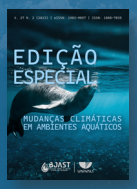
BJAST
BRAZILIAN JOURNAL OF AQUATIC SCIENCE AND TECHNOLOGY
V 27
N 02
2023



SENSITIVITY OF MARINE INVERTEBRATES TO ACIDIFICATION OF SEAWATER BY AN ATMOSPHERE ENRICHED WITH CO_2

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Cite as: Peytoureau, A. P., Arribas, A. J. M., Vieira, D. C. & Resgalla, C. (2023). Sensitivity of marine invertebrates to acidification of seawater by an atmosphere enriched with CO_2. Braz. J. Aquatic. Sci. Technol. 27(2):33-38. ISSN 1983-9057. DOI: 10.14210/bjast.v27n2.20464

Received: 16/06/2024. **Revised:** 25/07/2024. **Accepted:** 25/07/2024. **Published:** 07/10/2024.

Document type: Research article.

Funding: The authors received no specific funding for this work.

Competing interest: The authors declare no competing interest.

ABSTRACT

Ocean acidification, a consequence of increasing atmospheric CO_2, results from oceans absorbing about one-third of CO_2 emissions, leading to a decrease in pH. This phenomenon can cause sublethal or lethal damage to marine organisms some of which are crucial for coastal ecosystems and human economies. Understanding how to reproduce acidified seawater in a laboratory is essential for assessing potential impacts. Our protocol uses a CO_2-enriched atmosphere to determine the pH tolerance of *Mysidopsis juniae*, exposed at pH 6.8 and 7.0, and larvae of *Arbacia lixula*, exposed at pH 7.2. *M. juniae* showed lethal responses at pH 6.8 ± 0.3, while *A. lixula* exhibited delayed larval development as a sublethal reaction at pH 7.2 ± 0.3. Other studies have indicated negative sublethal effects on similar organisms at higher pH levels than those observed in this study that are indeed extreme and outside of the pH levels predicted for future ocean conditions. The strength of this study opens up the possibility of other types of more in-depth experiments in which pH is a determining factor in identifying other contaminants in the oceans. Therefore, further studies with more appropriate physiological parameters are needed in order to correctly and safely assess the dangers that ocean acidification can pose to marine organisms.

Keywords: Ocean Acidification. *Mysidopsis Juniae*. *Arbacia Lixula*. CO_2-enriched Atmosphere.

1 Introduction

The surface of our planet is mostly covered by oceans (approximately 70%). These are of fundamental importance in biogeochemical cycles and biodiversity of the planet. For instance, oceans act as carbon sinks for anthropogenic atmospheric carbon dioxide (Henley et al., 2020). Carbon dioxide, a greenhouse gas, absorbs heat from the Earth's surface and scatters it in all directions, initially helping to keep the Earth's temperature above freezing. However, the current excessive CO_2 levels, which have increased by nearly 40% since industrialization, are overloading the atmosphere and leading to increased global temperatures, with oceans absorbing one-third of this added carbon. (Lindsey, 2023; Doney et al., 2009; Hurd et al., 2018).

From postglacial times until the beginning of the 19th century, atmospheric CO_2 concentrations remained relatively constant at between 260 and 290 ppm (Hashimoto, 2019; Trabalka, 1985). During this period, the human impact on the carbon cycle was moderate, and atmospheric CO_2 variations were mostly due to natural causes, around 0.1–0.5 ppm/year (this figure varies between studies) (Trabalka, 1985) and after 1800 CO_2 fluctuations were mostly due to anthropogenic causes (Leseurre, 2022). The fluctuation varied from 0.6 ± 0.3 ppm/year between 1935–1940 to 1.5 ± 0.2 ppm/year between 1976 and 1982 (Trabalka, 1985). In 2010, the annual average CO_2 concentration in the atmosphere was approximately 389 ppm, and by February 2022, it had reached 419 ppm (Ang et al., 2022), which represents a yearly increase of 2 ppm (Lindsey, 2023). This rise is mainly due to fossil fuel combustion and deforestation (Gattuso & Hansson, 2017), with CO_2 emissions from fossil fuels having increased from 11 billion tons a year in 1960 to 36.6 billion tonnes in 2022 (Lindsey, 2023).

In view of these rapid fluctuations and their consequences, organizations such as the IPCC (2022) present various Shared Socioeconomic Pathways (SSPs) and Representative Concentration Pathways (RCPs), which are different scenarios that describe potential increases in global temperature up until 2100. RCPs explain where we could end up, and SSPs address this by defining how society leads to the consequences of RCPs. SSPy represents the level of radiative forcing, which is the change in energy flux measured in watts per square meter (W/m^2). Based on global warming of 1.5 °C since 1850–1900, the first two scenarios describe a situation of low greenhouse gas emissions (SSP1-2.6 and SSP1-1.9). The other scenarios presented intermediate, high, and very high greenhouse gas emissions (SSP2-4.5, SSP3-7.0, and SSP5-8.5, respectively) (IPCC, 2022 ; Rogelj et al., 2018).

Increasing global temperatures is not the only problem associated with increasing atmospheric carbon dioxide concentrations. Indeed ocean carbonate systems undergo several chemical reactions. It begins, as shown by the chemical equation below, when carbon dioxide (CO_2) reacts with water (H_2O) to form carbonic acid (H_2CO_3), which dissociates into bicarbonate ions (HCO_3^-) and carbonate ions (CO_3^{2-}), losing H^+ each time. An increase in hydrogen ions in water results in a decrease in pH and an increase in acidity, as shown in **Equation 01** (Doney et al., 2009; Bach, 2015).

$$CO_2\,(aq) + H_2O\,(l) \leftrightarrow H_2CO_3\,(aq) \leftrightarrow HCO_3^-\,(aq) + H^+\,(aq) \leftrightarrow CO_3^{2-}\,(aq) + 2H^+\,(aq) \quad \text{[Equation 01]}$$

Considering the scenario described by IPCC (2022) the pH of ocean surface will continue to decrease expect for the two lower emissions scenarios (SSP1-1.9 and SSP1-2.6).

Excessive ocean acidification has direct and indirect effects on marine organisms and their habitats (Hall-Spencer & Harvey, 2019). Direct effects include damage to all life stages of an organism caused by increasing CO_2 concentrations (Raven et al., 2005) or by weakening of the shells of calcareous organisms (Falkenberg et al., 2013). Indirect effects include changes in nutrient availability and/or composition that can affect trophic chains (Raven et al., 2005), problems during metamorphosis of fish larvae, or poor physical condition after metamorphosis (Espinel-Velasco et al., 2018). Interspecies interactions can also be affected by ocean acidification. Such is the case of crustacean *Carcinus maenas*, which has shown an increase in their claw strength in an acidic environment compared with its prey, mostly mollusks, which have not developed any advantage in this altered environment (Landes & Zimmer, 2012). Another example of this phenomenon is the interaction between the brown alga

Durvillaea antarctica, which shows a decrease in its protein and organic contents, and the amphipod *Orchestoidea tuberculata*, which presents a low preference for these changed algae (Duarte et al., 2016).

Changes in biological processes in surface waters also affect deep-sea ecosystems, which are highly dependent on the products generated by surface life forms (Raven et al., 2005; Sweetman et al., 2017). Studies have shown that the responses to a decrease in pH are mostly negative or neutral. Very few photosynthetic organisms such as plants, phytoplankton, and algae have positive effects (Gattuso & Hansson, 2017).

Various experiments and protocols of seawater acidification in the laboratory have been carried out with marine organisms to study the effects of ocean acidification. However, methodological problems remain when these tests are conducted. One of the problems observed is the inability to acidify the seawater and stabilize pH. Several paths are proposed by the scientific community, such as the report "Guide to best practices for ocean acidification research and data reporting" by Riebesell et al. (2010). One of the most intuitive solutions is to add strong acids and bases to seawater, such as HCl or NaOH, but this does not alter the concentration of dissolved inorganic carbon (DIC), which changes the total alkalinity (At) of the seawater. Another option is to use CO_3^{2-} and/or HCO_3^- to alter the the dissolved inorganic carbon DIC of the water; however, carbonate chemistry variables fluctuate significantly (Riebesell et al., 2010).

Another method involves changing the DIC while maintaining constant At. This can be achieved by bubbling seawater with a composition of gases that manipulate the carbonate chemistry until it reaches the desired pH or by adding high-CO_2 seawater to a portion of normal seawater (Riebesell et al., 2010). For example, this technique was used in an experiment on the effects of ocean acidification on pelagic communities in their natural environments. It consisted of a giant floating tube called the KOSMOS system, which isolates a part of the marine ecosystem. It had several bags containing a water column and the desired plankton community. Seawater enriched with CO_2 was pumped into the bags through small tubes until the desired pH was reached (BIOACID, 2024).

An alternative option to induce acidity in seawater is to expose it to a CO_2 enriched small atmosphere (Burgess et al., 1996). The latter can be useful for determining whether a pH-dependent pollutant such as ammonia (NH_3) is responsible for visible toxicity (Burgess et al., 1996). Ammonia in its un-ionized form is significantly toxic in sediments, and according to Greenstein et al. (1995) and Reidenbach et al. (2022), interstitial ammonia concentration increases with increasing pH and decreases with decreasing pH. This has a significant impact on the epipelagic zone of the ocean (Cooley et al., 2022). This can have an impact on the competition between species of microalgae and therefore allowing certain species to dominate, such as *Phaeodactylum tricornutum* (Bautista-Chamizo et al., 2019).

Based on the knowledge, experiments and protocols described above, this study aims to investigate the sensitivity of juvenile larvae of *Mysidopsis juniae* (da Silva, 1979) and *Arbacia lixula* (Linnaeus, 1758) to pH variation. For this purpose, it is necessary to develop a system and protocol for acidifying seawater with a CO_2-enriched atmosphere in a low-cost system, which will enable the lethal tolerance of *M. juniae* juveniles and the sublethal tolerance of A. *lixula* larvae to different pH levels to be assessed.

2 Material and Methods

2.1 Acidification system installation

Gaseous mixtures of air and CO_2 (99% air and 1% CO_2; and 98% air and 2% CO_2) were pumped into a hermetic enclosure to achieve seawater acidification with a low-cost system. The CO_2 present in the atmosphere was absorbed by seawater and gradually acidifying it until the desired pH was reached. This technique was based on the study by Burgess et al. (1996).

A plastic tray, small pump, air compressor, CO_2 tank, and pH meter were used to set up the system. An air compressor and CO_2 tank (ONU 1013 (ABNT 209-1)) were connected to the mixing recipient. The gases were mixed in the recipient and sent to a tray covered with a plastic film to prevent the gas from leaking, thus creating an artificial atmosphere (Figure 1). The CO_2 solution in seawater was accelerated using a water pump (HJ-111), agitating the water.

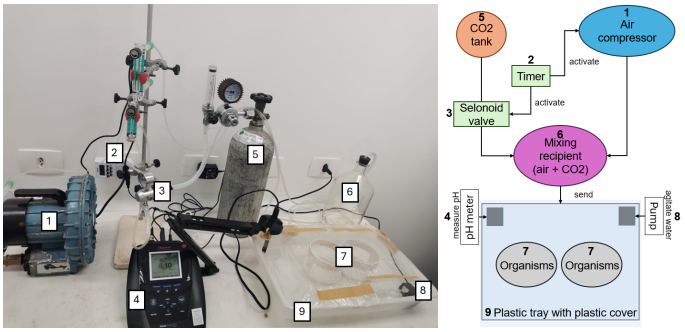

Figure 1. Image (left) and scheme (right) of the apparatus for pH reduction and ecotoxicological tests. 1, Air compressor; 2, Timer; 3, Valve; 4, pH meter; 5, CO_2 tank; 6 Mixing recipient; 7, Container for organisms; 8, Small pump; 9, Plastic tray.

The CO_2 was introduced at a constant rate (0.1 and 0.2 L/min) several times per day at regular intervals. The pH variations were monitored for 24 h. A timer (Weekly Digital Timer B-MAX) and a solenoid valve (Special CO_2/Coil Plants) were used to set up the whole system so that it would run for 24 h or more without human intervention. For *Mysidopsis juniae*, if the results did not show toxicity after 24 h than the experiment was extended or reproduced to 48 h (Burgess et al., 1996). The water was controlled by a pH meter (Thermo Scientific/Orion Star A211), which was programmed to record the pH value every half hour, in order to monitor changes in pH overnight.

To develop a work protocol, several pH stabilization experiments were carried out to determine the working time of the system (gasification) required for the pH to decrease and remain at the desired range. During these experiments, the behaviour of other parameters, such as oxygen, conductivity, salinity, and temperature, was monitored to determine how they varied and were affected by the system.

2.2 Acute tests with *Mysidopsis juniae*

M. juniae cultures were supplied by the Ecotoxicology Laboratory (Letox) of the Polytechnic School of the Universidade do Vale do Itajaí (UNIVALI), Itajaí, Brazil. The mysids were cultivated in six aquariums at a ratio of 40:12 females/males to optimize fecundation rates, and the juvenile mysids used were less than a week old. Each week, adult mysids were separated from juveniles, and females and males were counted to ensure that the ratio was respected for the next week. The mysids were fed with *Artemia sp*.

The plastic tray for *M. juniae* juveniles had a capacity of 8 L, and the volume of seawater to acidify was 5 L of water and 3 L of the atmosphere. The mysids were placed in two custom-madecylindrical containers equipped with 300-micron filter paper, each with a diameter of 10 cm and a height of 9.5 cm, resulting in a volume of roughly 0.75 L. Ten individuals of *M. juniae* were set up in each container, making a total of twenty.

Two types of experiments were conducted with *M. juniae*. During the first experiment, the mysids were inserted only when the desired pH was reached, creating a pH shock. Later experiments involved the introduction of organisms within the enclosure from the beginning of the acidification process, creating a pH acclimatization. Two other containers with mysids were placed under the same conditions, but without additional CO_2, as a control experiment.

After managing and stabilizing the variation in pH, *Mysidopsis juniae* were introduced along with their food (*Artemia naupliu*) in a plastic tray for 24 h, and their mortality was recorded. The first experiment was

carried out at 6.8 ± 0.3 (Burgess et al., 1996) to evaluate the sensitivity, and the control experiment was carried out at pH 8 ± 0.3. If mortality did not occur, the pH range was altered to obtain the minimum pH tolerance for *M. juniae*.

2.3 Chronic tests with *Arbacia lixula*

The larvae of *A. lixula* were also provided by the Letox (UNIVALI). Adult urchins were obtained from a scuba diving in the municipality of Penha, Brazil, and transported to the laboratory. They were stored in an aquarium and induced to release gametes by electrical shock, wet eggs and dry sperm were acquired from three females and three males Fertilization was achieved by activating the sperm with seawater and confirmed by the formation of the fertilization membrane under a microscope (ABNT/NBR, 2020).

The plastic tray for sea urchins, *A. lixula* larvae, contained 1.5 L water. Approximately 300 embryos were placed in four custom-made cylindrical containers equipped with 50-micron filter paper, each with a diameter of 3 cm and a height of 7.5 cm, giving a volume of approximately 0.05 L.

The experiment with sea urchin larvae were carried at 7.2 ± 0.3 (Burgess et al., 1996) to evaluate their sensibility. Sea urchin larvae are less tolerant to pH reduction than mysids because they it has a sublethal impact, compared to *Mysidopsis. juniae*, which has a lethal impact. The system acidified the seawater to a pH of 7.2 ± 0.3. Another plastic tray without additional CO_2 was used as a control. Organisms were introduced at pH = 7.2 in four small containers for each plastic tray. After 24 h, the larvae were removed from the containers with a syringe and placed in a 10 mL beaker containing 4% formalin (1.5 mL). Sedgewick Rafter chambers were used under a microscope to count the number of larvae with and without developmental delay.

2.4 Statistical analysis

Data processing for *M. juniae* and mortality percentages were established considering the two containers, and the acceptable control mortality was set at 10% (DA SILVA et al., 2020). For *Arbacia lixula*, 100 larvae were quantified based on the number of larvae with developmental delays and normal development in the Sedgewick Rafter Chamber. Next, the average of the four containers was calculated. The acceptable control effect was set to below 20% (ABNT/NBR, 2020).

Statistical analysis was performed using the GraphPad Prism 7 software. A normality test was used to determine whether the data followed a normal distribution and based on the results the chi-square test was selected and used to evaluate the significance association of the survival of *Mysidopsis juniae* and the significant impact on *Arbacia lixula* both at different pH levels. The same software was used to create the graphs.

3 Results e Discussion

3.1 Validating the acidification system

The different experiments realized by the system established a change in how the pH varies depending on various factors. It may be directly linked to the installation of the system or changes that occur when the system is activated and deactivated. Variations in pH fluctuated between experiments. The rate of pH variation changed in the experiments before and after the use of the solenoid valve; thus, the amount of CO_2 entering the atmosphere was reduced, increasing the time required to reach the desired pH.

The pH stabilization test was performed without any organisms for 6 h and 45 min, and the system was activated for 15 min each 2h15 so fit into the range of pH = 6.8 ± 0.3. Figure 2 shows the variation in pH over time. It shows a decrease in pH, which varies rapidly over 9 min from 8.05 to 7.10. A constant pattern appears when the pH again reaches 7.10 again and the CO_2 is reactivated.

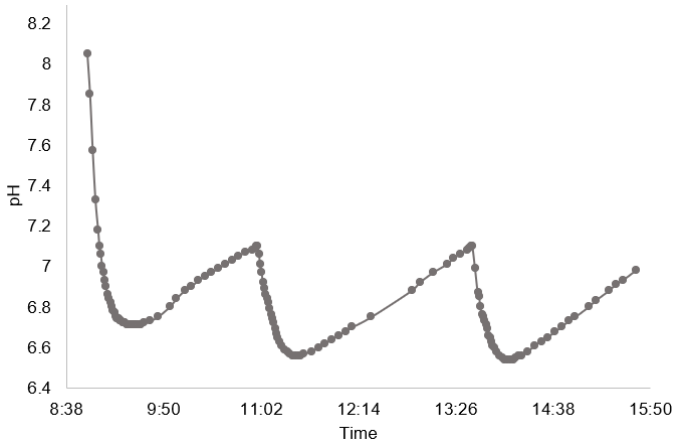

Figure 2. Variation in pH in the stabilization test, showing oscillation as a function of time (20/10/2023).

Other parameters were also monitored for 7 h in the initial experiments to confirm that they did not exhibit any variation due to the acidification system or had a negative impact on the organisms. The oxygen concentration in the water decreased slightly from 7.24 mg/L to 6.87 mg/L(Table 1). It could fall a little further, but this would not affect test organisms, such as sea urchin larvae or small crustaceans, because physiological stress begins to affect aquatic life only at levels below 5 mg/L (Haddout et al., 2022). It was observed that the temperature increased from 22.3 °C to 24.7 °C (Table 1), due to the plastic film, which retains heat. The ideal temperature for *Mysidopsis juniae* is 24 ± 1 °C (Oliveira et al., 2011), and *Arbacia lixula* is thermophilic (Gianguzza et al., 2014); therefore, none of them will be negatively impacted. The relation between conductivity and pH variation was directly proportional. For salinity, conductivity remained between 34 and 35.

Table 1. Oxygen concentration (mg/L) and temperature (°C) in the seawater at the beginning and final of the experiment.

Parameter	Initial Value	Final Value
Oxygen concentration (mg/L)	7.24	6.87
Temperature (°C)	22.3	24.7

For each of the experiments, human presence was necessary for, at least, a cycle to predict how the pH would change in seawater. Depending on the time taken for the pH to increase again to a value slightly above the desired pH, the timer was set so that the system could work overnight.

3.2 pH acclimation vs shock mortality with *Mysidopsis juniae*

The first pH shock test was performed with the living organism *M. juniae* at pH 6.8 ± 0.3, and the valve permitting control of the CO_2 output without human intervention. The experiment lasted for 27.5 h. The timer was set to activate CO_2 inflow for 15 min every 2 h. It took 25 min for the pH to fall to 7.10, after which it did not fall below 6.8 as expected. Therefore, it was necessary to readjust the experiment by reactivating the CO_2 again for 15 min. After CO_2 deactivation, the pH continued to decrease until it reached pH < 6.8.

Figure 3 shows that 2.5 h after the beginning of the experiment, the pH levels remained constant between 6.92 and 6.73. It was also noticed that after 19 h of experimentation, the pH no longer fell below 6.8 and seemed to recover a few hours later.

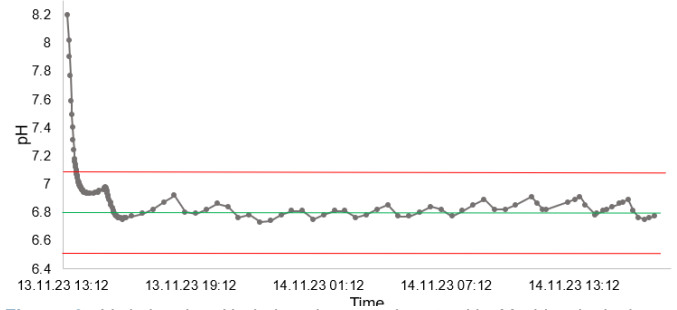

Figure 3. Variation in pH during the experiment with *Mysidopsis juniae* on 13/11/2023. The red lines indicate the maximum and minimum limits of the set pH and the green line indicates the target pH.

For *Mysidopsis juniae*, all individuals in the control test (C1) survived and showed a significant difference from the results for the tested organisms (P <0.0001) (Figure 5A). Nine of the 20 mysids in the experimental test survived, three survived in the container closest to the CO_2 air outlet, and six survived in the other container.

To determine the pH at which all mysids survived, the next experiment was performed at pH 7 ± 0.3. After 50 min, a pH of 7 was reached. The entire experiment lasted 24 h. The pH changed very slowly; therefore, the gasification process was set for 10 min of gasification every 5 h. This experiment only performed five cycles (Figure 4) of activation and deactivation of CO_2 in total, compared to the 11 cycles of the previous experiment (Figure 3).

Eleven of the twenty mysids survived. Three mysids present in one of the container out of ten, compared to eight in the other container. In the control (C2), all organisms survived, and there was a significant difference compared with the acidified experiment (P = 0.0036) (Figure 5B).

The proportions of mortality and survival between the two experiments with pH shock at pH 6.8 and 7 did not differ significantly (P = 0.3406). There was still more mortality at pH 6.8 compared to pH 7 (55% and 35%, respectively) (Figure 5).

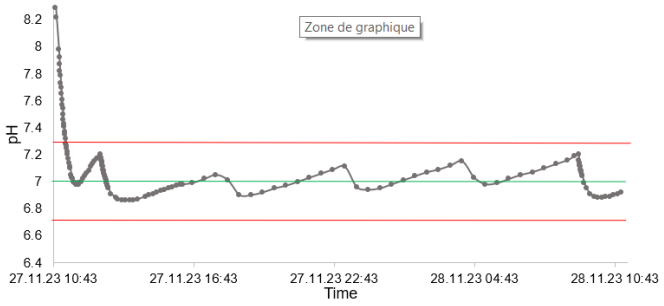

Figure 4. Variation in pH during the experiment with *Mysidopsis juniae* on 27/11/2023. The red lines indicate the maximum and minimum limits of the set pH and the green line indicates the target pH.

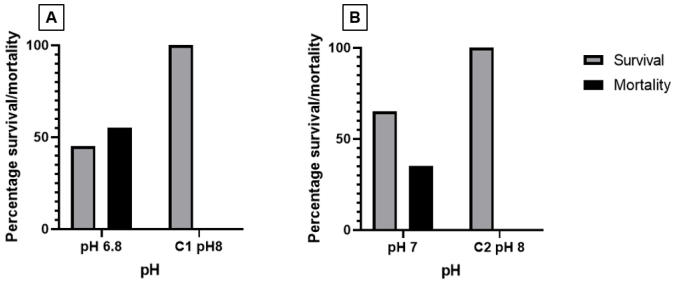

Figure 5. Percentage of survival and mortality of Mysidopsis juniae during pH shock along with their controls (C1 and C2) at pH 6.8 (A) and pH 7 (B).

After observing the mortality in the two shock pH experiments, the first experiment with pH acclimation was performed at pH 7 ± 0.3. Gasification was performed for 15 min at 5 h intervals (Figure 6). The test lasted for 24 h, and all organisms survived under both the acidified water and control (C3) conditions (Figure 8A).

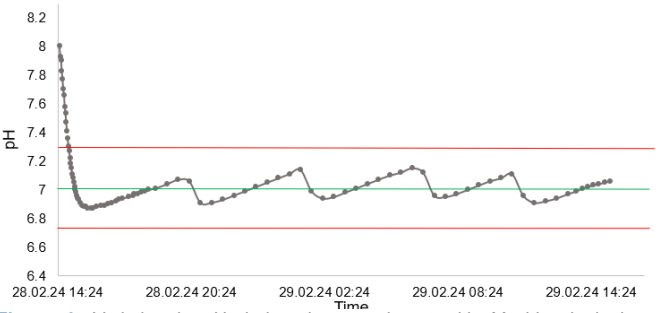

Figure 6. Variation in pH during the experiment with *Mysidopsis juniae* on 28/02/2024. The red lines indicate the maximum and minimum limits of the set pH and the green line indicates the target pH.

The last experiment was conducted at pH 6.8 ± 0.3 with 15 min of gasification every 4 h (Figure 7). Thirteen out of twenty survived, six in the left container and nine in the right. In the control (C4) group, all mysids survived, which was significantly different from the test experience (P = 0.0177) (Figure 8B). Both pH acclimation experiments resulted in significant differences (P = 0.0083) between survival and mortality at pH 6.8 and pH 7 (35% and 0% mortality, respectively) (Figure 8).

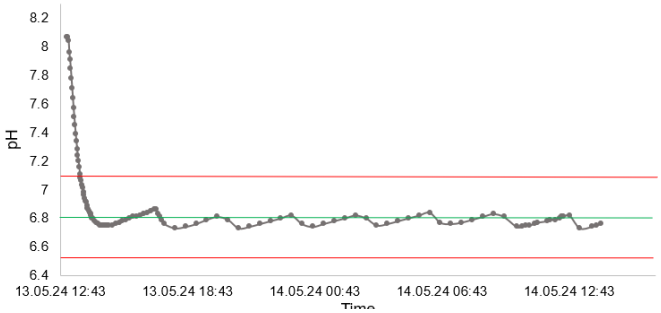

Figure 7. Variation in pH during the experiment with *Mysidopsis juniae* on 13/05/2024. The red lines indicate the maximum and minimum limits of the set pH and the green line indicates the target pH.

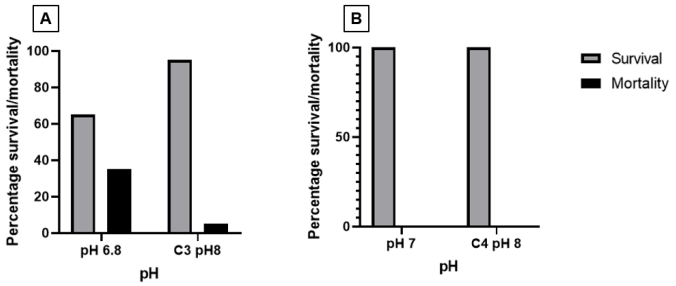

Figure 8. Percentage of survival and mortality of *Mysidopsis juniae* during pH acclimation along with their controls (C3 and C4) at pH 6.8 (A) and pH 7 (B).

In summary, the tests conducted with pH shock resulted in higher mortality in organisms compared to tests with pH acclimatization (Table 2), but the results showed higher significance during the test with pH acclimation.

Table 2. Survival and Mortality of M. juniae in pH shock and pH acclimatation and survival in the control experiment

pH	Survival	Mortality	Control (pH 8)
pH 6.8 (shock)	9	11	20
pH 7 (shock)	13	7	20
pH 6.8 (acclimation)	13	7	19
pH 7 (acclimation)	20	0	20

In general, experiments at pH 6.8 show more mortality than experiments at pH 7, as shown in Figure 9. In fact, between the two tests performed at pH 6.8 (pH shock and acclimatization), there was no significant difference (P = 0.3406) compared with tests performed at pH 7, in which a significant difference was observed (P = 0.0083). Future experiments should be conducted only with pH acclimatization to obtain meaningful results in view of the higher mortality when using pH shock.

Other species of mysids also show an increase in mortality at higher pH levels, such as *Neomysis awatschensis*, which showed a reduced survival rate at pH 7 after 10 days of exposure (Lee et al., 2020). Although *N. awatschensis* displays elevated mortality at a higher pH than *Mysidopsis juniae*, it was subjected to an acidic environment for a longer duration of 10 days than just 1 day. It would be interesting to conduct further experiments to determine whether *M. juniae* exhibits a similar reaction to prolonged exposure to acidified water at pH 7.

Even in the worst-case scenario described by IPCC (2022), there is no prediction that the pH could reach a value as low as pH 6.8 or even pH 7, however, different sublethal reactions were also observed within the order Mysida. For example, *Praunus flexuosus* was exposed to three pH levels (pH 7.72, 7.61, and 7.50) in addition to the control (pH 7.94), and changes were identified in the number of molts between the ambient and acidified environments. There was a significantly higher number of molts in the non-CO_2 enriched seawater; moreover, the period between molts increased with the age of the mysids and the decreasing pH (Sperfel et al., 2017).

In a study by Lee et al. (2020), experience with *N. awatschensis* also showed that the total length of the mysids and other growth parameters were slightly reduced at pH 7 compared to pH 8. Furthermore, decreasing pH has also been proven to have a negative impact on neonate production in the mysid *Americamysis bahia* between pH 7.8 and 7.6 (Grear, 2016).

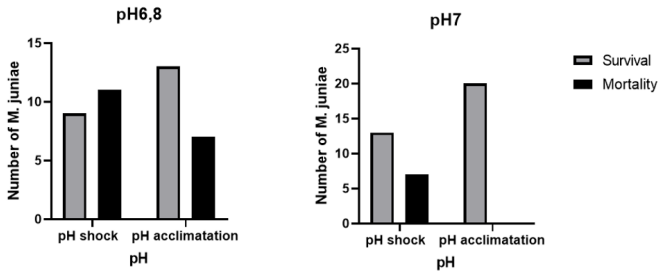

Figure 9. Number of survival and mortality of *M. juniae* at pH 6.8 (left) and pH 7 (right) with pH shock and acclimatation

3.3 Chronical exposure in *Arbacia lixula*

After several adjustments, the gasification of the experiment was adjusted to 20 min every 50 min at pH 7.2 ± 0.3. Unfortunately, a technical problem prevented experiment from being completed: the Carbon Dioxide cylinder ran out of CO_2 at approximately 02:00 in the morning. Figure 10 shows the first 5 h of the experiment. At the end of the 24 hours, the final pH was 8.25. For the control, a delay in development was observed in 6% of the larvae, which was below the 20% acceptance control effect.

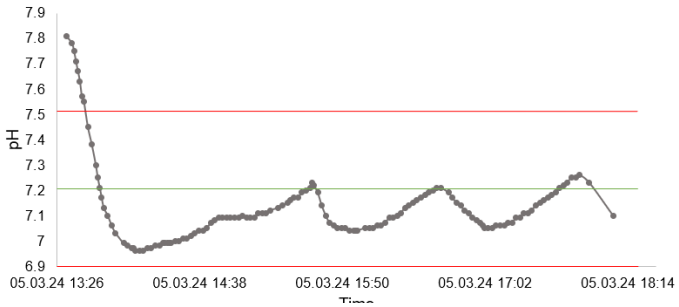

Figure 10. Variation in pH during the experiment with *Arbacia lixula* on 05/03/2024. The red lines indicate the maximum and minimum limits of the set pH and the green line indicates the target pH.

Despite these results, some interesting observable effects still exist. As shown in Figure 11A, the urchin larvae managed to fully develop in the control experiment, which was not the case with the other experiments. As shown in Figure 11B, a decrease in pH was observed during late development of urchin larvae, and all larvae showed signs of developmental delay. The post-oral length developed little or very little in the larvae, as shown in Figure 11B, and the body length began to take on the characteristic triangular shape, as shown in Figure 11A.

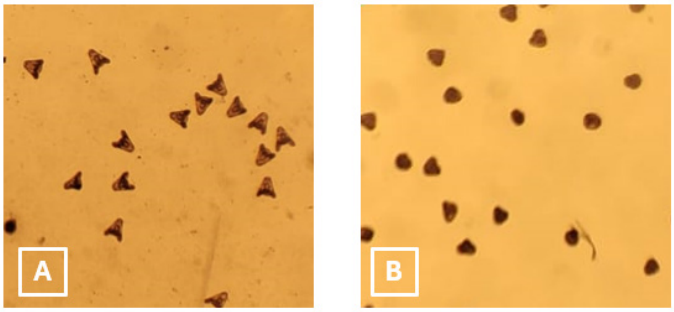

Figure 11. A) Urchin larvae on control experiment; B) Urchin larvae in acidify seawater.

The tolerance of sea urchin larvae was set at pH of 7.2 by Burgess et al. (1996); nevertheless, effects have already been observed at pH of 7.9 in the study by Visconti et al. (2017). In general, post-oral length was shorter and asymmetrical in larvae exposed to acidified water. The effect of pH on larval development has also been linked to temperature (Visconti et al., 2017). Further experiments with *A. lixula* could be performed to determine whether temperature has an impact on the effect of pH at 7.2.

A study by Passarelli et al. (2017) observed the impact of pH reduction in the sediment elutriate on the larval development of *Paracentrotus lividus* from Spain and *Lytechinus variegatus* from Brazil. A developmental delay was observed at pH 7 or below, but normal development was observed at pH 7.5. In comparison, the experiment at pH 7.2 showed a more similar tolerance pattern to *P. lividus* from Spain than to *L. variegatus* from Brazil. *Arbacia lixula* appears to be the least tolerant when compared to *Lytechinus variegatus*; some results show a 80% success rate in larval development at pH 7 and 20% at pH 6.5.

The research found that ocean acidification may have an impact on organisms such as *A. lixula larvae*. In the I20PCC RCP8.5, the spatial distribution of the ocean surface pH could reach levels below 7.9 by 2100 (Jiang et al., 2019). This may cause developmental problems, such as changes in the mobility of metals in these organisms and in organisms with similar responses, such as *Paracentrutos lividus* and *Lytechinus variegatus* (Passarelli et al., 2017).

4 Conclusions

From the research and results previously presented, we conclude that there are different seawater acidification techniques for use in the laboratory, depending on the objectives of the experiments. However, each of these techniques has its own constraints when it comes to setting up or stabilizing the pH over the long term. The seawater acidification system with a small CO_2-enriched atmosphere works well but requires human presence at the beginning to calibrate the equipment compared to other techniques that are more automatized.

Lethal effects were observed on *Mysidopsis juniae* at pH 6.8 ± 0.3 and greater mortality when the organisms were introduced at pH shock rather than pH acclimation, and sublethal effects, such as late development of larvae, were observed at pH 7.2 ± 0.3 in *Arbacia lixula*.

Although the two cases of ocean acidification reproduced in the laboratory do not correspond to any future scenarios, this does not prevent these experiments from serving as a warning and as support for the development of other types of research, such as toxic identification evaluation that uses confounding factors, such as pH, to eliminate the effects of ammonia in sediment.

In the framework of this research, it would be interesting to repeat the experiments of this study with longer exposure to acidified water for both *M. juniae* and *A. lixula*, to identify whether the tolerance limit changes. Additional experiments could focus on determining the sublethal effects of ocean acidification on *M. juniae* by considering the pH levels predicted by the IPCC across various scenarios, as well as identifying the pH threshold at which *A. lixula* begins to experience sublethal effects. More realistic chronic studies should be conducted with pH variations to understand how they could affect marine organisms.

Ocean acidification and its impacts, both lethal and sublethal, is a vast field that must be studied if we are to prevent, as far as possible, the consequences it will have on ecosystems and consequently on human beings.

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