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INVESTIGATING THE TOXIC AND ACIDIFYING EFFECT OF SCRUBBER EFFLUENT ON *STRONGYLOCENTROTUS DROEBACHIENSIS* LARVAE

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Front page photo: Ida Vartia, Strongylocentrotus droebachiensis larvae, Kristineberg Marine Research Station, April 7, 2022

ABSTRACT
To meet the new environmental requirements of reduced sulfur emissions from ships, many shipowners have installed an open-loop scrubber to clean the exhaust. In this scrubbing process, seawater is pumped from the ocean and combined with the emission gas, creating an acidic and toxic effluent that is directly discharged into the ocean, potentially damaging the marine environment. This study aims to understand the effects of different concentrations of scrubber effluent (toxicant mixtures, pH and alkalinity decrease) on green sea urchin larvae (*Strongylocentrotus droebachiensis*). The central hypothesis was that the combination of toxins and low pH/alkalinity in scrubber effluent would negatively impact survival, growth, and morphology of sea urchin larvae. Moreover, it was hypothesized that part of the negative effect driven by low pH and low alkalinity could be minimized by correcting these parameters using a strong base. However, some negative effects would still be observed due to the toxins present in the scrubber effluent. The experiment was conducted at the Kristineberg Marine Research Station. Larvae were cultured for 14 days in different concentrations of scrubber effluent in filtered seawater. In the treatments with highest concentrations, pH and alkalinity were corrected to a similar level as filtered seawater to only evaluate the effect of the mixture of toxicants. We found severe effects on body length growth rate and development caused by the scrubber effluent in the treatment with the highest concentration (10% ~ pH 7.3). When corrected for pH and alkalinity (10%AT ~ pH 7.9), we observed a decrease of the harmful effects enabling the larvae to develop and grow. However, toxins hindered the larvae from reaching the same growth rate as the larvae in the water without scrubber effluent. In conclusion, the scrubber effluent harms the green sea urchin larvae development and growth. While this effect can be reduced by treating the effluent for its increased acidity, sea urchin larvae still suffer from toxicity of the chemical cocktail.

SUMMERING

För att möta de nya miljökraven på minskade svavelutsläpp från fartyg har många redare installerat en open-loop skrubber för att rengöra avgaserna. I denna process pumpas havsvatten upp från havet och kombineras med utsläppsgasen, vilket skapar ett surt och giftigt vatten som direkt släpps ut i havet och potentiellt skadar den marina miljön. Den här studien syftar till att förstå effekten som olika koncentrationer av skrubbervatten (blandning av gifter och minskning i pH och alkalinitet) har på larver av tistelsjöborre *(Strongylocentrotus droebachiensis).* Den centrala hypotesen var att kombinationen av gifter och minskning i pH och alkalinitet i skrubbervattnet skulle negativt påverka överlevnad, tillväxt och morfologi hos sjöborrelarverna. Dessutom antogs det att en del av den negativa effekten som drivs av låg pH och alkalinitet kunde minimeras genom att korrigera dessa parametrar med en stark bas. Vissa negativa effekter skulle dock fortfarande observeras på grund av de gifter som finns i skrubbervattnet. Experimentet genomfördes på Kristinebergs marina forskningsstation. Larver behandlades under 14 dagar i olika koncentrationer av skrubbervatten i filtrerat havsvatten. I de högsta koncentrationerna korrigerades pH och alkalinitet till samma nivå som filtrerat havsvatten för att endast utvärdera effekten av blandningen av giftiga ämnen. Vi fann allvarliga effekter på larverna i den högsta koncentrationen av skrubbervatten (10 % ~ pH 7,3), minskad tillväxthastighet av kroppslängden och utveckling av armar och mage förekom inte. I behandlingen där vi korrigerade pH och alkalinitet (10%AT ~ pH 7.9) observerade vi en reduktion av de skadliga effekterna, det medförde att larver kunde tillväxa och utvecklas. Gifterna som var närvarande hindrade dock larverna från att nå samma tillväxt som hos larver i behandlingen utan skrubbervatten. Sammanfattningsvis skadar skrubbervatten utvecklingen och tillväxten av tistelsjöborrar. Även om den här effekten kan minskas genom att behandla skrubbervattnet för dess ökade surhet, så lider sjöborrelarver fortfarande av toxiciteten hos den kemiska cocktailen.

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Introduction

In 2020, the International Maritime Organization (IMO), an agency of the United Nations, implemented global standards on sulfur content in marine fuels via the framework of the international convention for preventing pollution from ships (MARPOL), Regulation 14 of Annex VI. This annex regulates air pollution from shipping, mainly focusing on limiting the maximum sulfur oxides (SO_X) emission from 3.5% to 0.5% in 2020 (MEPC, 2016). The primary purpose of this regulation was to reduce adverse impacts on human health and the acidification of freshwater and terrestrial ecosystems (Hassellöv et al., 2013; Turner et al., 2018; Ytreberg et al., 2021). Ship emissions also contain other substances such as nitrogen oxides (NO_X), particulate matter, polycyclic aromatic hydrocarbons (PAHs), and metals, which result in additional health and environmental issues (Ytreberg et al., 2021).

To reduce air emissions of SO_X, it is mandatory for ships to either change to fuels with lower sulfur content or install a scrubber, officially known as an Exhaust Gas Cleaning System (EGCS) (ICES, 2020). Scrubbers are efficient in removing SO_X (MEPC, 2019). Hence, with them installed, ships can use cheap fuels with high sulfur content, such as heavy fuel oil (ICES, 2020). There are three types of scrubbers; open-loop, closed-loop, and hybrid systems. To meet the requirements of MARPOL Annex VI, the most common solution for shipowners was to install the open-loop scrubber (DNV GL, 2022).

In an open-loop system, seawater is pumped from the ocean and combined with exhaust gas (Linders et al., 2019); in the text, this will be referred to as the scrubbing process. Contaminants in the gas are washed out and transferred into the seawater as dissolved or particulate chemical species (Linders et al., 2019; Winnes et al., 2020). The resulting scrubber effluent officially known as Exhaust Gas Scrubber Effluent (EGSE), (Thor et al., 2021) is then directly discharged into the water around the ship (ICES, 2020; Linders et al., 2019) continuously at $45 m^3/MWh$ (IMO, 2008).

Every scrubber effluent has a unique chemical composition. These depend on scrubber design, contaminant removal efficiency, fuel composition, etc. (ICES, 2020; Linders et al., 2019). They differ in the ratio of sulfuric compounds (from absorption of SO_X), heavy metals, persistent organic pollutants (mainly polycyclic aromatic hydrocarbons (PAHs)), nitrogen compounds (from absorption of NO_X) (ICES, 2020), along with soot and ash (Linders et al., 2019). Some characteristics of the substances in the scrubber effluent have the potential to negatively impact marine biota (acidification, persistency, high toxicity, bioaccumulation, and eutrophication) with an overall damaging effect on the structure and function of marine ecosystems (Hassellöv et al., 2020; ICES, 2020). The scrubber effluent is highly acidic due to the presence of sulfuric acid (H_2SO_4) and the release of hydrogen ions (H^+) as a result of absorbed SO_X in the scrubbing process (Linders et al., 2019).

Finally, ship traffic intensity, environmental factors (hydrographic conditions), and organisms that live in the aquatic environment all modulate the effluent's effect (Linders et al., 2019). Advocates for scrubbers imply that the pollution problem will disappear by dilution. However, this argument has been firmly disproven, and the shipping community's wide-scale use of the open-loop system is of serious concern (Hassellöv et al., 2020). Moreover, allowing scrubbers thwarts innovation and the development of more environmentally friendly fuels (Thor et al., 2021).

Distilled fuels (Marine Gas Oil (MGO), Liquefied Natural Gas (LNG), or biofuels) do not contain the same high concentrations of contaminants compared to residual heavy sulfur fuel oils (HSFO) (Thor et al., 2021; Turner et al., 2017). Nevertheless, they are costlier and are usually incompatible with the lubrication system used with HSFO (Turner et al., 2017). Therefore, in connection with the IMO 2020 regulation, economic factors fully decide the prospect of more investments in scrubbers (Deere-Jones, 2016; Thor et al., 2021; Turner et al., 2017). In addition,

consider the likely event of fuel spills from HSFO. They bear more critical economic and ecological ramifications than more environmentally friendly fuels (Deere-Jones, 2016). As the ideal course of action, ICES (2020) recommends a rapid and complete transition to low-sulfur fuels to erase the use of scrubbers.

Scrubber Contaminants

Sulfur oxides

The water absorbs SOx during the scrubbing process. The product of the reaction is independent of the SOx present in the reactors. In contact with seawater, sulfur dioxide (SO_2) hydrolyzes to form sulfurous acid (H_2SO_3), which is later oxidized to the strong acid sulfuric acid (H_2SO_4), which further dissociates into sulfate ions (SO_4^{2-}), releasing two hydrogen ions (H^+), creating an acidic effect (Linders et al., 2019).

$$SO_2\ (g) + 2H_2O + ½O_2\ (g) \leftrightarrow H_2SO_4\ (aq) \leftrightarrow SO_4^{2-} - + 2H^+$$

With a few exceptions, carbonate and other ions make seawater naturally alkaline (pH>7.0), and it has a natural buffer capacity (total alkalinity) against acidification (Linders et al., 2019; The Royal Society, 2005). In open-loop systems, the seawater's natural buffer capacity partly neutralizes the acidity and raises pH again, allowing for further SO_X absorption. However, this eventually leads to a decrease in alkalinity and pH (Linders et al., 2019).

Nitrogen oxides

NO_X are formed by the reaction of nitrogen and oxygen gas (N_2 and O_2) under high-temperature combustion (Linders et al., 2019; Turner et al., 2018). Scrubber removal of NO_X depends on the exhaust gas ratio between nitric oxide (NO) (solubility is neglectable) and nitrogen oxide (NO_2). NO_2 reacts readily with the seawater to form the strong acid nitric acid (HNO_3), which dissociates completely to one H^+ and one nitrate ion (NO^{3-}), creating an acidic effect (Linders et al., 2019; Turner et al., 2017). Like SO_X, it leads to a decrease in alkalinity and pH. The NO/NO_2 ratio in emissions also influences the scrubber effluents' addition to eutrophication. NO_X removal varies a lot; therefore, the scrubbers' source of nutrients also varies (ICES, 2020).

Total Acidification Effect

The ocean absorbs carbon dioxide (CO_2) from the atmosphere and acts as the world's most powerful carbon sink, a buffer in the fight against climate change. When gaseous atmospheric CO_2 dissolves in seawater, it forms the weak acid carbonic acid (H_2CO_3) that readily undergoes hydrolysis and forms bicarbonate ions (HCO_3^-) followed by carbonate ions (CO_3^{2-}), in the process releasing hydrogen ions (H^+). Adding H^+ to a solution reduces the pH and has an acidifying effect (The Royal Society, 2005).

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+ \text{ (Equilibrium)}$$

As mentioned earlier, seawater has a natural buffering capacity against acidification. Nevertheless, the ocean's total alkalinity is limited. Furthermore, when significant amounts of excess anthropogenic CO_2 ends up in the ocean's surface, it loses its ability to withstand large changes in pH (Hunter et al., 2011; The Royal Society, 2005). CO_2-induced acidification of seawater is called Ocean Acidification (OA) (Doney et al., 2009; The Royal Society, 2005). OA is already negatively impacting marine ecosystems and species (IPCC, 2022).

The scrubbing process produces strong acids (H_2SO_4 and HNO_3) compared to the weak acid H_2CO_3 formed by dissolved CO_2 in seawater (Hassellöv et al., 2013). All acids result in reduced pH of the seawater and reduced carbonate concentrate. However, the input of strong acids is not reversible without adding a strong base (Turner et al., 2018). A strong base that effectively neutralizes acidification caused by H_2SO_4 is sodium carbonate (Na_2CO_3) (Davison & House, 1988). Acidification due to CO_2 keeps total alkalinity constant. However, each mole of H_2SO_4 and HNO_3 decreases total alkalinity with two and one equivalent, respectively (Hunter et al., 2011).

Over an extended period, with steady acidification by the strong acids, CO_2 moves from the ocean to the atmosphere. Stips et al. (2016) conclude that for each ton of SO_2 discharged by scrubbers, the ocean uptake of atmospheric CO_2 reduces by half a ton. In conclusion, this reduces the ocean's effectiveness as a carbon sink and adds to enhanced climate change and ocean acidification. (Doney et al., 2007; Dulière et al., 2020; Hassellöv et al., 2013; Hunter et al., 2011; ICES, 2020; Stips et al., 2016).

PAHs and Metals

A scrubber easily removes polycyclic aromatic hydrocarbons (PAHs) from emissions (IMO, 2006). PAHs are persistent substances that can accumulate in the marine environment (e.g., sediments and the water column) (Thor et al., 2021; Turner et al., 2017). Furthermore, PAHs are carcinogens documented as global contaminants and are on the list of priority pollutants in regional frameworks (EPA, 2014; EU, 2013).

Metals that have been measured in scrubber effluents include arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), mercury (Hg), molybdenum (Mo), nickel (Ni), vanadium (V), and zinc (Zn) (ICES, 2020). High acidity in scrubber effluent increases metals' solubility, increasing bioavailability and causing issues in coastal communities (ICES, 2020; Linders et al., 2019). The combined effects of the mixture of chemicals in the scrubber effluent (PAHs and metals) can lead to enhanced toxicity (Endres et al., 2018; Thor et al., 2021).

Biological Responses to Scrubber Effluents

Scrubber effluents have the potential to impact marine species and ecosystems. For example, Thor et al. (2021) found that effluents from open-loop scrubber systems are highly toxic for the copepod species *Calanus helgolandicus*. Copepods exposed to open-loop effluent for 14 days experienced reduced survival and delayed development at the lowest concentration of 1% in FSW, amplified in concentrations 5%, 10%, and 40%. Koski et al. (2017) showed increased adult copepod mortality, reduced feeding, and delayed development among the copepod *Acartia tonsa* exposed to scrubber effluent. In the Koski et al. (2017) and Thor et al. (2021) studies, the individual effects of PAHs and metals could not explain the increase in mortality as the concentration of these compounds was much lower than previously demonstrated lethal concentrations for these organisms.

In conclusion, the combined effects of contaminants in the scrubber effluent were higher than the mathematical addition of individual effects of each contaminant. The adverse effects on the marine zooplankton community are worth noting because they are the food web's base and can reduce ecosystems' productivity (Koski et al., 2017). NO_X concentration in scrubber effluent stimulates microbial plankton growth, indicating scrubbers' addition to eutrophication (Koski et al., 2017; Ytreberg et al., 2019; Ytreberg et al., 2021) and pH alone had a negligible impact on the micro planktonic community (Ytreberg et al., 2021).

Contaminants in the scrubber effluent (mainly PAHs and metals in dissolved bioavailable form) also have the potential to bioaccumulate in food webs (ICES, 2020). In turn, it finds its way into filtering organisms, fish, and marine mammals (Hassellöv et al., 2020; ICES, 2020). Presently, scrubber effluent has no reported direct effects on fish or mammals; however, considerable studies of single compound contaminants (PAHs and metals) show detrimental effects

(Hassellöv et al., 2020). In adult fish, PAHs cause a decrease in growth, edema, cardiac dysfunction, lesions and tumors of the skin and liver, damage to immune systems, trophic transfer, etc. (Logan, 2007). Marine mammals can accumulate toxins in their tissues. Severe and extended exposure to toxicity by both PAHs and metals have been shown to cause renal damage and systematic suppression of immune functions, introducing infectious and noninfectious diseases (DeGuise et al., 1996; Hassellöv et al., 2020; ICES, 2020; Lavery et al., 2009; Thompson et al., 2007). In conclusion, contaminants in scrubber effluent can harm vital functions and population productivity in filtering organisms, fish, and marine mammals (Hassellöv et al., 2020; ICES, 2020). Moreover, by bioconcentration and biomagnification, humans risk being exposed to these toxins due to their presence in food (Linders et al., 2019).

The concentration and distribution of the contaminants affect the scrubber's actual impact on the marine environment (Hassellöv et al., 2020; Ytreberg et al., 2021). A particular concern is the broad use of scrubbers in heavily trafficked nearshore coastal areas (ICES, 2020) with immense biodiversity (Tittensor et al., 2010). These waters often hold higher concentrations of contaminants, a smaller chance of dilution, and poorer dispersal potential to the open sea (Linders et al., 2019).

Legislation

The IMO's MARPOL Annex VI mandatory regulation of SO_X has increased the number of ships with scrubbers (ICES, 2020). Scrubbers decrease the input of SO_X into the atmosphere, redirecting atmospheric pollution to the marine environment (Hassellöv et al., 2020; ICES, 2020; Koski et al., 2017; Thor et al., 2021). Article 195 of the United Nations Convention on the Law of the Sea (UNCLOS) states that "In taking measures to prevent, reduce and control pollution of the marine environment, States shall act so as not to transfer, directly or indirectly, damage or hazards from one area to another or transform one type of pollution into another" Hence, using scrubbers violates the article.

Legislation regarding scrubber effluent is lagging in protecting the marine environment. While this method reduces pollutant emissions to the atmosphere, it inadequately regulates the pollutants that enter the marine environment (MEPC, 2015). There are limits to some components of scrubber effluents proposed in IMO guidelines. However, they are not legally binding but instead viewed as an invitation to individual member states to implement the guidelines in national legislation (MEPC, 2008); this creates inconsistent legislation of scrubbers between countries (ICES, 2020). Under Article 211 (3) (UNCLOS), port states have full authority over their ports: i.e., there is no limit on the level of stringency of their regulations (Endres et al., 2018). Per this liberty, ports or regions worldwide have banned scrubber systems from operating (Nepia, 2022).

The IMO guidelines require that scrubber effluent has a minimum pH of 6.5 at a distance of 4 m from the point source (Linders et al., 2019). However, the effluent is seldom treated before discharge to reduce the acidity (ICES, 2020; Thor et al., 2021), and the actual effluent can reach a pH of 3 or less (Linders et al., 2019). It is analytically challenging to measure pH in seawater accurately (Kulinski et al., 2017). In addition, the guidelines exception criteria permit a maximum difference of 2 pH units amidst measurements of ship inlet and overboard discharge during maneuvering and transit. With substantial amounts of ships operating scrubbers in a closed area, the inlet pH can be below the natural pH of that area. The standard for acceptable pH of the discharge water when using the exception criteria instead of minimum standards can lead to a scrubber effluent with lower pH entering the ocean (Hassellöv et al., 2020). Furthermore, the guideline requires limits for other scrubber contaminants, including turbidity, PAHs, and NO_X emissions' uptake do not exceed 12% (Turner et al., 2018). There is no discharge limit for the remaining amount of potentially harmful substances, including heavy metals (Hassellöv et al., 2020; Turner et al., 2017).

The Scrubber system's inputs of contaminants and their effects on the marine environment make it challenging to achieve good environmental status under marine environmental management and to meet the objectives of international agreements and regulations (Hassellöv et al., 2020). These include regional conventions (HELCOM; OSPAR) and European Directives such as the prevention of deterioration in the EU Water Framework Directive (EC, 2000), the Environmental Quality Standards, and environmental targets of the EU Marine Strategy Framework Directive (EC, 2008, 2017). The main objective of the Marine Strategy Framework Directive is to reach a good environmental status for the marine environment, and each member state is obligated to ensure that its waters achieve this (Turner et al., 2017). Similarly, the Water Framework Directive requires that the member states shall implement essential efforts to prevent deterioration of the status of surface water bodies but also to reach good ecological status or potential, and good chemical status (EC, 2000). However, the IMO regulates atmospheric emissions from shipping and discharge from scrubbers, while the EU does not. Because of this, scrubber effluent is not held to the same standards as other potentially polluting discharges within the EU, such as the Environmental Risk Assessment (Turner et al., 2017).

Green Sea Urchin

Echinoderms are marine calcifying phyla of marine invertebrates, including sea urchins, brittle stars, and sea stars (Dupont et al., 2013). The green sea urchin *(Strongylocentrotus droebachiensis)* is a grazing species, commonly associated with kelp, (Scheibling & Hatcher, 2013) that has an essential role in affecting the structure of rocky subtidal communities (Hagen, 1995) impacting boreal coastal ecosystems (Jager et al., 2016). *S. droebachiensis* are commercially valued (Jager et al., 2016) for example because of their roe (Scheibling & Hatcher, 2013). Sea urchins have complex life cycles (Thorson, 1950) with six life-history stages (i.e., sperm/egg, zygote, blastula/gastrula, pluteus larvae, juvenile, adult). Pelagic zygotes evolve into pluteus larvae that develop skeleton-supported pairs of arms and feed in the water column. After a while, the pluteus metamorphoses, transforming into a benthic juvenile and settle on a fitting substrate. Finally, sea urchins develop into adults when juveniles sexually mature (Dorey, 2013) (Figure 1).

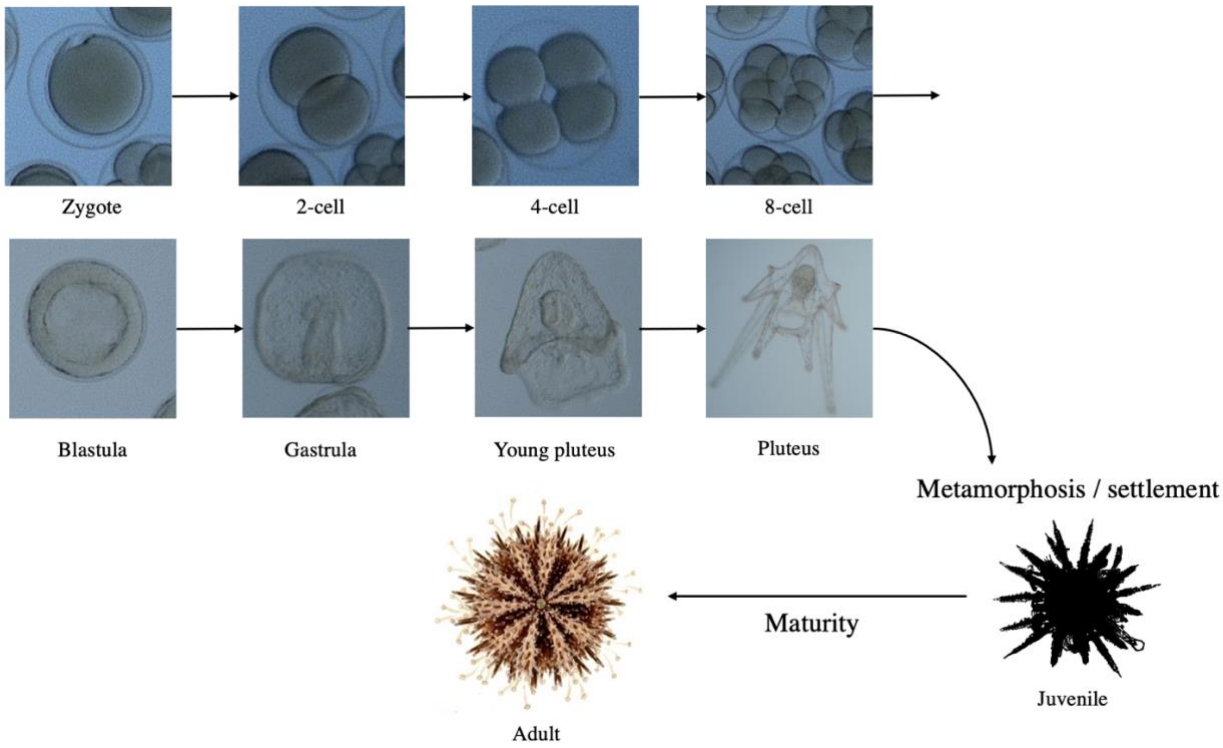

Figure 1. S. droebachiensis pelagic embryogenesis and larval development until pluteus stage followed by larvae metamorphosis and transformation into a benthic juvenile that settles and finally sexually mature, forming an adult sea urchin. Image source: Photos of larvae stages are taken by Ida Vartia, Kristineberg Marine Research station, 2022. Painting of juvenile by Ida Vartia. Image of adult urchin; image source from freely licensed media Aquascope 2000.

S. droebachiensis has been used as a model to investigate the impact of CO_2-induced OA. The larvae of *S. droebachiensis* have a physiological tipping point at $pH_T = 7.0$ (Dorey et al., 2013). In their study, Dorey et al. (2013) found that larvae respiration, mortality, and asymmetry increase while growth rate decreases when pH is lowered within the 7.0-8.0 pH range. Furthermore, larvae also tended to have smaller arms. Therefore, lowered pH leads to a delay in larvae development, affecting their chance of survival. Marine sediments polluted by PAHs and heavy metals have shown to be toxic to sea urchin spermatozoa. Both PAHs and heavy metal sediments were toxic for embryotoxicity, but the sediment polluted by heavy metals had a more substantial toxic effect (Geffard et al., 2001). Other studies have also shown that PAHs (Bellas et al., 2008) and heavy metals (Xu et al., 2011) are toxic to sea-urchin embryos.

Ocean acidification driven by CO_2 does not affect seawater alkalinity. However, acidification via a strong acids such as H_2SO_4 or hydrogen chloride (HCl) causes a decrease in both pH and alkalinity. Kurihara and Shirayama (2004) saw effects on larvae from two sea urchin species (*Hemicentrotus pulcherrimus* and *Echinometra mathaei*) of decreased pH and altered carbon chemistry because of the HCl in seawater affecting early development and life history. The effects were on cleavage rate, developmental speed, and pluteus larval morphology, comparable to the effects of treatments with CO_2-related acidification. When exposed to acidification because of a strong acid, larvae of the sea urchin *Paracentrotus lividus* experienced severe developmental defects at pH 7.5. The damage seemed to be early and irreversible (Pagano et al., 1985). This indicates that acidification by a strong acid has a more substantial effect than CO_2-induced acidification, potentially related to the decrease in alkalinity. When sea urchin embryos of *Sphaerechinus granularis* were cultivated in seawater acidified with HCl or H_2SO_4, pH <6.5 caused mitotic abnormalities (Cipollaro et al., 1986). Pagano et al. (1985) also found mitotic abnormalities with the decreased pH.

Aim

Currently, no studies have been made on the effect of scrubber effluent on sea urchins. The consequence of strong acid-induced acidification for *S. droebachiensis* is also unknown. In addition, the relative contribution of the mixture of toxicants and the change in pH on the biological response to scrubber effluent is currently unknown. This thesis aims at filling these knowledge gaps using the green sea urchin larvae as a model. The possibility of correcting the carbonate chemistry using a strong base after exposure to scrubber effluent and the consequence for the biological response will be explored.

The main goal of this project is to better understand the effect of different concentrations of scrubber effluent from an open-loop system on the sea urchin *S. droebachiensis* during embryonic and larval development. Using an original design, the relative contribution of the toxicant mixture plus pH and alkalinity decrease on the sea urchin's response to scrubber effluent will be evaluated. At the highest concentrations of the toxicant mixture, pH and alkalinity will be corrected to focus only on the effect of the mixture of toxicants. Several endpoints will be assessed: early larvae survival, growth rates, and morphometrics via allometries. Allometries are measurements of morphological traits in relation to body size (Voje & Hansen, 2013). The results of this study will be combined with the information published in Dorey et al. (2013) documenting the effects of low pH on *S. droebachiensis*.

The central hypothesis is that the combination of toxins and low pH/alkalinity in scrubber effluent will negatively impact survival, BL growth rate and morphology of sea urchin larvae. Further hypothesized is that part of the adverse effects driven by low pH and low alkalinity could be minimized using a strong base (Na_2CO_3), correcting the pH and alkalinity of the seawater after the addition of scrubber effluent. However, some adverse effects would still be observed due to the toxins in the scrubber effluent.

Material and Method

Scrubber Effluent

The scrubber effluent used for this experiment was provided by IVL Svenska Miljöinstitutet at the Kristineberg Marine Research station, they performed the chemical analysis of the effluent.

Animal Collection and Larval Culture

Adults of the green sea urchin *S. droebachiensis* originating from Tromsö, Norway was collected and transported to the Kristineberg Marine Research Station in February 2022. After arriving at Kristineberg, they were acclimated to a continuous flow of natural filtered seawater (FSW) following natural fluctuations to avoid stress factors. As a nutrition they were given the microalgae *Saccharina latissima.*

To trigger spawning, the physiological stressor potassium chloride (KCl) (1 ml at 0.5 M in FSW) was injected across the peristomal membrane of one male and one female urchin on March 24, 2022. For the male gametes, a pipette was used to gather the sperm and then to transfer it to Eppendorf tubes. For approximately one hour, the tubes with the sperm were kept on ice. Eggs were released by the female urchin directly into FSW with $pH_T \approx 8.0$ at 10 °C (Figure 2). 40 µl of sperm was added to 1 L egg solution and fertilized the eggs for 15 minutes. To ensure that the fertilization was successful, embryos were observed under a microscope. Finally, they were kept in a thermo-constant room at 10°C for 3 h. This allowed them to divide and develop before they were transferred to the treatment bottles (Figure 3). 1.4 ml of the solution of embryos were transferred to 18 bottles (≈ 615 ml each) with water corresponding to each treatment (see below); in every bottle there was approximately 10 embryos per ml. Bottles were kept in the thermo-constant room at 10°C to meet the preferred water temperature for the larvae.

The larvae were given 100 µl (≈3000 cells per ml) of a solution of the microalgae *Rhodomonas sp.* on day 5 (they now had a stomach). No food was given day 6 and 7. Starting on day 8, the food was administered daily in connection to the daily sampling and the amount was doubled (200 µl) compared to day 5.

Figure 2. Female S. droebachiensis releasing eggs into filtered seawater. Photo taken by Sam Dupont, Kristineberg Marine Research Station, 2022.

Figure 3. Embryos of S. droebachiensis. Photo taken by Ida Vartia, Kristineberg Marine Research Station, 2022.

Set-up of Biological Experiment and Seawater Carbonate Chemistry

The experiment was conducted at the Kristineberg Marine Research Station of the University of Gothenburg in Fiskebäckskil from March 22, 2022 – April 8, 2022.

To assess the impact of the scrubber effluent on the survival, growth, and development of *S. droebachiensis,* larvae were raised in 18 bottles representing six different treatments with 3 replicates per treatment. Aerated filtered seawater with air at a CO_2 concentration of approximately 440 ppm was used to dilute 100% scrubber effluent into solutions with different concentrations. The six treatments were: (1) 0% (control), (2) 0,1%, (3) 1 % and (4) 10%, (5) 1%AT, (6) 10%AT. A summary of treatments is presented in Table 1. All bottles were kept in a thermo-constant room with a temperature held at 10°C. In treatments 1%AT and 10%AT, the pH and alkalinity were corrected to the same level as the control using a sodium carbonate (Na_2CO_3) solution.

Table 1. Summary of the six treatments that were applied to larvae over a 14-day period. For two of six treatments pH and alkalinity were corrected to the same level as the control using a Na_2CO_3 solution.

	Treatment	Replicates	Concentration of scrubber effluent in FSW	Corrected for pH and alkalinity using a Na2CO3 solution
	0% (control)	3	0%	No
	0.1%	3	0.1%	No
	1%	3	1%	No
	1%AT	3	1%	Yes
	10%	3	10%	No
	10%AT	3	10%	Yes
Total	6 treatments	18 bottles		

The scrubber effluent used for this study was very acidic compared to seawater. A pilot study was conducted to determine the possibility of increasing pH and alkalinity in the AT treatments. In the pilot study, 1 M solution of Na_2CO_3 and ddH_2O was used to increase the carbonate concentration in the FSW. Control FSW with different concentration of scrubber effluent was prepared, and the correct volume of Na_2CO_3 solution was determined to correct the change in pH and alkalinity.

Twice a week, water was replaced in each replicate with aerated filtered seawater with a CO_2 concentration of approximately 440 ppm with the corresponding concentrations of scrubber effluent and corrected pH and alkalinity when relevant. Before every water renewal, seawater pH, alkalinity, salinity, and temperature were monitored following the recommendations of Dickson et al. (2007). mV and temperature of each treatment were measured after calibration using TRIS (Tris/HCl) buffer solution with a salinity of 32‰ and used to calculate pH on the total scale (pH_T). A titration system was used for assessing total alkalinity (TA) following recommendations by Dickson et al., 2007. Other parameters of the carbonate system (pCO_2, Ω_c, Ω_a) were calculated from pH_T, temperature (T; °C), total alkalinity (TA; mmol kg^1), and salinity (‰) using CO_2sys with the dissociation constants from Mehrbach et al. (1973) as refitted by Dickson & Millero (1987), following Dorey et al. (2013).

Biological Measurements

Mortality

Every morning, 20 ml from each replicate of each treatment were sampled with a pipette into two subsamples of 10 ml each (Figure 4). Larvae in each subsample were directly fixed with drops of paraformaldehyde solution (4% in filtered seawater) after controlling with a microscope that the individuals were alive and moving. By looking in each subsample with a microscope, larvae were counted and the average number of larvae for each subsample was used to calculate relative density (RD) for each replicate. RD was calculated each day by dividing the counted average number of larvae with the maximum larvae counted of the experiment. A correcting factor was applied to correct for the 20 ml daily dilution. Mortality rate (day^{-1}) was calculated as the regression coefficient of the significant linear relationship between relative density and time (day): RD = -MR * day + intercept (Figure 5).

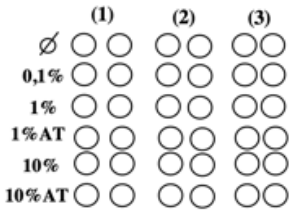

Figure 4. Two subsamples of 10 ml for each replicate of each treatment.

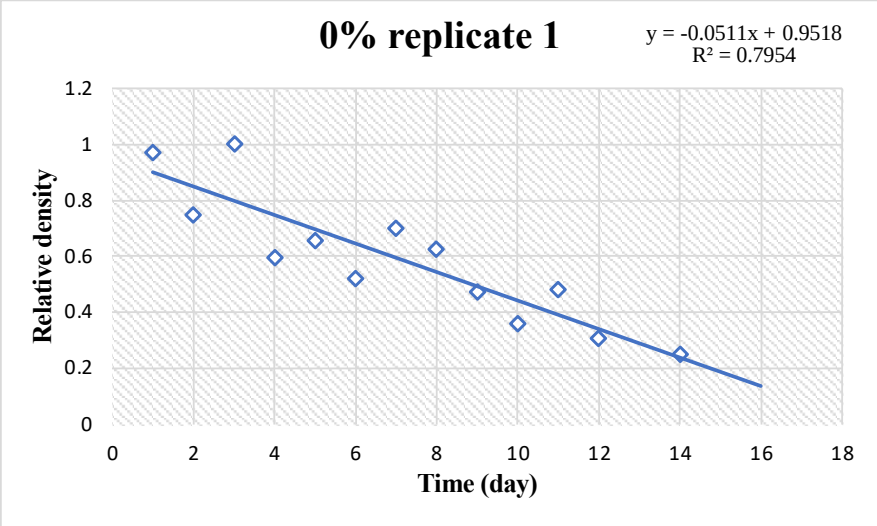

Figure 5. Example of mortality rate (day^{-1}) calculated as the coefficient (-0.0511 day^{-1}) from the significant linear regression between relative density and time (day).

Body Length and Morphometry

From each replicate, when the larvae in the subsamples had been counted, 50 larvae were positioned on a slide and moved to a microscope with an attached camera. This was to be able to get enough good pictures of larvae but also to count the number of abnormal larvae. It was decided that abnormality was too subjective to include in the experiment. Pictures were taken every day of the experiment (Days 0-14) for later measurements of larvae body length and other morphometric parameters using the software ImageJ. For each replicate, ten larvae were photographed and for each larva, 9 measurements were made. These measurements were: Body length (BL), post-oral rod (POR), posterolateral rod (PLR), and stomach diameter (S) both horizontal and vertical (

Figure 6). For all rod parameters, both pair of rods were measured. Prior to day 5, the larvae were not developed enough to enable measuring of other parameters than body length. For the 10% treatment, only BL was measured due to arrested development of other parameters. Data from 2158 photographed and measured larvae was used for later analysis.

Body length growth rate (GR in µm $\ln(day)^{-1}$) were calculated as the regression coefficient of the significant logarithmic relationship between measured BL (µm) and development time (day): BL = GR * ln(day) + intercept. Allometry was calculated against BL to distinguish the direct effect of the treatments and exclude an indirect effect (due to delayed development) (Stumpp et al., 2011). Allometries (µm μm^{-1}) for the rod parameters (BR-, PLR-, and POR length) were calculated as the coefficient of the significant linear relationship between the longest rod of each parameter (µm) with BL (µm). Stomach volume (SV) was calculated as $SV = \frac{4}{3}\pi \times [\frac{S1+S2}{4}]^3$, where S1 and S2 are the two measured diameters of the larvae's stomach. Allometry (μm^3 µm-1) was calculated as the coefficient of the significant linear relationship between SV and BL.

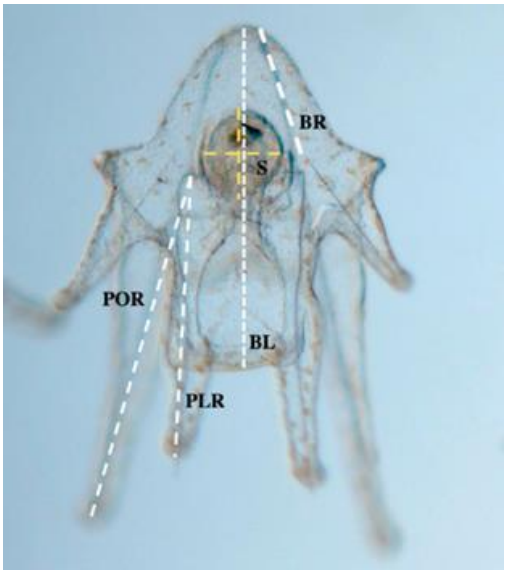

Figure 6. S. droebachiensis larvae with 5 morphological parameters: Body Length (BL), Body Rod (BR), Post-Oral Rod (POR), Posterolateral Rod (PLR), and Stomach (S) Photo taken by Ida Vartia, Kristineberg Marine Research Station, 2022.

Statistical Analysis

General linear models (GLM) were run using the statistical software SAS and R. Graphs were made using excel and R. The level applied for significance for all statistical analyses was 5%. All data were checked for normal distribution and homoscedasticity visually by looking at plots: Residuals vs. fitted plot, Normal Q-Q plot, Scale-location plot, Residuals vs. Leverage plot, and a histogram. The relationships between parameters were tested using logarithmic or linear regressions.

GLM was conducted to compare differences in the average BL growth rate per concentration of scrubber effluent (treatments 0%, 0.1%, 1%, and 10%). Another GLM was conducted to compare the effect of concentration of scrubber effluent and correction of pH and alkalinity (AT) on BL growth rate (treatments 1%, 1%AT, 10%, and 10%AT). This was because only the 1% and 10% treatments were corrected for pH and alkalinity. Additional GLM was conducted to compare differences in average BL growth rate between the control (0%), the 1%AT and the 10%AT treatments separately without the AT as a factor.

Similarly, GLMs were conducted to compare differences in average mortality rate per treatment separately for the concentration of scrubber effluent and AT. For all four allometries (body rod (BR), posterolateral rod (PLR), post-oral rod (POR), stomach volume (SV)), GLM were conducted separately for the concentration of scrubber effluent (excluding the 10% treatment because of no development of these parameters) and the AT (excluding the 10% and 10%AT treatments). Only the significant GLM results were tested for further analysis with Scheffé's post-hoc test to understand which treatments that were significantly different from each other. For the parameters of the chemistry, effects of the treatments were also tested with a GLM followed by a post-hoc Scheffé's test.

Results

Seawater Chemistry

Carbonate chemistry for each replicate of each treatment is summarized in Table 2. There was a significant difference in measured pH between the treatment pHs (One-way ANOVA, $F_{5,12}$=602.9, p<0.0001) (Figure 7). There was a significant difference between the alkalinity measurements (One-way ANOVA, $F_{5,12}$=34.83, p<0.0001), CO_2 measurements (One-way ANOVA, $F_{5,12}$=560.3, p<0.0001), calcite saturation state (Ω_c) (One-way ANOVA, $F_{5,12}$=148.4, p<0.0001) and aragonite saturation state (Ω_a) (One-way ANOVA, $F_{5,12}$=128.5, p<0.0001) of treatments. In summary, there was a significant effect by treatment for all parameters (Figure 7). All tested parameters were followed by a post-hoc Scheffé's test to reveal which treatments were different from each other. The post-hoc test revealed for the 10% treatment, all parameters where significantly different from all other treatments. Alkalinity and pH were lower in the 10% treatment compared to the other treatments, and CO_2 was very high. Seawater was undersaturated regarding calcite and aragonite for the 10% treatment. In the same concentration but corrected for pH and alkalinity (10%AT), CO_2 levels and alkalinity did not have significantly different levels from control. The 10%AT treatment was neither undersaturated regarding calcite nor aragonite, and the pH level was higher than the 10% treatment. However, for these parameters' levels are significantly different from the control.

Between the 1% and 1%AT treatments there is only a significant difference in pH_T but not for other parameters. Between the control and the 1%AT treatments there is a significant difference in pH but not for other parameters. Lastly, between the control and the 1% treatment there is a significant difference in levels of the pH and calcite and aragonite saturation. However, there is no significant difference for alkalinity or CO_2.

Table 2. Seawater carbonate chemistry parameters presented as Mean±SE; pH and alkalinity were measured a total of 4 times over the course of the experiment. pH is presented on the total scale (pH_T) and this along with alkalinity was used to calculate CO_2 partial pressure (pCO_2; µatm), as well as calcite and aragonite saturation states (Ω_c and Ω_a respectively), for a salinity of 32 and a temperature of 10°C.

		Measured		Calculated		
Treatment	Replicate	pH_T	Alkalinity (µmol/kgSW)	pCO_2 (µatm)	Ω_c	Ω_a
0%	1	8.02±0.06	2411±33.45	553±88.38	2.85±0.30	1.81±0.19
	2	7.99±0.04	2398.75±31.30	577.7±58.2	2.72±0.2	1.72±0.13
	3	7.99±0.04	2593±190.30	622.9±53.85	3.04±0.44	1.93±0.28
0.1%	1	7.97±0.03	2359.5±23.51	601.4±43.09	2.42±0.15	1.5±0.1
	2	7.98±0.03	2492.75±137.13	612±26.39	2.72±0.35	1.7±0.22
	3	7.99±0.03	2379.5±37.44	578.8±42.88	2.64±0.22	1.7±0.14
1%	1	7.87±0.02	2441.25±92.45	672.6±33.64	2.33±0.167	1.47±0.1
	2	7.86±0.03	2341.75±34.21	664.3±41.93	2.25±0.21	1.43±0.14
	3	7.82±0.06	2371.75±39.83	761.2±110.37	2.18±0.31	1.38±0.2
1%AT	1	7.9±0.01	2492.75±122.68	635.3±24.84	2.54±0.17	1.61±0.1
	2	7.93±0.02	2343±59.34	558.1±40.02	2.55±0.06	1.61±0.04
	3	7.94±0.02	2375.75±25.67	556±24.87	2.69±0.12	1.71±0.08
10%	1	7.27±0.08	1671±42	2087.3±424.24	0.45±0.07	0.29±0.04
	2	7.26±0.08	1683±36.69	2176.7±461.6	0.44±0.06	0.28±0.04
	3	7.28±0.05	1835±187.85	2202.1±335.29	0.5±0.05	0.32±0.03
10%AT	1	7.88±0.06	2355±55.43	648.6±77.7	2.28±0.32	1.44±0.2
	2	7.91±0.04	2210±161.07	567.5±80.35	2.34±0.19	1.48±0.13
	3	7.93±0.03	2275.25±89.42	550.9±60.32	2.60±0.24	1.65±0.155

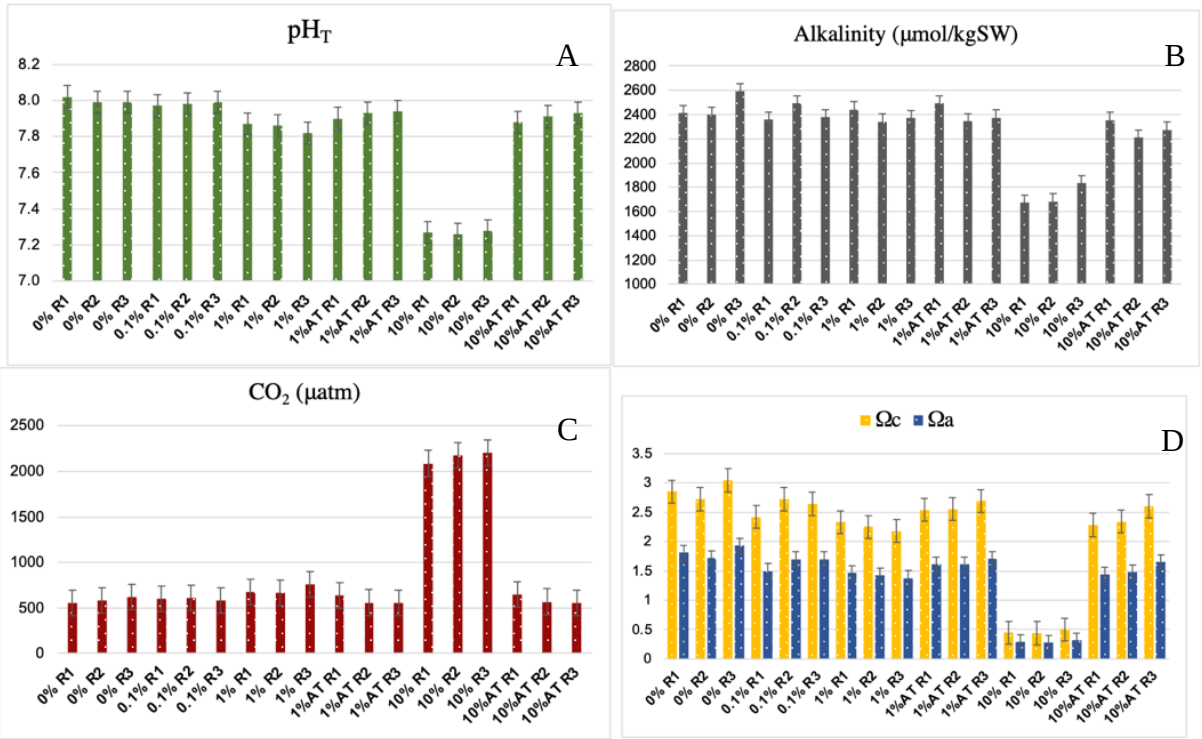

Figure 7. Seawater carbonate chemistry parameters presented in bar plots. pH on the total scale (pH_T) [A]. Alkalinity on the total scale (µmol/kgSW) [B]. CO_2 partial pressure (pCO_2; µatm) [C]. Calcite and Aragonite saturation states (Ω_c and Ω_a respectively) [D].

Biological Measurements

Body Length Growth Rate per Treatment

The scrubber effluent concentration (0%, 0.1%, 1% and 10%) has a significant effect on BL growth rate (One-way ANOVA; $F_{3, 8} = 847.13$, $p < 0.0001$). A Scheffé's test revealed that all treatments were significantly different from each other. The 0% treatment had the larvae with highest BL growth rate, followed by the 0.1%, 1%, and then 10% treatments. The growth rate of the 10% treatment was 9 times lower than the 1% treatment (Figure 9).

The effect of the alkalinity correction was tested for the concentrations 1% and 10%. BL growth rate was significantly impacted by both concentration (Two-way ANOVA, concentration effect: $F_1 = 474.88$, $p < 0.0001$) and AT correction ($F_1 = 254.84$, $p < 0.0001$), there was also a significant interaction ($F_1 = 155.48$, $p < 0.001$). A Scheffé's test comparing the 4 different treatments revealed a significant difference between all treatments except from the 1% and 1%AT treatments. The 1% and 1%AT treatments had the highest BL growth rate, followed by the 10%AT and then the 10% treatments, with the 10%AT treatment having 8 times higher BL growth rate than the 10% treatment (Figure 9).

There was significant difference in BL growth rate between the 0% and 1%AT treatments (One-way ANOVA; $F_{1,4} = 58.46$, $p = <0.0001$) and between the 0% and 10%AT treatments (One-way ANOVA; $F_{1,4} = 64.68$, $p < 0.0001$). Neither the 1%AT nor the 10%AT treatments reached the same BL growth rate as the control treatment when corrected for pH and alkalinity (Figure 9).

The differences in BL growth rate between the treatments 0%, 10%, and 10%AT on day 14 of the experiment are visualized in Figure 8. In the control treatment, larvae had a sound development, while in the 10% treatment the larvae development arrested for the duration of the experiment. However, at the same concentration but corrected for pH and alkalinity (10%AT), larvae were able to reach the pluteus stage, but with a different phenotype compared to the control treatment.

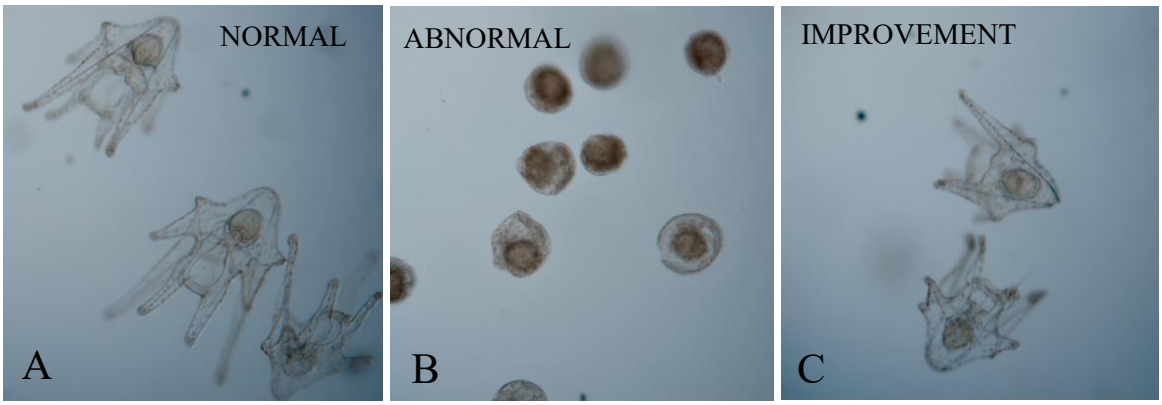

Figure 8. Images taken with microscope of S. droebachiensis larvae on day 14. Control treatment (0%), pluteus stage larvae in FSW [A], larvae from treatment 10% scrubber effluent in an abnormal blastula/gastrula stage [B]. Pluteus stage larvae in treatment 10%AT (10% scrubber effluent corrected for pH and alkalinity) [C]. Photos taken by Ida Vartia, Kristineberg Marine Research station, April 7, 2022.

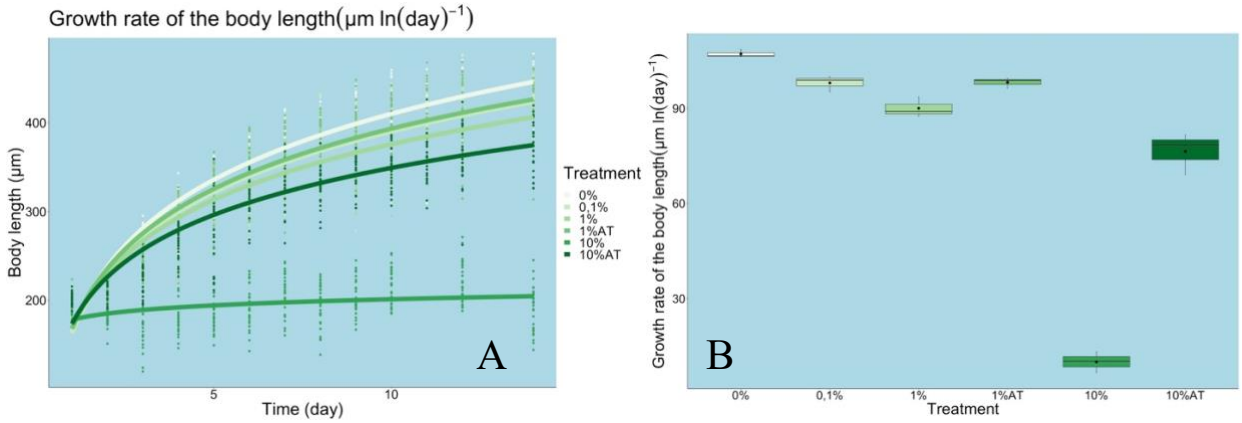

Figure 9. Logarithmic regression lines of the body length (µm ln(day)$^{-1}$) for all treatments, the relationship between time (day) and body length is positive, as days increase the body length increases. Each dot represents data points taken each day per treatment [A]. Boxplot of body length (µm ln(day)$^{-1}$) per treatment, 10% has a much lower growth rate then all the other treatments except for 1%AT which was not tested. Each box represents the regression coefficient extracted from the logarithmic relationship of BL and time for the three replicates per treatment. Median and mean is presented [B].

Mortality Rate

The effect of the scrubber effluent concentration on the mortality rate was significant with the treatments 0%, 0.1%, 1% and 10% (One-way ANOVA; $F_{3,8} = 10.7$, $p = 0.0036$). A Scheffé's test revealed that the only significant difference in mortality rate was between the 10% and the other treatments. The mortality rate was around -0.05 except for in the 10% treatment, where the mortality rate was 0.02 units higher, thus the larvae had a greater survival (Figure 10).

For treatments 1%, 1%AT, 10% and 10%AT there was a significant difference in average mortality rate by both concentration (Two-way ANOVA, concentration: $F_1 = 31.15$, $p = 0.0005$) and AT correction ($F_1 = 41.82$, $p < 0.0002$), there was also a significant interaction ($F_1 = 37.98$, $p < 0.0003$). A Scheffé's test revealed that the only significant difference in mortality rate was between the 1% and the other treatments. The mortality rate was around -0.05 in the 1% treatment and -0.03 in the other treatments, thus the larvae had a greater survival in both the corrected treatments and the 10% treatment (Figure 10).

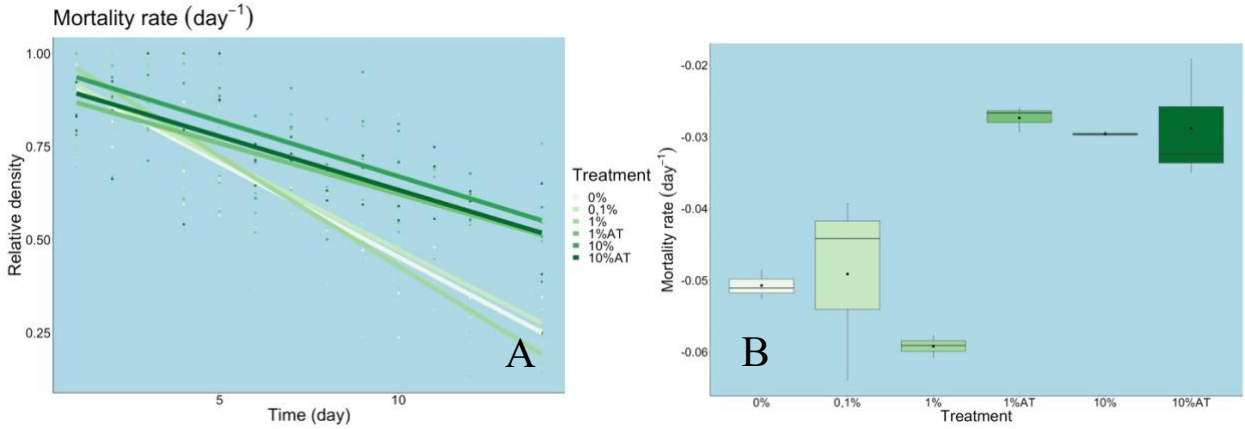

Figure 10. Linear regression lines of the mortality rate (day^{-1}) for all treatments, the relationship between time (day) and relative density is negative, as days increase the relative density decreases. Each dot represents data points taken each day per treatment [A]. Boxplot of mortality rate (day^{-1}) per treatment. Each box represents the regression coefficient extracted from the linear relationship of relative density and time for the three replicates per treatment. Median and mean is presented [B].

Allometry

BR allometry was not significantly impacted by the treatments (0%, 0.1% and 1%; no data could be collected for the 10% treatment as the larvae did not develop calcified structures; One-way ANOVA; $F_{2, 6} = 0.99$, $p = 0.4238$). However, when comparing with the AT corrected treatments, BR allometry was significant between the 1% and 1%AT treatments (One-way ANOVA; $F_{1, 4} = 8.67$, $p = 0.0422$). The larvae in the 1% treatment grew longer BR in relation to the BL compared to the 1%AT treatment (Figure 11).

POR allometry was not significantly impacted by the treatments (0%, 0.1% and 1%; no data could be collected for the 10% treatment as the larvae did not develop calcified structures; One-way ANOVA; $F_{2, 6} = 1.7$, $p = 0.2592$). Similarly, when comparing with the AT corrected treatments, POR allometry was not significant between the 1% and 1%AT treatments (One-way ANOVA; $F_{1, 4} = 6.29$, $p = 0.0662$) (Figure 12).

PLR allometry was significantly impacted by the treatments (0%, 0.1% and 1%; no data could be collected for the 10% treatment as the larvae did not develop calcified structures; One-way ANOVA; $F_{2, 6} = 31.30$, $p = 0.007$). A Scheffé's test comparing the 3 treatments revealed that the 0.1% treatment is significantly different from other treatments, larvae grew shorter PLR in relation to the BL. Similarly, when comparing with the AT corrected treatments, PLR allometry was significant between the 1% and 1%AT treatment (One-way ANOVA; $F_{1, 4} = 25.68$, $p = 0.0071$). Compared to the 1%AT treatment, larvae in the 1% treatment grew longer PLR in relation to the BL (Figure 13).

SV allometry was not significantly impacted by the treatments (0%, 0.1% and 1%; no data could be collected for the 10% treatment as the larvae did not develop calcified structures; One-way ANOVA; $F_{2, 6} = 0.54$, $p = 0.6098$). However, when comparing with the AT corrected treatments, SV allometry was significant between the 1% and 1%AT treatments (One-way ANOVA; $F_{1, 4} = 15.68$, $p = 0.00167$). Larvae in the 1% treatment grew larger SV in relation to the BL than in the 1%AT treatment (Figure 14).

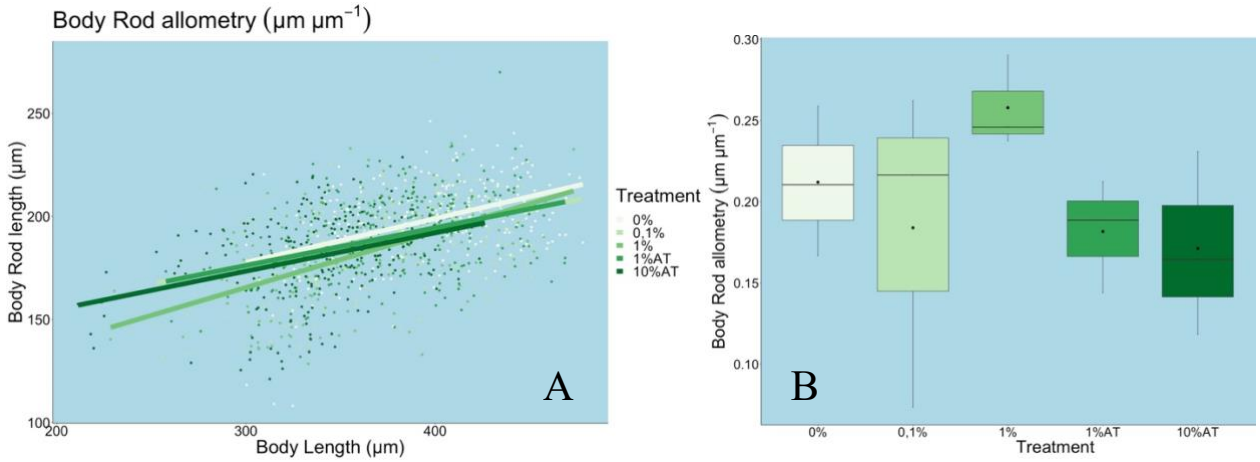

Figure 11. Linear regression lines of the body rod allometry (µm µm^{-1}) for all treatments, the relationship between body length (µm) and body rod length (µm) is positive, as body length increase the body rod length increases. Each dot represents data points taken each day per treatment [A]. Boxplot of body rod allometry (µm µm^{-1}) per treatment. Each box represents the regression coefficient extracted from the linear relationship of body rod length and body length for the three replicates per treatment. Median and mean is presented [B].

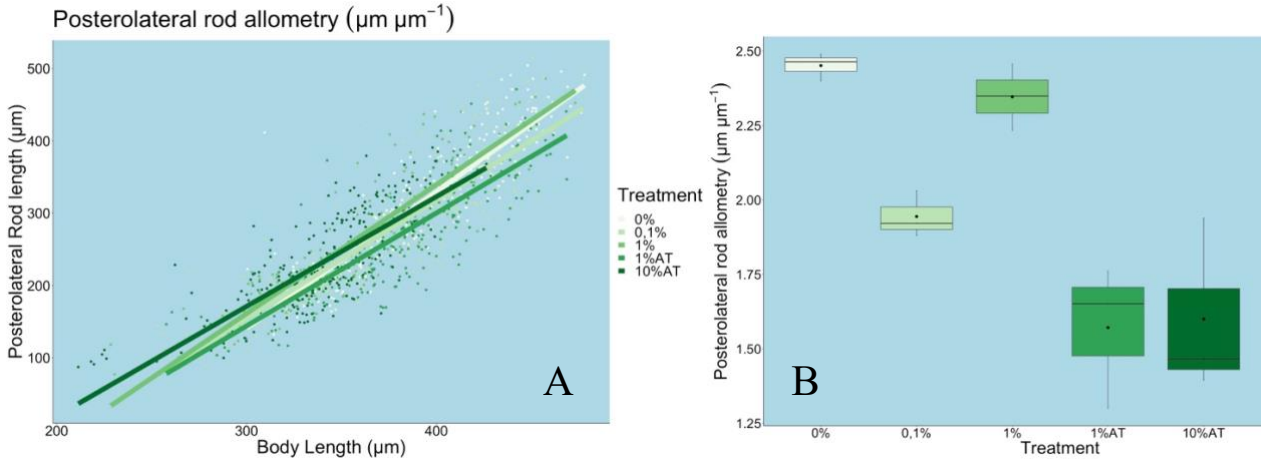

Figure 12. Linear regression lines of the posterolateral rod allometry (µm µm^{-1}) for all treatments, the relationship between body length (µm) and posterolateral rod length (µm) is positive, as body length increase the posterolateral rod length increases. Each dot represents data points taken each day per treatment [A]. Boxplot of posterolateral rod allometry (µm µm^{-1}) per treatment. Each box represents the regression coefficient extracted from the linear relationship of posterolateral rod length and body length for the three replicates per treatment. Median and mean is presented [B].

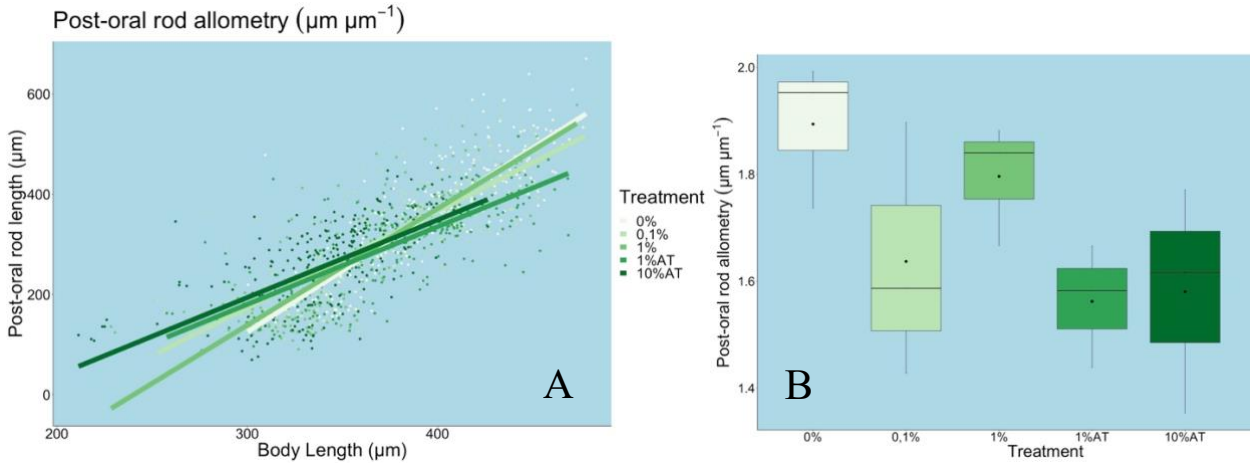

Figure 13. Linear regression lines of the post-oral rod allometry (µm µm^{-1}) for all treatments, the relationship between body length (µm) and post-oral rod length (µm) is positive, as body length increase the post-oral rod length increases. Each dot represents data points taken each day per treatment [A]. Boxplot of post-oral rod allometry (µm µm^{-1}) per treatment. Each box represents the regression coefficient extracted from the linear relationship of post-oral rod length and body length for the three replicates per treatment. Median and mean is presented [B].

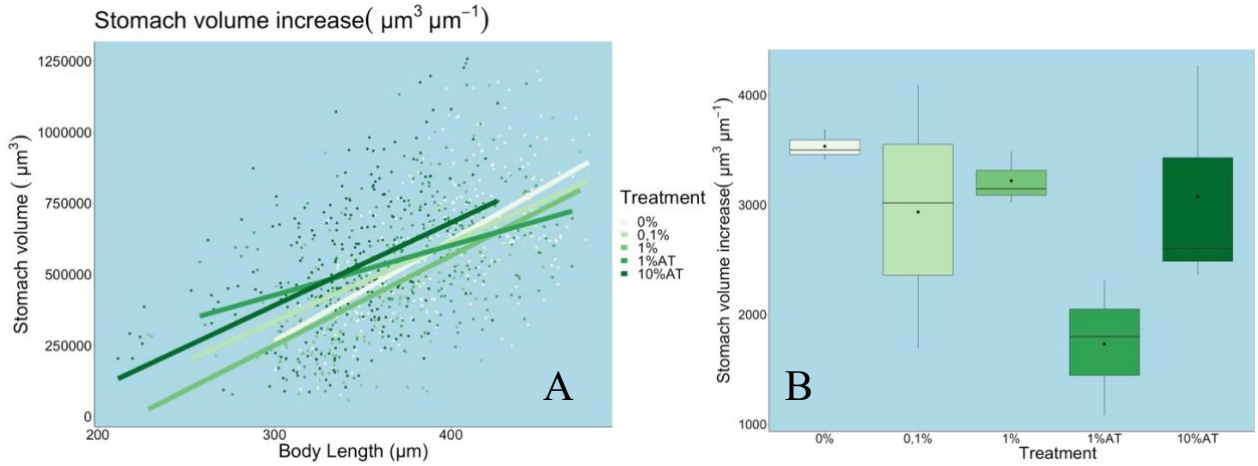

Figure 14. Linear regression lines of the stomach volume increase (µm^3 µm^{-1}) for all treatments, the relationship between body length (µm) and stomach volume (µm^3) is positive, as body length increase the stomach volume increases. Each dot represents data points taken each day per treatment [A]. Boxplot of stomach volume increase (µm^3 µm^{-1}) per treatment. Each box represents the regression coefficient extracted from the linear relationship of stomach volume and body length for the three replicates per treatment. Median and mean is presented [B].

Discussion

The central hypothesis was that the combined effect of toxins, low pH and low alkalinity in scrubber effluent would negatively impact survival, BL growth rate, and morphology of green sea urchin larvae. Moreover, it was hypothesized that the negative effect of low pH and low alkalinity could be minimized by using a strong base (Na_2CO_3). Furthermore, it was expected that some adverse effects would still be observed due to the toxins present in the scrubber effluent.

Using a Na_2CO_3 solution, we successfully increased pH and alkalinity in the treatments of 1% and 10% scrubber effluent, restoring the seawater carbonate chemistry to normal levels.

Survival

The larvae survival was higher in the 10%, 1%AT, and 10%AT treatments compared to the other treatments, including the control. This contradicts the central hypothesis. The 10% treatment, followed by the 1% treatment, was predicted to have the lowest survival. It is also unexpected that larvae in the 1%AT and 10%AT treatments survived better than the control. To fully interpret these observations, it is essential to look at the other parameters. For example, it is obvious that the larvae in the 10% treatment were abnormal and failed to reach the pluteus stage (Figure 8). In this treatment, larvae were in a state of arrested development from the first days, without arms and stomach, and could not ingest any food. However, despite the arrested development, they survived for the duration of the experiment. Because of the absence of ingestion, we can reasonably predict that those larvae would eventually die. A longer lasting experiment would surely reveal their actual mortality rate. In Dorey et al. (2013), authors excluded treatments where development was arrested from their mortality rate calculation. In their study, the individuals with the same pH level as in our 10% treatment did not survive after day 13. Similarly, the mortality of the other treatments with high survival over the two weeks of the experiment may have been underestimated. Our work suggests that when exposed to high concentrations of scrubber effluent, the larvae BL growth rate decreases and it may lead to a decreased mortality over a period of two weeks.

The long-term impact of scrubber effluent over different experimental durations could be tested in future experiments, as well as the reversibility of the process (e.g., by transferring the larvae into control FSW). This would be relevant outside of experimental settings, where exposure to scrubber effluent on marine life is likely to be transient. Russo et al. (2003) found that embryos cultured in cadmium (Cd) for 15 hours and then transferred to control seawater were able to develop normally while harmful effects due to exposure to Cd for more than 24 hours were not reversible.

A lowered pH raises energy costs for calcifying organisms (Gaylord et al., 2015); this combined with the toxins, could explain why the larvae in the 1% treatment experienced decreased survival compared to those in the 1%AT treatment.

The Growth Rate of Body Length

For BL growth rate, the results supported the hypothesis. The BL growth rate decreased with increased concentration of scrubber effluent. AT correction had a significant effect at the 10% treatment, partly correcting the adverse effects of the scrubber effluent. No effect was observed for the AT correction for the 1% treatment, probably because the difference in pH was too low. There was a considerable improvement in BL growth rate when correcting the 10% treatment for pH and alkalinity (10%AT). At the 10% concentration, the pH was 7.3, a pH level well known to affect growth negatively (Dorey et al., 2013). However, the BL growth rate at the 10%AT, 1%, and 1%AT treatments were not as high as the control. The adverse effects of toxins could explain this.

The larvae in the 1% treatment developed faster than those in both the 10% and 10%AT treatments but they still had a higher mortality rate.

A delay in development makes pelagic larvae spend more time in the water column before settling to become a juvenile. In addition, this increases mortality by increased exposure to predation during the planktonic period (Christiansen & Fenchel, 1979; Vaughn & Allen, 2010). However, in a study by Chan et al. (2015), authors made an intriguing observation. They found that the green sea urchin larvae do not suffer a decreased swimming ability because of their size. This is because their smaller size is compensated by a change in shape and swimming technique due to selective pressure and phenotypic plasticity (Chan et al., 2015). This indicates that the larvae in the 10%AT treatment, with a significantly lower BL growth rate, may not be destined to have less survival than in other treatments. Instead, they can potentially acclimate to the environment and change their shape for increased survival.

Morphology

There was no difference between treatments for POR allometry. However, for BR, PLR, and SV the 1% treatment had greater allometric relationships than the 1%AT treatment. Furthermore, the 0.1% treatment had a greater allometric relationship than the 0% and 1% treatments regarding PLR. For the 10% treatment, there was nothing to measure because the development was arrested, which is a result in of itself. One of the most sensitive stages in the life history of sea urchins is larval development (Limatola et al., 2020). Sea urchin larvae require arms to catch food, swim and protect themselves against predators (Emlet, 1983; Strathmann et al., 1992). Changes in skeleton development could increase mortality because of its important role in protection (Emlet, 1982). The range of pH where development was possible (swimming larvae) in Dorey et al. (2013) was between pH 7.0-8.0. It was below pH 7 that development was arrested, and all embryos died within 13 days of fertilization. For our 10% treatment, an arrested development was observed in pH 7.3. This could be explained as the combined effect of low pH, low alkalinity, and toxins. Another difference with Dorey et al. (2013) is that scrubber effluent decreases not only pH but also the alkalinity, potentially amplifying the negative effect. CO_2-induced acidification keeps the alkalinity constant.

Russo et al. (2003) conducted a study where embryos of the sea urchin *P lividus* were cultured in different concentrations of $CdCl_2$. Cd-exposed embryos developed with gut and skeleton abnormalities and an arrest of development after 48-50 h. In the highest concentration, 94 % of embryos had an abnormal blastula. In our 10% treatment the larvae had a similar morphology, and because Cd is a heavy metal often present in scrubber effluents, this indicates that Cd could have played a significant role in damaging the development.

Two arm rods grew longer in the 1% treatment compared to the 1%AT treatment. A change in echinoderm larvae arm length affects their ability to catch food (Hart & Strathmann, 1994) where longer arms increase feeding efficiency (Hart & Strathmann, 1994). However, longer arms also reduces swimming ability in the water column (Clay & Grunbaum, 2011; Strathmann & Grunbaum, 2006). One parameter for arm length is food availability; henceforth, the arm to BL ratio depend on changes in food abundance (Soars et al., 2009). In the 1% treatment, arms were longer, and the stomach was larger compared to the 1%AT treatment. This could be explained by both the fact that when the environment is rich in nutrition, the plutei generate a large stomach and short arms (Sewell et al., 2004; Strathmann et al., 1992) also, as previously noted, calcifying organisms lose energy efficiency in low pH. In green sea urchins this effect is coupled with decreased digestive efficiencies which cues increased consumption to compensate (Stumpp et al., 2013) leading to increased armlength. There is a trade-off between long arms and large stomachs in different environments (Miner, 2005) and our results fail to reveal why the larvae in the 1% treatment both had long arms and a large stomach compared to those in the 1%AT treatment. Juvenile development is induced earlier with larvae with short arms compared to long arms

(Strathmann et al., 1992), indicating that larvae with shorter arms survive better and live a shorter amount of time in the pelagic environment before settling. This tells us that the larvae in the 1%AT treatment is less stressed and has better fitness than those in the 1% treatment.

Toxins and Multiple Stressors

Single-compound studies show that PAHs and heavy metals cause detrimental effects on sea urchin larvae. In Pillai et al. (2003), authors noticed that when exposed to PAHs, sea urchin embryos experienced a disruption in axial development because of effects on genetic pathways leading to exogastrulation. Similar effects have been seen with sea urchin larvae exposed to Lithium (Li) (Nocentemcgrath et al., 1991). Exogastrulation was not observed in any of our treatments. Moreover, Xu et al. (2011) exposed green sea urchin embryos to 11 combinations of four heavy metals (Cu, Pb, Zn, and Cd). Embryonic toxicity was observed for the four heavy metals individually, and the toxic effects decreased in the order: Cu > Pb > Zn > Cd. Additionally, the authors found that the interaction between metals in the different combinations was complex. The authors established that the combined effects of heavy metals should be contemplated in the risk assessment of heavy metal pollution in the ocean. Toxins are more harmful in the ocean than in laboratory studies, perhaps due to large amounts of toxins combining (Russo et al., 2003). The study of scrubber effluents could progress by investigating its components interactions and effect on biological responses outside of laboratory settings.

The multiple stressors in our experiment (Boyd et al., 2018), makes it challenging to interpret the isolated effect that each stressor had on the biological response of the model organism. In addition to the pH, alkalinity, and toxins effect, in treatments where pH and alkalinity were corrected, we introduced the strong base Na_2CO_3. Moreover, when clustering the toxins as one stressor, there is a risk of missing what precisely is driving the biological response. This is because there are many different contaminants that could be damaging. However, when other organisms were exposed to scrubber effluents, the scrubber acted as a "witches' cauldron" (Koski et al., 2017; Thor et al., 2021). Non-lethal concentrations of contaminants showed lethal effects when combined but not when alone.

In our study, this combined effect was conspicuous. Meaning, the combined effect of the several stressors in our experiments on the biological response was more significant than the totality of the individual stressors (Boyd et al., 2018). Moreover, it is difficult to know if those effects could have been increased due to enhanced toxicity in acidic environments (ICES, 2020; Linders et al., 2019). Each scrubber effluent possess unique composition of stressors (ICES, 2020; Linders et al., 2019), this acts as a bias in the results because it is challenging determine whether another effluent composition would have triggered the same biological responses. Finally**,** soot was detected in the 10% and 10%AT treatments (Figure 15). Future studies could investigate the relative contribution of the different aspects of the scrubber effluent (pH, alkalinity, toxicants) and their interactions on the biological response of green sea urchins. It would be interesting to see the eventual impact that scrubber effluents could have on the green sea urchin larvae combined with the changing climate and its additional stressors such as increasing water temperatures and CO_2-induced OA.

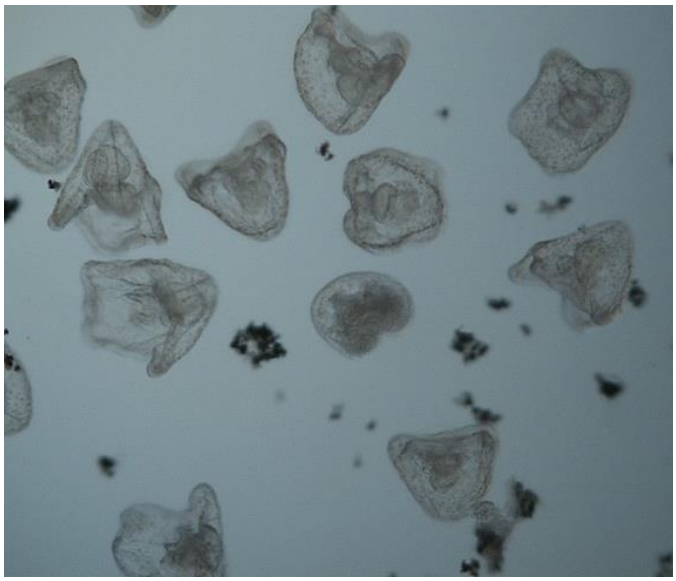

Figure 15. Soot particles in the 10%AT treatment on experimental day 5. Photo taken by Ida Vartia, Kristineberg Marine Research station, March 29, 2022.

Consequences and Next Step

Our results suggest that correcting pH and alkalinity in high concentrations of scrubber effluent partly corrects the adverse effects observed on the tested organisms. Perhaps adding Na_2CO_3 to the scrubber effluent could be a cheap and easy mitigation mechanism for open-loop scrubbers. Currently, in closed-loop scrubber systems, bases are used for neutralizing the scrubber effluent (Linders et al., 2019). However, in open-loop systems, the pH levels are poorly regulated, and at the same time, it is not easy to monitor the acidity onboard. Future studies should confirm our findings on other organisms and test the potential impacts of high alkalinity.

Our results also demonstrate that correcting the low pH and low alkalinity can only partly mitigate the harmful effects of scrubber effluents. Some adverse effects are likely attributed to toxins that can bioaccumulate or magnify. At best AT correction could be used as a short-term alternative with the prospect of better alternatives and technologies in the future. Nevertheless, the optimal situation would be one where shipowners change to more environmentally friendlier fuels, both for the marine environment and for the climate.

In conclusion, our results confirm the harmful effects of scrubber effluent on the marine environment. Besides the increased usage of scrubbers, lagging and inconsistent legislation by IMO regarding scrubber use threatens EU countries' efforts to reach good environmental status, good ecological status, and good chemical status. There is a need for restrictive regulations on the release of scrubber effluent into seawater as a counter measurement against the future increase of installed scrubbers. To do this, either the IMO needs to change their regulations or, the EU and Regional frameworks must govern independently. Obviously, the latter would help increase efficient implementation of the regulations because the regulators would need to oversee the regulations in a much smaller capacity. Moreover, EU legislations are more strict than international law thanks to its robust legal order. Today, most papers written about scrubber effluents' effect on marine organisms focus on the toxicants, neglecting acidification. Ultimately, our results show that the acidification and decreased alkalinity play a significant role in the biological response, and that there is a potential development of some partial mitigation strategies.

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