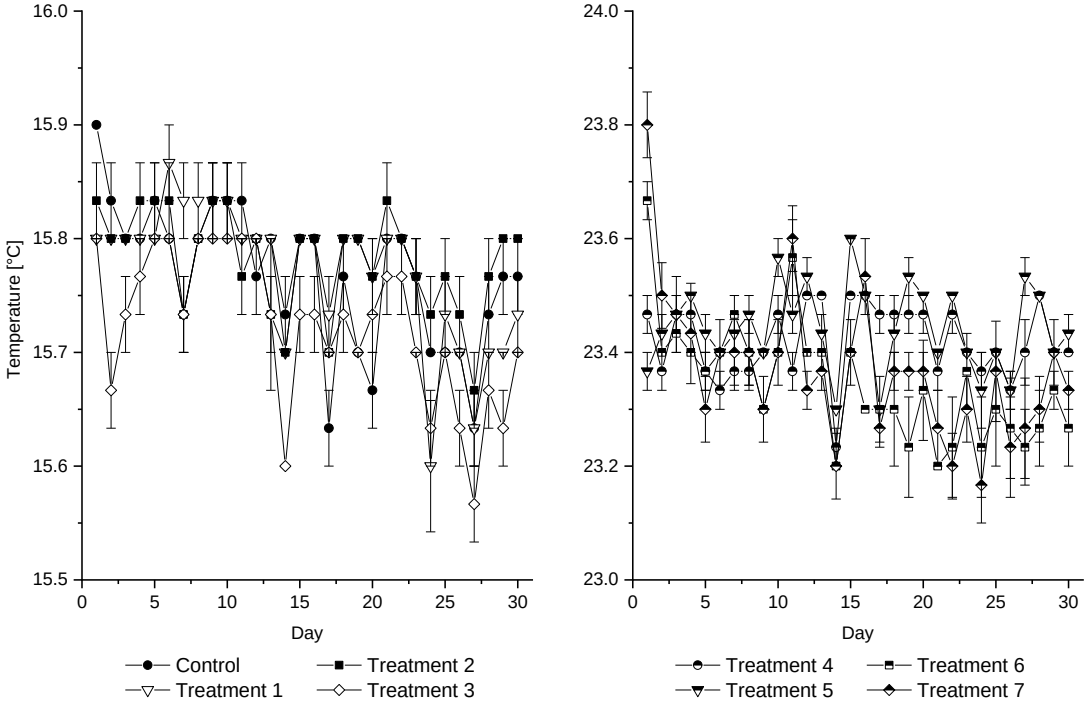


Figure 17 Temperature trend of *G. salinus* (Kiel). Control (400 ppm, 16°C); Treatment 1 (1600 ppm, 16°C) ; Treatment 2 (2700 ppm, 16°C); Treatment 3 (3500 ppm, 16°C); Treatment 4 (400 ppm, 24°C); Treatment 5 (1600 ppm, 24°C); Treatment 6 (2700 ppm, 24°C); Treatment 7 (3500 ppm, 24°C)).Values are mean ± SE.

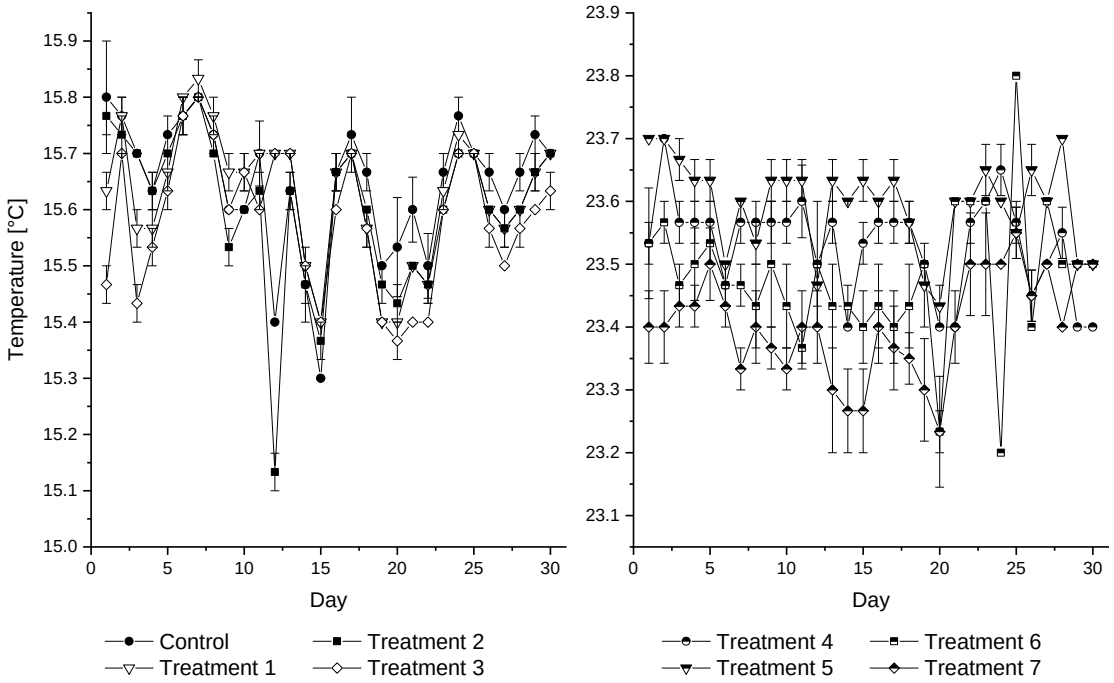


Figure 18 Temperature trend of *G. salinus* (Maasholm). Control (400 ppm, 16°C); Treatment 1 (1600 ppm, 16°C) ; Treatment 2 (2700 ppm, 16°C); Treatment 3 (3500 ppm, 16°C); Treatment 4 (400 ppm, 24°C); Treatment 5 (1600 ppm, 24°C); Treatment 6 (2700 ppm, 24°C); Treatment 7 (3500 ppm, 24°C)).Values are mean ± SE.

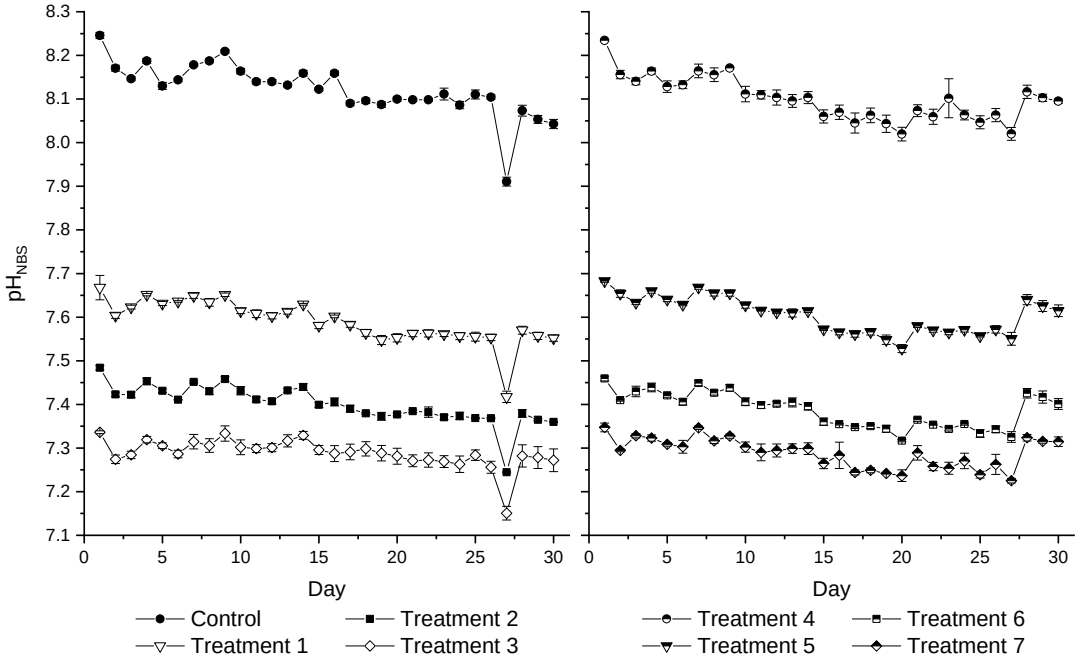


Figure 19 pH_{NBS} trend of *G. salinus* (Kiel). Control (400 ppm, 16°C); Treatment 1 (1600 ppm, 16°C) ; Treatment 2 (2700 ppm, 16°C); Treatment 3 (3500 ppm, 16°C); Treatment 4 (400 ppm, 24°C); Treatment 5 (1600 ppm, 24°C); Treatment 6 (2700 ppm, 24°C); Treatment 7 (3500 ppm, 24°C)).Values are mean ± SE.

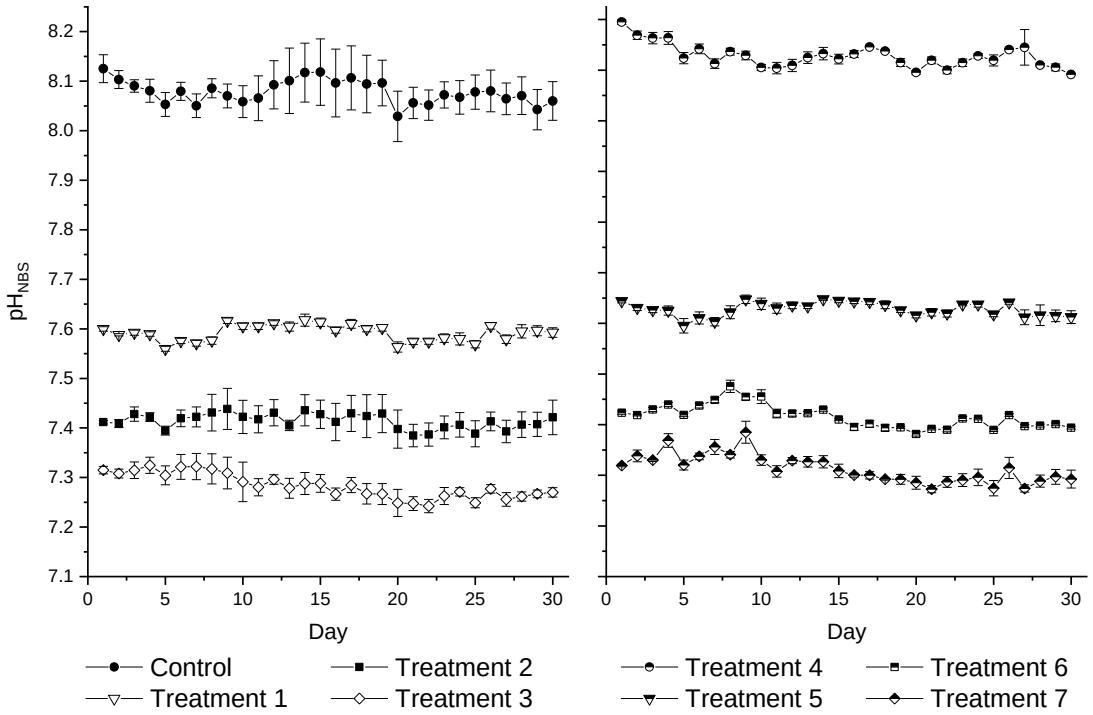


Figure 20 pH_{NBS} trend of *G. salinus* (Maasholm). Control (400 ppm, 16°C); Treatment 1 (1600 ppm, 16°C) ; Treatment 2 (2700 ppm, 16°C); Treatment 3 (3500 ppm, 16°C); Treatment 4 (400 ppm, 24°C); Treatment 5 (1600 ppm, 24°C); Treatment 6 (2700 ppm, 24°C); Treatment 7 (3500 ppm, 24°C)).Values are mean ± SE.

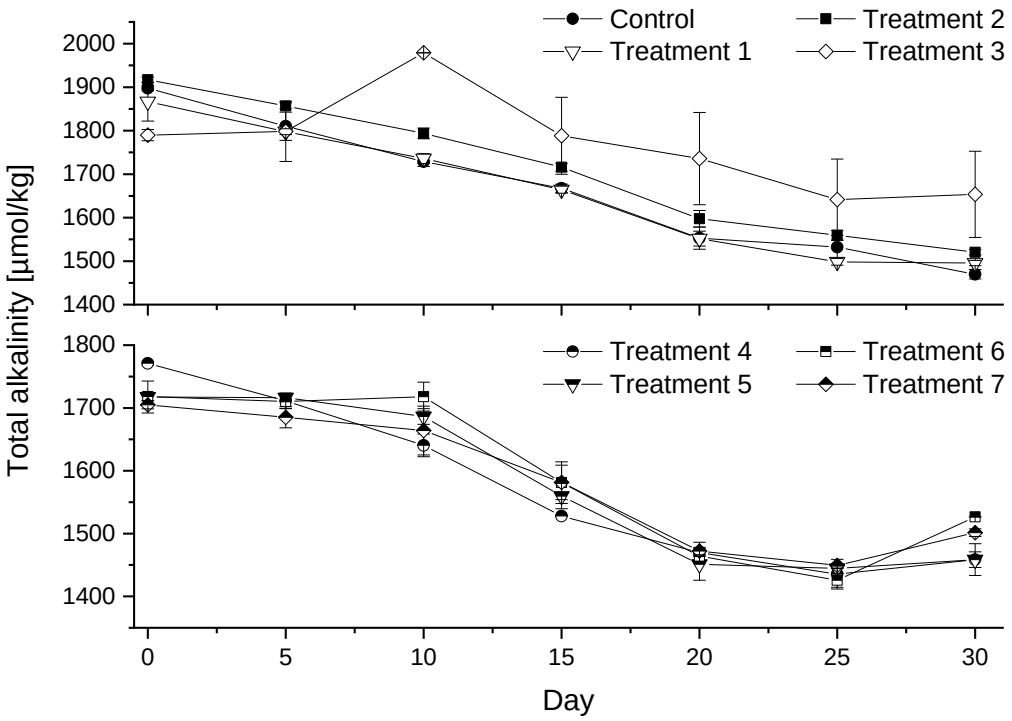


Figure 21 Total alkalinity trend of *G. salinus* (Kiel). Control (400 ppm, 16°C); Treatment 1 (1600 ppm, 16°C); Treatment 2 (2700 ppm, 16°C); Treatment 3 (3500 ppm, 16°C); Treatment 4 (400 ppm, 24°C); Treatment 5 (1600 ppm, 24°C); Treatment 6 (2700 ppm, 24°C); Treatment 7 (3500 ppm, 24°C)).Values are mean ± SE.

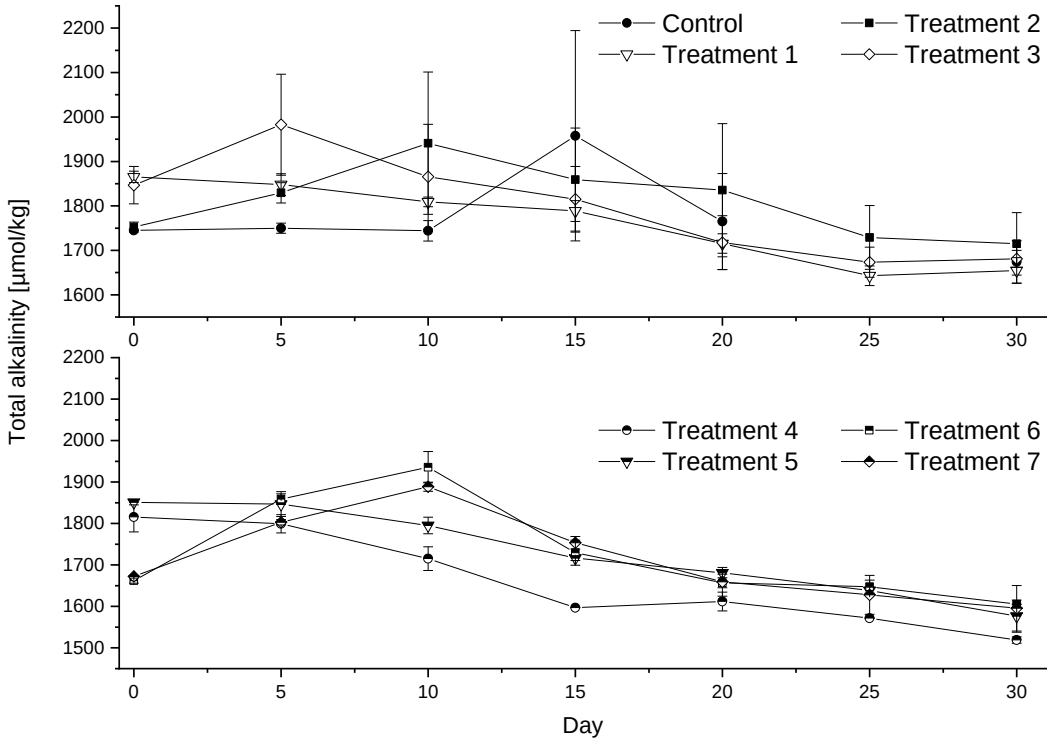


Figure 22 Total alkalinity trend of *G. salinus* (Maasholm). Control (400 ppm, 16°C); Treatment 1 (1600 ppm, 16°C) ; Treatment 2 (2700 ppm, 16°C); Treatment 3 (3500 ppm, 16°C); Treatment 4 (400 ppm, 24°C); Treatment 5 (1600 ppm, 24°C); Treatment 6 (2700 ppm, 24°C); Treatment 7 (3500 ppm, 24°C)).Values are mean ± SE.

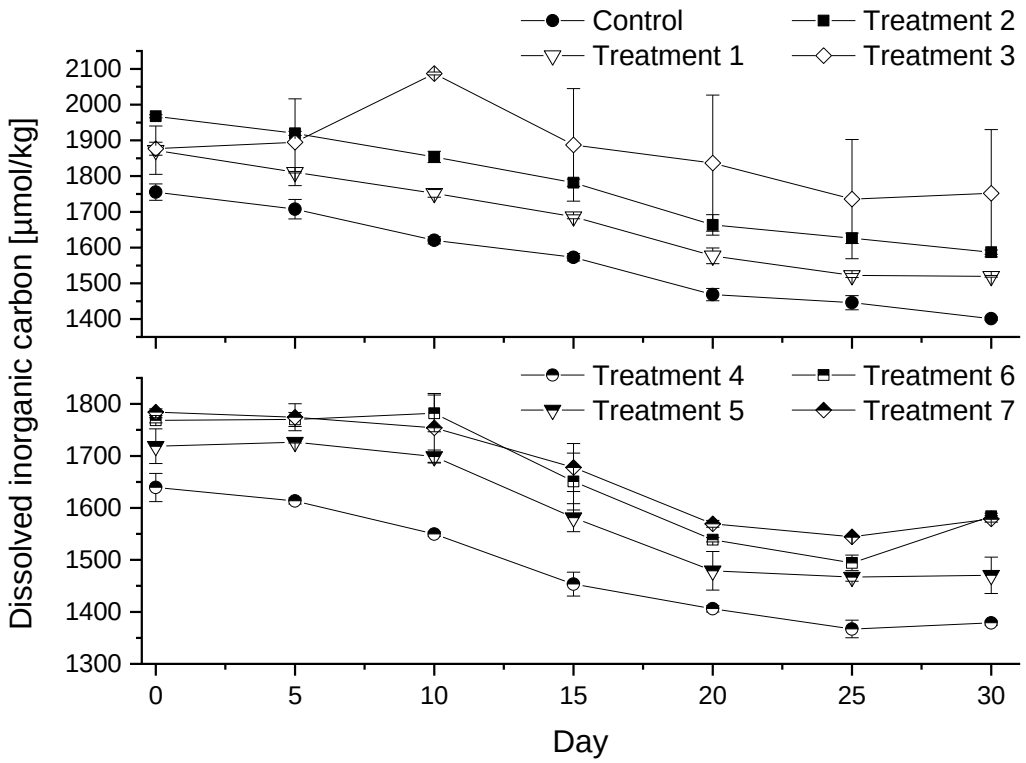


Figure 23 Dissolved inorganic carbon trend of *G. salinus* (Kiel). Control (400 ppm, 16°C); Treatment 1 (1600 ppm, 16°C); Treatment 2 (2700 ppm, 16°C); Treatment 3 (3500 ppm, 16°C); Treatment 4 (400 ppm, 24°C); Treatment 5 (1600 ppm, 24°C); Treatment 6 (2700 ppm, 24°C); Treatment 7 (3500 ppm, 24°C)).Values are mean ± SE.

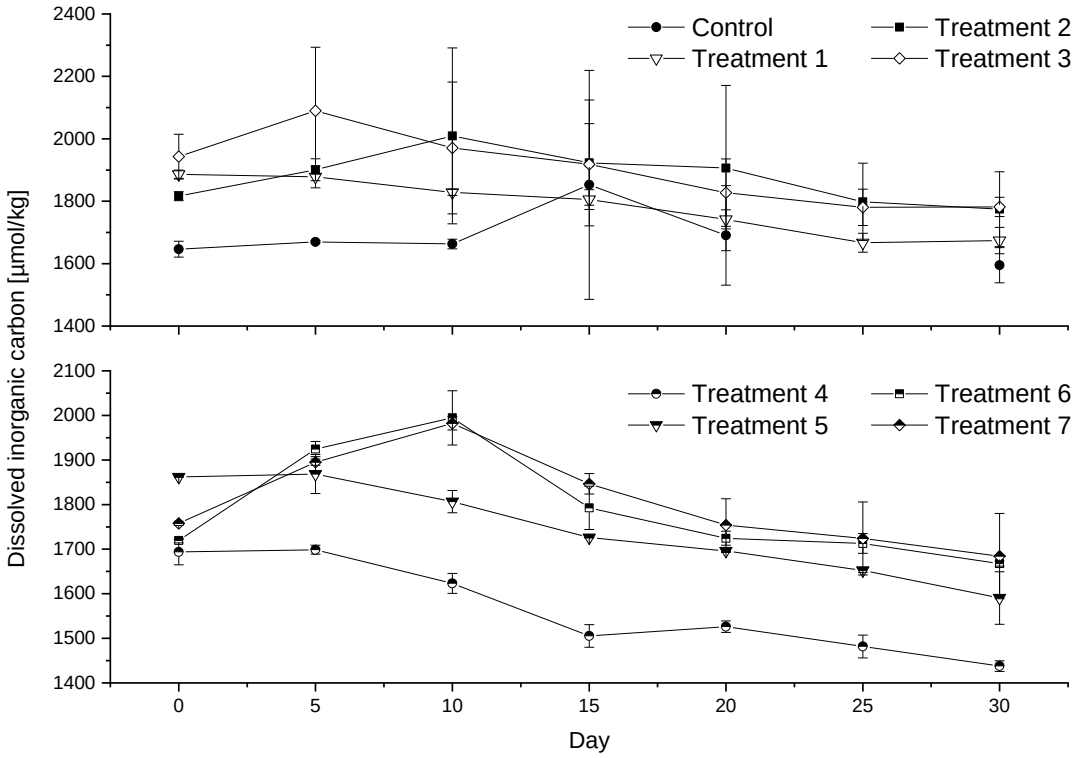


Figure 24 Dissolved inorganic carbon trend of *G. salinus* (Maasholm). Control (400 ppm, 16°C); Treatment 1 (1600 ppm, 16°C) ; Treatment 2 (2700 ppm, 16°C); Treatment 3 (3500 ppm, 16°C); Treatment 4 (400 ppm, 24°C); Treatment 5 (1600 ppm, 24°C); Treatment 6 (2700 ppm, 24°C); Treatment 7 (3500 ppm, 24°C)).Values are mean ± SE.

Table 7 Survival of individuals of *G. locusta* (Falckenstein) shown for three replicates (1-3), for Control and 7 treatments (Treatment 1- Treatment 7); pH and temp (mean values) represent pH_{NBS} and temperature (in °C).

	Control (400 ppm, 16°C)					**Treatment 1 (1600 ppm, 16°C)**					**Treatment 2 (2700 ppm, 16°C)**				
Day	**Individuals**			**pH**	**Temp**	**Individuals**			**pH**	**Temp**	**Individuals**			**pH**	**Temp**
	1	2	3			1	2	3			1	2	3		
1	10	10	10	8.118	15.6	10	10	10	7.604	15.5	10	10	10	7.459	15.5
2	9	10	10	7.987	15.5	10	10	10	7.555	15.4	10	9	10	7.403	15.4
3	9	10	10	8.030	15.4	10	10	10	7.575	15.3	10	9	10	7.431	15.4
4	9	10	9	8.031	15.5	10	10	10	7.586	15.5	10	9	10	7.449	15.6
5	9	10	9	8.060	15.7	9	10	10	7.604	15.7	10	9	10	7.458	15.7
6	9	10	9	8.051	15.8	9	10	10	7.594	15.8	10	9	10	7.452	15.9
7	8	10	9	8.051	15.8	9	10	10	7.594	15.7	9	9	10	7.466	15.8
8	8	10	8	8.059	15.8	9	9	10	7.601	15.8	9	9	10	7.468	15.9
9	8	10	8	8.043	15.8	9	9	10	7.607	15.7	9	9	9	7.474	15.7
10	8	10	8	8.029	15.8	9	9	9	7.586	15.6	9	8	9	7.440	15.8
11	8	10	7	8.077	15.5	8	9	9	7.591	15.5	9	7	8	7.419	15.5
12	8	10	7	8.075	15.5	7	9	9	7.594	15.5	9	6	8	7.441	15.5
13	8	10	6	8.090	15.5	7	9	9	7.605	15.5	8	6	8	7.443	15.5
14	8	10	6	8.099	15.4	7	9	9	7.621	15.5	8	6	8	7.462	15.4
15	8	9	6	8.074	15.3	7	8	9	7.604	15.4	8	6	8	7.465	15.3
16	7	9	6	8.062	15.3	7	8	9	7.602	15.3	8	6	8	7.443	15.3
17	7	9	6	8.064	15.3	7	7	8	7.607	15.4	8	6	6	7.458	15.3
18	7	9	6	8.067	15.6	7	6	8	7.618	15.6	8	6	6	7.462	15.6
19	7	9	5	8.058	15.5	6	5	8	7.620	15.5	8	6	6	7.463	15.5
20	7	9	5	8.075	15.6	6	5	8	7.637	15.5	8	6	6	7.275	15.6
21	7	9	4	8.040	15.7	6	5	8	7.597	15.5	8	6	6	7.430	15.7
22	7	9	4	8.062	15.5	5	5	8	7.601	15.3	8	6	6	7.443	15.5
23	7	9	3	8.056	15.3	5	5	8	7.589	15.2	8	6	5	7.443	15.4
24	7	9	3	8.028	15.3	4	5	8	7.581	15.2	8	6	5	7.430	15.3
25	6	9	3	8.044	15.5	4	5	8	7.606	15.3	8	6	4	7.442	15.5
26	6	9	2	8.005	15.5	4	5	8	7.609	15.4	8	6	4	7.466	15.5
27	6	9	2	8.035	15.6	4	5	8	7.592	15.5	8	6	4	7.444	15.6
28	6	9	2	8.025	15.9	4	4	8	7.597	15.8	8	6	4	7.433	15.9
29	6	8	2	7.999	16.0	4	4	8	7.609	15.9	8	6	3	7.459	16.0
30	6	8	2	8.045	16.0	4	4	7	7.622	16.0	8	6	3	7.476	16.0

	Treatment 3 (3500 ppm, 16°C)					Treatment 4 (400 ppm, 24°C)					Treatment 5 (1600 ppm, 24°C)				
Day	Individuals			pH	Temp	Individuals			pH	Temp	Individuals			pH	Temp
	1	2	3			1	2	3			1	2	3		
1	10	10	10	7.331	15.4	10	10	10	8.211	24.5	10	10	10	7.697	24.6
2	10	10	10	7.290	15.3	10	9	10	8.098	24.4	10	10	10	7.668	24.6
3	9	10	10	7.329	15.3	10	9	10	8.103	24.5	10	9	10	7.686	24.5
4	9	10	10	7.329	15.4	10	8	9	8.084	24.5	10	7	9	7.688	24.5
5	9	9	10	7.344	15.6	10	6	8	8.117	24.5	7	4	9	7.705	24.6
6	9	9	10	7.343	15.8	9	5	6	8.100	24.6	4	4	6	7.685	24.6
7	9	9	10	7.345	15.7	6	3	5	8.080	24.6	3	3	5	7.694	24.5
8	9	9	10	7.339	15.8	6	3	5	8.086	24.6	3	0	5	7.679	24.8
9	9	9	10	7.338	15.7	5	2	5	8.058	24.6	1	0	5	7.706	24.7
10	9	9	9	7.329	15.6	2	1	4	8.046	24.8	1	0	3	7.689	24.9
11	9	9	9	7.325	15.5	1	0	2	8.093	24.5	1	0	1	7.661	24.7
12	9	9	9	7.318	15.5	1	0	2	8.136	24.6	1	0	1	7.680	24.7
13	9	9	9	7.331	15.5	1	0	1	8.140	24.6	1	0	0	7.682	24.7
14	9	8	9	7.350	15.5	0	0	1	8.154	24.6	1	0	0	7.703	24.7
15	9	8	9	7.408	15.3	0	0	1	8.141	24.6	0	0	0	7.697	24.7
16	9	7	9	7.328	15.3	0	0	1	8.098	24.3	0	0	0	-	-
17	9	7	9	7.338	15.3	0	0	0	8.101	24.7	0	0	0	-	-
18	9	7	9	7.336	15.6	0	0	0	-	-	0	0	0	-	-
19	8	7	9	7.354	15.5	0	0	0	-	-	0	0	0	-	-
20	8	7	8	7.350	15.5	0	0	0	-	-	0	0	0	-	-
21	8	7	8	7.305	15.5	0	0	0	-	-	0	0	0	-	-
22	8	7	8	7.309	15.3	0	0	0	-	-	0	0	0	-	-
23	7	7	7	7.286	15.2	0	0	0	-	-	0	0	0	-	-
24	7	7	6	7.289	15.1	0	0	0	-	-	0	0	0	-	-
25	7	7	6	7.297	15.3	0	0	0	-	-	0	0	0	-	-
26	7	7	6	7.316	15.4	0	0	0	-	-	0	0	0	-	-
27	7	6	6	7.292	15.5	0	0	0	-	-	0	0	0	-	-
28	7	6	6	7.293	15.8	0	0	0	-	-	0	0	0	-	-
29	7	6	5	7.317	15.9	0	0	0	-	-	0	0	0	-	-
30	7	6	5	7.319	16.0	0	0	0	-	-	0	0	0	-	-

	Treatment 6 (2700 ppm, 24°C)					Treatment 7 (3500 ppm, 24°C)				
Day	Individuals			pH	Temp	Individuals			pH	Temp
	1	2	3			1	2	3		
1	10	10	10	7.563	24.5	10	10	10	7.430	24.2
2	9	10	9	7.535	24.5	9	10	10	7.388	24.4
3	8	10	9	7.548	24.5	8	9	9	7.428	24.4
4	7	10	9	7.572	24.5	8	9	9	7.441	24.5
5	7	9	8	7.585	24.6	7	8	8	7.463	24.4
6	7	6	7	7.556	24.5	6	8	8	7.452	24.4
7	6	6	6	7.563	24.5	6	5	8	7.442	24.4
8	4	6	6	7.562	24.5	5	4	8	7.441	24.4
9	3	5	5	7.554	24.6	3	3	6	7.457	24.4
10	2	4	4	7.550	24.5	3	2	4	7.446	24.4
11	1	2	3	7.503	24.4	3	2	3	7.405	24.5
12	1	2	2	7.530	24.4	3	2	3	7.402	24.3
13	1	0	1	7.548	24.4	3	2	1	7.415	24.5
14	1	0	0	7.565	24.3	3	2	0	7.470	24.3
15	1	0	0	7.540	24.4	3	2	0	7.433	24.3
16	1	0	0	7.539	24.2	0	2	0	7.423	24.3
17	1	0	0	7.565	24.3	0	2	0	7.436	24.3
18	1	0	0	7.522	24.2	0	2	0	7.443	24.3
19	1	0	0	7.527	24.3	0	1	0	7.448	24.3
20	1	0	0	7.468	24.2	0	0	0	7.488	24.2
21	1	0	0	7.507	24.4	0	0	0	-	-
22	1	0	0	7.523	24.3	0	0	0	-	-
23	0	0	0	7.520	24.6	0	0	0	-	-
24	0	0	0	-	-	0	0	0	-	-
25	0	0	0	-	-	0	0	0	-	-
26	0	0	0	-	-	0	0	0	-	-
27	0	0	0	-	-	0	0	0	-	--
28	0	0	0	-	-	0	0	0	-	-
29	0	0	0	-	--	0	0	0	-	-
30	0	0	0	-	-	0	0	0	-	-

Table 8 Survival of individuals of *G. locusta* (Maasholm) shown for three replicates (1-3), for Control and 7 treatments (Treatment 1- Treatment 7); pH and temp (mean values) represent pH_{NBS} and temperature (in °C).

	Control (400 ppm, 16°C)					**Treatment 1 (1600 ppm, 16°C)**					**Treatment 2 (2700 ppm, 16°C)**				
Day	**Individuals**			**pH**	**Temp**	**Individuals**			**pH**	**Temp**	**Individuals**			**pH**	**Temp**
	1	2	3			1	2	3			1	2	3		
1	10	10	10	8.184	16.0	10	10	10	7.617	16.0	10	10	10	7.440	16.0
2	10	9	10	8.054	16.0	10	9	9	7.562	16.0	9	10	10	7.423	16.0
3	10	9	10	8.070	15.8	9	9	9	7.574	15.9	8	10	10	7.428	15.9
4	9	8	10	8.022	15.7	9	9	9	7.571	15.7	7	10	9	7.424	15.7
5	9	8	9	8.061	15.6	9	9	9	7.597	15.6	6	8	9	7.443	15.6
6	9	8	8	8.036	15.6	9	8	9	7.585	15.6	6	8	9	7.450	15.6
7	9	7	8	8.021	15.8	9	8	9	7.595	15.8	6	8	8	7.457	15.8
8	8	7	8	8.023	15.6	8	7	9	7.587	15.6	6	8	8	7.459	15.6
9	8	7	6	7.987	15.4	8	7	9	7.555	15.4	6	8	8	7.434	15.4
10	8	7	6	7.992	15.4	8	7	9	7.565	15.4	6	8	8	7.454	15.4
11	8	7	6	7.984	15.9	7	7	9	7.530	15.9	6	8	8	7.426	15.9
12	8	7	6	7.986	15.9	7	5	8	7.535	15.9	5	8	7	7.420	15.9
13	5	7	6	7.902	16.0	7	3	8	-	-	4	6	6	-	-
14	5	7	6	7.782	15.8	7	3	7	7.522	15.9	4	5	6	7.421	15.8
15	5	7	6	7.962	15.9	6	3	6	7.526	15.9	3	4	6	7.444	15.8
16	5	7	6	7.954	15.9	6	3	6	7.523	15.9	1	3	6	7.429	15.8
17	5	7	5	8.088	15.8	6	2	6	7.536	15.9	1	1	6	7.439	15.9
18	5	7	5	8.051	15.7	6	2	6	7.533	15.8	0	1	4	7.451	15.7
19	5	7	5	8.041	15.7	6	2	6	7.541	15.8	0	0	4	7.443	15.7
20	3	6	5	7.999	15.5	6	2	6	7.513	15.5	0	0	2	7.439	15.5
21	3	6	5	7.933	15.6	4	2	6	7.538	15.6	0	0	2	7.397	15.6
22	3	6	5	7.993	15.7	4	2	5	7.579	15.8	0	0	1	7.390	15.9
23	3	5	5	8.099	15.8	3	0	5	7.551	15.9	0	0	1	7.407	15.9
24	3	5	5	8.055	15.7	3	0	5	7.476	15.8	0	0	1	7.337	15.8
25	2	4	5	8.030	15.6	2	0	5	7.488	15.8	0	0	1	7.303	15.8
26	2	4	5	8.041	15.7	1	0	4	7.495	15.8	0	0	1	7.390	15.8
27	2	3	5	8.064	15.7	1	0	4	7.487	15.8	0	0	1	7.325	15.8
28	2	3	5	8.029	15.7	1	0	3	7.475	15.7	0	0	0	7.331	15.7
29	1	3	5	8.032	15.7	1	0	2	7.484	15.7	0	0	0	-	-
30	1	3	4	8.018	15.7	1	0	1	7.474	15.8	0	0	0	-	-

	Treatment 3 (3500 ppm, 16°C)					Treatment 4 (400 ppm, 24°C)					Treatment 5 (1600 ppm, 24°C)				
Day	Individuals			pH	Temp	Individuals			pH	Temp	Individuals			pH	Temp
	1	2	3			1	2	3			1	2	3		
1	10	10	10	7.287	16.0	10	10	10	8.173	24.7	10	10	10	7.652	24.7
2	9	10	8	7.276	15.9	10	9	9	8.122	23.8	8	9	9	7.604	23.7
3	9	9	8	7.296	15.8	10	9	8	8.119	23.8	7	9	8	7.605	23.8
4	8	9	7	7.297	15.7	9	9	8	8.129	23.7	7	9	7	7.609	23.7
5	8	9	7	7.310	15.5	7	7	8	8.141	23.8	4	8	4	7.619	23.8
6	8	9	7	7.316	15.5	6	5	8	8.140	23.7	3	7	2	7.634	23.7
7	8	9	5	7.317	15.8	6	2	5	8.154	23.6	2	3	2	7.595	23.7
8	8	8	5	7.326	15.6	6	2	5	8.155	23.6	1	3	1	7.582	23.7
9	7	8	4	7.302	15.4	4	1	5	8.126	23.7	1	1	1	7.539	23.8
10	6	7	4	7.329	15.4	4	1	4	8.143	23.7	1	1	1	7.556	23.7
11	6	6	4	7.302	15.8	2	1	4	8.124	23.7	1	1	1	7.574	23.8
12	6	6	4	7.308	15.9	2	0	3	8.118	23.5	1	1	1	7.542	23.7
13	6	6	4	-	-	1	0	2	-	-	1	1	1	-	-
14	6	6	4	7.305	15.9	0	0	2	8.177	23.6	1	0	1	7.621	23.7
15	6	6	4	7.308	15.8	0	0	2	8.193	23.6	1	0	1	7.597	23.7
16	6	5	3	7.298	15.8	0	0	1	8.136	23.6	1	0	1	7.613	-
17	6	4	3	7.336	15.9	0	0	1	8.131	23.4	1	0	1	7.670	-
18	5	4	3	7.353	15.7	0	0	1	8.156	-	1	0	1	7.658	-
19	4	4	3	7.356	15.7	0	0	1	8.150	-	1	0	0	7.641	-
20	3	4	3	7.336	15.5	0	0	1	8.154	-	1	0	0	7.647	-
21	2	4	2	7.316	15.6	0	0	1	8.218	-	1	0	0	7.656	-
22	2	4	2	7.361	15.8	0	0	1	8.243	-	1	0	0	7.738	-
23	1	4	2	7.340	15.9	0	0	1	8.217	-	1	0	0	7.692	-
24	1	4	2	7.276	15.8	0	0	1	8.089	-	1	0	0	7.585	-
25	1	3	2	7.270	15.7	0	0	1	8.057	-	1	0	0	7.569	-
26	1	2	2	7.249	15.8	0	0	1	8.089	-	1	0	0	7.585	-
27	1	1	2	7.240	15.8	0	0	1	8.112	-	1	0	0	7.571	-
28	0	1	2	7.239	15.7	0	0	1	8.063	-	0	0	0	7.559	-
29	0	1	2	7.214	15.7	0	0	1	8.077	-	0	0	0		-
30	0	1	1	7.238	15.7	0	0	0	8.053	-	0	0	0	7.554	-

	Treatment 6 (2700 ppm, 24°C)					Treatment 7 (3500 ppm, 24°C)				
Day	Individuals			pH	Temp	Individuals			pH	Temp
	1	2	3			1	2	3		
1	10	10	10	7.444	24.6	10	10	10	7.356	24.6
2	9	9	10	7.439	24.7	9	10	7	7.352	24.6
3	7	8	8	7.455	23.6	7	8	5	7.369	23.5
4	5	7	5	7.464	23.6	5	6	2	7.371	23.5
5	1	4	2	7.478	23.5	2	3	1	7.374	23.5
6	1	2	0	7.470	23.4	2	2	0	7.370	23.4
7	1	0	0	7.460	23.6	2	2	0	7.343	23.6
8	1	0	0	7.445	23.6	2	2	0	7.337	23.6
9	0	0	0	7.405	23.6	2	1	0	7.304	23.5
10	0	0	0	-	-	2	0	0	7.316	23.6
11	0	0	0	-	-	2	0	0	7.317	23.8
12	0	0	0	-	-	2	0	0	-	-
13	0	0	0	-	-	2	0	0	-	-
14	0	0	0	-	-	2	0	0	7.361	23.5
15	0	0	0	-	-	2	0	0	7.364	23.5
16	0	0	0	-	-	2	0	0	7.363	23.7
17	0	0	0	-	-	2	0	0	7.361	23.6
18	0	0	0	-	-	2	0	0	7.377	23.5
19	0	0	0	-	-	1	0	0	7.397	23.6
20	0	0	0	-	-	1	0	0	7.362	23.7
21	0	0	0	-	-	0	0	0	7.401	-
22	0	0	0	-	-	0	0	0	7.446	-
23	0	0	0	-	-	0	0	0	-	-
24	0	0	0	-	-	0	0	0	-	-
25	0	0	0	-	-	0	0	0	7.292	-
26	0	0	0	-	-	0	0	0	-	-
27	0	0	0	-	-	0	0	0	-	-
28	0	0	0	-	-	0	0	0	-	-
29	0	0	0	-	-	0	0	0	-	-
30	0	0	0	-	-	0	0	0	-	-

Table 9 Survival of individuals of *G. salinus* (Kiel) shown for three replicates (1-3), for Control and 7 treatments (Treatment 1- Treatment 7); pH and temp (mean values) represent pH_{NBS} and temperature (in °C).

	Control (400 ppm, 16°C)					**Treatment 1 (1600 ppm, 16°C)**					**Treatment 2 (2700 ppm, 16°C)**				
Day	**Individuals**			**pH**	**Temp**	**Individuals**			**pH**	**Temp**	**Individuals**			**pH**	**Temp**
	1	2	3			1	2	3			1	2	3		
1	10	10	10	8.246	15.9	10	10	10	7.668	15.8	10	10	10	7.484	15.8
2	10	10	10	8.171	15.8	10	10	10	7.604	15.8	10	10	10	7.423	15.8
3	10	10	10	8.146	15.8	10	10	10	7.623	15.8	9	10	10	7.422	15.8
4	10	9	9	8.187	15.8	10	10	9	7.652	15.8	9	10	10	7.453	15.8
5	10	9	8	8.130	15.8	10	10	9	7.631	15.8	9	10	10	7.431	15.8
6	10	9	8	8.144	15.8	10	10	9	7.636	15.9	9	9	10	7.411	15.8
7	10	9	8	8.178	15.7	10	10	9	7.649	15.8	9	9	10	7.452	15.7
8	10	9	8	8.187	15.8	10	10	9	7.634	15.8	9	9	10	7.430	15.8
9	10	9	8	8.209	15.8	10	10	9	7.651	15.8	9	9	10	7.458	15.8
10	10	9	8	8.164	15.8	10	10	9	7.614	15.8	9	8	10	7.431	15.8
11	10	9	8	8.140	15.8	10	10	9	7.608	15.8	9	8	9	7.411	15.8
12	10	9	8	8.140	15.8	10	10	9	7.603	15.8	8	8	9	7.407	15.8
13	10	9	8	8.132	15.8	10	10	9	7.613	15.8	8	8	9	7.432	15.7
14	10	9	8	8.159	15.7	10	10	8	7.629	15.7	8	8	9	7.440	15.7
15	9	9	8	8.122	15.8	10	10	8	7.581	15.8	8	8	9	7.399	15.8
16	8	9	8	8.159	15.8	10	10	8	7.602	15.8	8	8	9	7.406	15.8
17	8	9	8	8.090	15.6	10	10	8	7.583	15.7	8	8	9	7.390	15.7
18	8	9	8	8.096	15.8	10	10	8	7.564	15.8	8	8	9	7.380	15.8
19	8	9	8	8.087	15.7	10	9	8	7.548	15.8	8	8	9	7.373	15.8
20	8	9	8	8.100	15.7	10	9	8	7.553	15.8	8	8	9	7.377	15.8
21	8	9	8	8.098	15.8	10	9	8	7.562	15.8	8	8	9	7.384	15.8
22	8	9	8	8.098	15.8	9	9	8	7.563	15.8	8	8	9	7.382	15.8
23	8	9	8	8.111	15.8	9	9	8	7.561	15.8	8	8	9	7.370	15.8
24	8	9	8	8.086	15.7	9	9	8	7.557	15.6	8	8	9	7.374	15.7
25	8	9	8	8.110	15.7	9	9	8	7.555	15.7	8	8	9	7.369	15.8
26	8	9	8	8.104	15.7	9	9	8	7.553	15.7	8	8	9	7.368	15.7
27	7	9	7	7.911	15.6	9	9	8	7.417	15.6	8	8	9	7.244	15.7
28	7	9	7	8.073	15.7	9	9	8	7.570	15.7	8	7	9	7.379	15.8
29	6	9	6	8.053	15.8	9	9	8	7.557	15.7	8	7	9	7.365	15.8
30	6	9	6	8.043	15.8	9	9	7	7.552	15.7	8	7	9	7.360	15.8

	Treatment 3 (3500 ppm, 16°C)					Treatment 4 (400 ppm, 24°C)					Treatment 5 (1600 ppm, 24°C)				
Day	Individuals			pH	Temp	Individuals			pH	Temp	Individuals			pH	Temp
	1	2	3			1	2	3			1	2	3		
1	10	10	10	7.336	15.8	10	10	10	8.234	23.5	10	10	10	7.683	23.4
2	10	10	10	7.274	15.7	10	10	10	8.156	23.4	10	9	10	7.654	23.4
3	10	10	10	7.284	15.7	10	10	9	8.140	23.5	10	9	10	7.633	23.5
4	10	9	10	7.319	15.8	10	10	9	8.164	23.5	10	9	10	7.660	23.5
5	10	9	10	7.305	15.8	10	10	9	8.128	23.4	8	9	10	7.641	23.4
6	10	9	10	7.286	15.8	10	10	9	8.133	23.3	8	9	10	7.629	23.4
7	10	9	10	7.315	15.7	10	10	9	8.164	23.4	8	8	10	7.668	23.4
8	10	9	10	7.306	15.8	10	10	9	8.156	23.4	8	8	10	7.655	23.5
9	10	9	10	7.333	15.8	10	10	9	8.171	23.4	8	8	10	7.655	23.4
10	10	9	10	7.302	15.8	10	10	9	8.111	23.5	8	8	10	7.627	23.6
11	10	9	10	7.299	15.8	10	10	9	8.110	23.4	8	8	10	7.615	23.5
12	10	9	10	7.301	15.8	10	10	9	8.103	23.5	8	8	10	7.611	23.5
13	10	9	10	7.317	15.7	10	10	9	8.096	23.5	8	8	10	7.610	23.4
14	10	9	10	7.329	15.6	10	9	9	8.103	23.2	8	8	10	7.614	23.3
15	10	9	10	7.296	15.7	10	9	9	8.060	23.5	8	7	10	7.572	23.6
16	10	9	10	7.287	15.7	10	9	9	8.070	23.5	8	7	10	7.566	23.5
17	10	9	10	7.291	15.7	10	9	9	8.045	23.5	8	7	10	7.561	23.3
18	10	9	10	7.298	15.7	10	9	9	8.063	23.5	8	7	10	7.566	23.4
19	10	9	10	7.288	15.7	10	9	9	8.043	23.5	8	7	10	7.548	23.5
20	10	9	10	7.281	15.7	10	9	8	8.020	23.5	8	7	9	7.528	23.5
21	9	9	10	7.271	15.8	10	9	8	8.073	23.4	8	7	9	7.580	23.4
22	9	9	10	7.273	15.8	10	8	8	8.059	23.5	8	7	9	7.570	23.5
23	9	9	10	7.269	15.7	10	8	8	8.102	23.4	8	7	9	7.565	23.4
24	9	9	10	7.263	15.6	9	8	8	8.063	23.4	8	7	9	7.571	23.3
25	9	9	10	7.284	15.7	9	8	8	8.047	23.4	8	7	9	7.557	23.4
26	9	9	10	7.256	15.6	9	8	8	8.063	23.3	8	7	9	7.572	23.3
27	9	8	10	7.151	15.6	9	8	8	8.020	23.4	8	7	9	7.550	23.5
28	9	8	10	7.282	15.7	9	8	7	8.116	23.5	8	7	9	7.640	23.5
29	9	8	10	7.278	15.6	9	8	7	8.103	23.4	8	6	9	7.625	23.4
30	9	7	10	7.272	15.7	9	8	7	8.095	23.4	8	6	8	7.615	23.4

	Treatment 6 (2700 ppm, 24°C)					Treatment 7 (3500 ppm, 24°C)				
Day	Individuals			pH	Temp	Individuals			pH	Temp
	1	2	3			1	2	3		
1	10	10	10	7.459	23.7	10	10	10	7.347	23.8
2	10	10	10	7.410	23.4	10	10	10	7.295	23.5
3	10	10	10	7.430	23.4	10	10	10	7.328	23.5
4	9	10	10	7.439	23.4	10	10	10	7.323	23.4
5	9	10	10	7.421	23.4	10	9	10	7.309	23.3
6	9	10	10	7.406	23.4	10	9	10	7.303	23.4
7	9	10	10	7.449	23.5	10	9	10	7.346	23.4
8	9	10	10	7.427	23.4	10	9	10	7.317	23.4
9	9	10	10	7.438	23.3	10	9	10	7.327	23.3
10	9	10	10	7.406	23.4	10	9	10	7.302	23.4
11	9	10	10	7.398	23.6	10	9	9	7.290	23.6
12	9	9	10	7.401	23.4	10	9	9	7.294	23.3
13	9	9	10	7.405	23.4	10	9	9	7.299	23.4
14	9	9	10	7.396	23.2	10	9	9	7.299	23.2
15	9	9	10	7.360	23.4	10	9	9	7.265	23.4
16	8	9	10	7.355	23.3	10	9	9	7.283	23.5
17	8	9	10	7.348	23.3	10	9	9	7.244	23.3
18	8	9	10	7.350	23.3	10	9	9	7.249	23.4
19	8	9	10	7.344	23.2	9	9	9	7.242	23.4
20	8	9	10	7.317	23.3	9	9	9	7.237	23.4
21	8	9	10	7.365	23.2	9	9	9	7.289	23.3
22	8	9	10	7.354	23.2	9	8	9	7.258	23.2
23	8	9	9	7.344	23.4	9	8	9	7.254	23.3
24	8	9	9	7.355	23.2	8	8	9	7.271	23.2
25	8	9	8	7.333	23.3	8	8	9	7.239	23.4
26	7	9	7	7.343	23.3	6	7	9	7.263	23.2
27	7	8	7	7.325	23.2	5	7	9	7.225	23.3
28	5	8	6	7.426	23.3	5	6	9	7.324	23.3
29	5	8	6	7.417	23.3	4	6	9	7.315	23.4
30	5	8	6	7.401	23.3	4	6	9	7.315	23.3

Table 10 Survival of individuals of *G. salinus* (Maasholm) shown for three replicates (1-3), for Control and 7 treatments (Treatment 1- Treatment 7); pH and temp (mean values) represent pH_{NBS} and temperature (in °C).

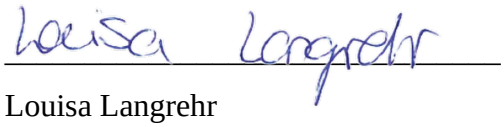
	Control (400 ppm, 16°C)					**Treatment 1 (1600 ppm, 16°C)**					**Treatment 2 (2700 ppm, 16°C)**				
Day	**Individuals**			**pH**	**Temp**	**Individuals**			**pH**	**Temp**	**Individuals**			**pH**	**Temp**
	1	2	3			1	2	3			1	2	3		
1	10	10	10	8.125	15.8	10	10	10	7.600	15.8	10	10	10	7.412	15.8
2	10	10	9	8.103	15.8	10	10	10	7.588	15.7	10	10	9	7.409	15.7
3	10	9	9	8.090	15.7	10	10	10	7.592	15.7	10	10	8	7.428	15.7
4	9	8	9	8.081	15.6	10	10	10	7.590	15.6	9	9	8	7.422	15.6
5	9	8	9	8.053	15.7	10	10	9	7.560	15.7	9	9	8	7.395	15.7
6	9	8	8	8.079	15.8	9	10	9	7.576	15.8	8	9	7	7.419	15.8
7	8	8	8	8.050	15.8	9	9	9	7.571	15.8	8	9	6	7.422	15.8
8	7	8	8	8.086	15.7	8	9	9	7.576	15.7	8	9	6	7.431	15.7
9	7	6	8	8.070	15.6	7	8	9	7.616	15.5	8	9	6	7.438	15.5
10	6	5	8	8.059	15.6	7	8	8	7.605	15.6	8	9	6	7.422	15.6
11	6	4	7	8.066	15.7	7	8	8	7.605	15.6	8	9	5	7.417	15.6
12	6	4	7	8.093	15.4	7	8	8	7.612	15.1	7	9	5	7.431	15.1
13	6	4	7	8.101	15.6	7	8	8	7.605	15.6	7	9	5	7.405	15.6
14	6	4	7	8.117	15.5	7	8	8	7.618	15.5	7	9	5	7.436	15.5
15	6	4	7	8.118	15.3	7	8	8	7.613	15.4	7	9	5	7.428	15.4
16	6	3	7	8.096	15.7	7	7	8	7.597	15.7	7	9	5	7.412	15.7
17	5	3	7	8.107	15.7	6	5	7	7.610	15.7	6	8	5	7.429	15.7
18	5	3	7	8.094	15.7	5	4	7	7.600	15.6	6	7	4	7.424	15.6
19	4	3	7	8.096	15.5	5	3	7	7.602	15.5	6	6	4	7.429	15.5
20	3	3	6	8.029	15.5	5	3	6	7.563	15.4	4	6	4	7.398	15.4
21	3	3	6	8.056	15.6	5	3	6	7.574	15.5	4	6	4	7.385	15.5
22	3	3	5	8.052	15.5	4	3	5	7.574	15.5	4	5	3	7.387	15.5
23	3	3	5	8.072	15.7	4	3	5	7.581	15.6	4	5	2	7.401	15.6
24	3	3	5	8.067	15.8	4	3	5	7.580	15.7	4	5	2	7.406	15.7
25	3	3	5	8.078	15.7	4	3	5	7.569	15.7	4	5	2	7.388	15.7
26	3	3	5	8.080	15.7	4	3	4	7.606	15.6	4	4	2	7.413	15.6
27	3	3	5	8.064	15.6	4	3	4	7.579	15.6	3	4	2	7.393	15.6
28	3	3	5	8.071	15.7	4	3	4	7.595	15.6	3	4	2	7.407	15.6
29	3	3	5	8.043	15.7	4	2	4	7.596	15.7	3	4	1	7.407	15.7
30	3	3	5	8.060	15.7	4	2	4	7.593	15.7	3	4	1	7.421	15.7

	Treatment 3 (3500 ppm, 16°C)					Treatment 4 (400 ppm, 24°C)					Treatment 5 (1600 ppm, 24°C)				
Day	Individuals			pH	Temp	Individuals			pH	Temp	Individuals			pH	Temp
	1	2	3			1	2	3			1	2	3		
1	10	10	10	7.315	15.5	10	10	10	8.195	8.195	10	10	10	7.644	23.5
2	9	10	10	7.308	15.7	9	8	10	8.169	8.169	10	9	10	7.631	23.7
3	9	10	10	7.315	15.4	8	8	10	8.163	8.163	10	9	9	7.626	23.6
4	8	10	10	7.325	15.5	8	8	8	8.164	8.164	10	8	8	7.625	23.6
5	7	10	10	7.304	15.6	6	8	7	8.124	8.124	9	8	8	7.595	23.6
6	6	9	9	7.321	15.8	5	7	7	8.142	8.142	9	6	8	7.611	23.5
7	6	8	8	7.322	15.8	5	7	7	8.113	8.113	8	6	7	7.603	23.6
8	6	8	8	7.317	15.7	5	7	7	8.137	8.137	8	6	6	7.622	23.6
9	5	8	8	7.309	15.6	5	7	6	8.129	8.129	8	5	6	7.648	23.6
10	5	6	8	7.291	15.7	5	5	6	8.105	8.105	7	4	6	7.639	23.6
11	5	6	7	7.280	15.6	4	5	6	8.104	8.104	6	3	6	7.630	23.6
12	5	6	7	7.296	15.7	3	5	5	8.109	8.109	6	3	4	7.635	23.5
13	4	6	7	7.278	15.7	3	5	5	8.125	8.125	6	2	4	7.634	23.6
14	4	6	7	7.288	15.5	2	4	4	8.133	8.133	5	1	3	7.648	23.4
15	4	6	7	7.288	15.4	2	4	4	8.122	8.122	5	1	3	7.645	23.5
16	4	6	7	7.266	15.6	1	4	4	8.132	8.132	5	1	3	7.643	23.6
17	4	5	7	7.285	15.7	1	4	4	8.146	8.146	5	1	3	7.642	23.6
18	4	5	7	7.267	15.6	1	4	4	8.138	8.138	4	1	3	7.637	23.6
19	4	5	7	7.267	15.4	1	4	4	8.115	8.115	4	1	3	7.626	23.5
20	4	5	7	7.249	15.4	1	3	4	8.096	8.096	4	0	2	7.616	23.4
21	4	4	7	7.247	15.4	1	3	4	8.119	8.119	4	0	2	7.622	23.4
22	4	4	6	7.242	15.4	1	3	4	8.100	8.100	3	0	1	7.620	23.6
23	4	4	6	7.263	15.6	0	3	4	8.115	8.115	3	0	1	7.638	23.6
24	4	4	6	7.271	15.7	0	3	4	8.128	8.128	3	0	1	7.638	23.7
25	4	4	6	7.249	15.7	0	3	4	8.119	8.119	3	0	1	7.618	23.6
26	4	4	6	7.277	15.6	0	3	4	8.141	8.141	3	0	1	7.642	23.5
27	3	4	6	7.256	15.5	0	3	4	8.145	8.145	2	0	1	7.612	23.5
28	3	4	6	7.262	15.6	0	2	4	8.110	8.110	2	0	1	7.616	23.6
29	3	4	4	7.267	15.6	0	2	4	8.106	8.106	2	0	1	7.615	23.4
30	3	4	7	7.270	15.6	0	2	4	8.092	8.092	2	0	1	7.613	23.4

	Treatment 6 (2700 ppm, 24°C)					Treatment 7 (3500 ppm, 24°C)				
Day	Individuals			pH	Temp	Individuals			pH	Temp
	1	2	3			1	2	3		
1	10	10	10	7.424	23.5	10	10	10	7.319	23.4
2	9	10	10	7.419	23.6	9	10	10	7.338	23.4
3	9	9	10	7.430	23.5	7	9	8	7.330	23.4
4	8	9	10	7.440	23.5	6	8	8	7.369	23.4
5	8	9	9	7.419	23.5	5	8	8	7.320	23.5
6	7	8	7	7.438	23.5	5	8	8	7.337	23.4
7	6	7	7	7.449	23.5	5	7	6	7.356	23.3
8	5	7	6	7.476	23.4	5	6	6	7.341	23.4
9	4	6	5	7.455	23.5	3	7	6	7.385	23.4
10	3	6	4	7.455	23.4	2	6	6	7.330	23.3
11	3	5	4	7.422	23.4	2	5	5	7.308	23.4
12	3	5	4	7.422	23.5	2	4	5	7.329	23.4
13	3	5	2	7.423	23.4	2	3	5	7.327	23.3
14	3	4	1	7.430	23.4	1	3	5	7.327	23.3
15	3	3	1	7.410	23.4	1	2	5	7.309	23.3
16	2	2	1	7.395	23.4	1	2	5	7.300	23.4
17	1	1	1	7.402	23.4	0	2	5	7.300	23.4
18	1	0	1	7.394	23.4	0	2	5	7.293	23.4
19	1	0	1	7.395	23.5	0	2	5	7.292	23.3
20	1	0	1	7.382	23.2	0	2	4	7.285	23.2
21	1	0	1	7.392	23.6	0	1	4	7.273	23.4
22	1	0	1	7.390	23.6	0	1	4	7.287	23.5
23	1	0	1	7.412	23.6	0	1	4	7.291	23.5
24	1	0	0	7.412	23.2	0	1	4	7.296	23.5
25	1	0	0	7.390	23.8	0	1	4	7.275	23.6
26	1	0	0	7.419	23.4	0	1	4	7.315	23.5
27	1	0	0	7.397	23.6	0	1	4	7.274	23.5
28	1	0	0	7.398	23.5	0	1	4	7.289	23.4
29	1	0	0	7.401	23.5	0	0	4	7.297	23.5
30	0	0	0	7.394	23.5	0	0	4	7.293	23.5

Eidesstattliche Erklärung

Hiermit versichere ich an Eides statt, dass ich diese Arbeit selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Außerdem versichere ich, dass ich die allgemeinen Prinzipien wissenschaftlicher Arbeit und Veröffentlichung, wie sie in den Leitlinien guter wissenschaftlicher Praxis der Carl von Ossietzky Universität Oldenburg festgelegt sind, befolgt habe.



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