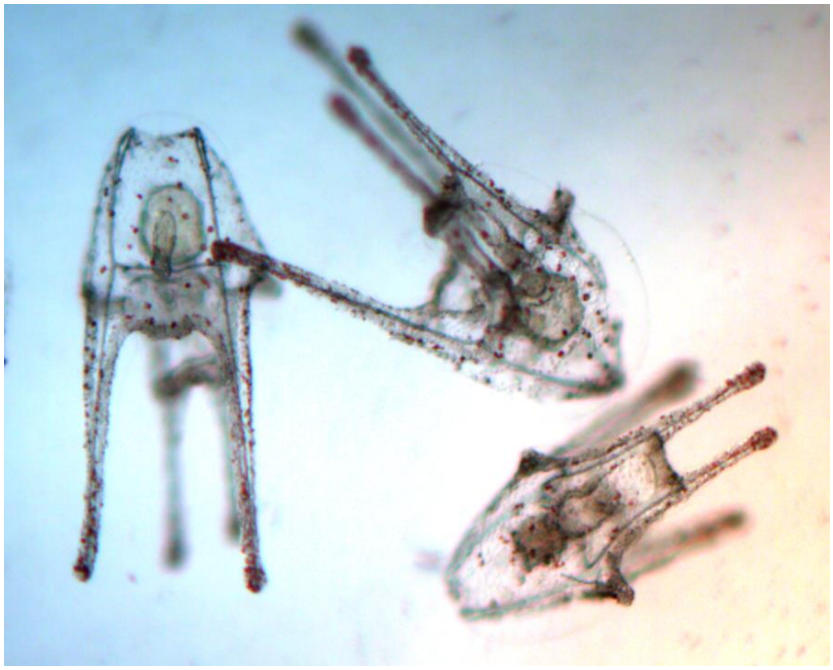
GÖTEBORGS UNIVERSITET

DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCINECE

Food modulates the response of sea urchin *Echinus esculentus* larvae to ocean acidification



Erica Ottosson

Degree of Bachelor of science with a major in environmental science

ES1510 examination course in environmental science, 15 hp

Term/year: Spring 2021

Supervisor: Sam Dupont, Department of Biological and Environmental Sciences

Examiner: Ingela Dahllöf, Department of Biological and Environmental Sciences

Abstract

The Increasing carbon emissions to the atmosphere from anthropogenic sources causes ocean acidification, decreasing oxygen levels and warming of the ocean. These changes are a global threat to the marine ecosystems. In coastal areas these alterations can have dire consequences since multiple stressors are acting together. These coastal areas host a large diversity of species and ecosystems where stressors such as ocean acidification can result in losses in biodiversity. This can affect all trophic levels and alter the entire food web.

Species are often adapted to the variability of their environmental niche. When exposed to conditions at the edge or outside of their niche, organisms require more energy to maintain vital functions and start experience stress. In the IPCCs Special Report (2019), food is mentioned as a key parameter modulating the effects of ocean acidification for rocky shore species. Ocean acidification can have larger impacts in areas where resources of food are limited providing less physiological plasticity in response to stress.

Sea urchins have been pointed out as sensitive group in response to ocean acidification, especially during their larvae stage. For this thesis, I analysed material and data from an experiment performed on sea urchin *Echinus esculentus* collected from the Gullmar Fjord (Sweden). The experiment was designed to investigate how different levels of food affects the response of *E. esculentus* larval stage to acidification. Previous experiments on sea urchin larvae have demonstrated that mortality and abnormal development increases with decreasing pH as a consequence of increased maintenance cost for organisms when they are exposed to stress. We hypothesized that *E. esculentus* sensitivity to low pH will decrease with increasing food concentration.

The results from this experiment demonstrated that the mortality was lowest in the treatment that received a low amount of food and growth rates increased with the increasing amount of food. While the response to low pH was depending on the level of food, increased levels of food did not compensate for the negative effect of low pH. This can be interpreted as local adaptation to low food conditions experienced during spawning season of this species and to large present pH variability.

Key words: Ocean acidification, sea urchin, *Echinus esculentus,* pH, food.

Sammanfattning

Ökade antropogena utsläpp av koldioxid till atmosfären leder till många negativa konsekvenser och havet påverkas starkt av dessa utsläpp dels genom försurning, minskat syreinnehåll samt uppvärmning. Dessa förändringar hotar marina ekosystem och den biodiversiteten som finns där. I kustområdena där biodiversiteten generellt sätt är högre kan detta leda till förödande konsekvenser då flera stressfaktorer samverkar. Denna förlust av biodiversitet kan resultera i en förenklad artsammansättningen och kan påverka hela näringskedjan.

Organismer utvecklas ofta för att anpassa sig till de variationer och förändringar som sker i deras habitat. När organismer utsätts för stress utöver den de anpassat sig till under en längre tid så kan de behöva lägga mer enerig på att bibehålla livsviktiga funktioner och därmed ökar deras energibehov. I IPCC:s Special Report (2019) så konstaterades det att tillgången på föda kan avhjälpa havsförsurningens negativa effekter. I områden med låg tillgång på föda kan effekterna därför bli mer uttalade till följd av ökad stress och en minskad plasticitet.

En grupp som utpekats som extra känsligt mot havsförsurning är sjöborrarna, framför allt i sitt larvstadie. I denna uppsats har data och material från ett experiment utfört på den ätliga sjöborren *Echinus esculentus* tagna från Gullmarsfjorden analyserats. Experimentet utformades för att studera hur *E. esculentus* i sitt larvstadie påverkas av försurning vid olika födokoncentrationer. I liknande studier med andra sjöborrearter har man kunnat bevisa att under förhållanden med lågt pH så ökar dödligheten och det leder dessutom till onormal utveckling i högre grad. Detta antas bero på ökade energikostnader för att upprätthålla vitala funktioner. Vår hypotes var att *E. esculentus* känslighet till lågt pH minskar med ökad mängd föda.

Resultaten från experimentet visar att dödligheten var lägst i de behandlingarna med låga mängder föda och att tillväxten tilltar med ökade födokoncentrationer. Effekterna till följd av lägre pH är beroende av mängden föda, men ökade koncentrationer av föda kompenserade inte för det låga pH-värdets negativa effekter. Detta kan tolkas som en lokal anpassning till låga mängder föda, eftersom de vid lek är vana vid låga födokoncentrationer och att de naturligt utsätts för stora pH variationer.

Nyckelord: Havsförsurning, Ätlig sjöborre, *Echinus esculentus, föda, pH.*

Acknowledgment

Working with this report has taught me a lot and I got to use my knowledge and skills that I have picked up during my three years of studying at the University of Gothenburg. To have actual use of my education in a real project have been fun and I have gotten a better understanding on how to use my knowledge in a practical way. I would especially like to thank my supervisor for this project, Sam Dupont how have been a great help during this work. I am very grateful that I got to be a part of their performed experiment and get a better feeling for how this works in real life. Sam has also helped me to find good papers on the subject and have been a huge help when I have had problems during my work. I would also like to thank my friend and classmate Johanna Bengtson that has helped me think of things I would not have figured out without her and for just being supporting during my work with the thesis.

Table of content

Introduction

Ocean acidification

71% of earth is made up of water and 97% of it is saline. The ocean constitutes 99 % of earth's biologically habitable space and is essential for life on earth. This large ecosystem contributes to a rich biodiversity and it provides numerous ecosystem services. The ocean is essential for human well-being, livelihood and food. The ocean stores excess carbon dioxide and heat produced from increasing concentrations of greenhouse gases. It slows down surface warming and is an important component of the global biogeochemical cycles (IPCC, 2019). Increasing carbon emissions to the atmosphere from anthropogenic sources leads to ocean acidification, warming and decreasing oxygen levels in the ocean. These global changes threaten marine ecosystems and anthropogenically induced environmental changes owing to the increasing global footprint of human activities and emissions of greenhouse gases are so important that the present time period is now called 'the Anthropocene' (Vargas, et al., 2017).

The acidification of the oceans has led to an alteration in the oceanic carbon chemistry cycle and a disturbance in the geochemical balance. Since pre-industrial times between 20-30% of the anthropogenically emitted carbon have been absorbed by the ocean. Growing evidence suggests that the ocean sink for anthropogenic carbon is dynamic over a decadal timescale and has increased the last two decades. Since the late 1980s the open ocean surface pH has declined by 0.017-0.027 pH units. Future changes are dependent on societal behaviour and different scenarios are considered. The IPCCs Representative Concentrations Pathways (RCPs) are possible future climate scenarios predicted until year 2100. They take into account time series of emissions and concentration of greenhouse gases, aerosols, and other chemically active gases, but also land use and coverage. If we follow the business-as-usual scenario (RCP8.5), surface pH in the open ocean is estimated to decrease by around 0.287-0.291 pH units by 2081-2100, compared to 2006-2015. If we manage to reduce the carbon emissions and follow the best case scenario (RCP2.6), the pH will drop 0.036-0.042 units. It is important to remember that pH is measured on a logarithmic scale. This implies that a decrease of 1 pH unit corresponds to a 10-fold increase of acidity or hydrogen ion (H^+) concentration (IPCC, 2019).

The carbon dioxide taken up by the ocean dissolves in the surface water and forms carbonic acid, which causes acidification, a reduction in pH and carbonate ion concentration (IPCC, 2019). If the partial pressure of carbon dioxide (pCO_2) in the surface ocean continues to increase in proportion to the rising emissions of atmospheric CO_2, a doubling of atmospheric CO_2 from preindustrial levels could cause a 60% increase in hydrogen ion concentration and a 30% reduction of carbonate ion concentration. An increased concentration of carbonate ions reduces the oceans ability to absorb more CO_2 from the atmosphere (Sabine, et al., 2004).

Spatial and temporal differences

Regional trends and changes on a local scale are the main drives for the impacts on ocean ecosystems and human societies. Regional and local conditions for pH, temperature, salinity, oxygen, and nutrient concentrations in the oceans can fluctuate (IPCC, 2019). For example, different levels of variability are observed between coastal areas and the open ocean. In coastal waters, biology plays a key role in driving variability in pH and pCO_2. As a consequence, large variability is observed in ecosystems such as sandy beaches, kelp forests and estuaries. These regions constitute a small part of the global oceans but provides up to 30% of the marine primary production and approximately 50% of the supplied organic carbon to the deep ocean. Numerous coastal areas have their own characteristic features, endemic species, and heterogeneous habitats. This makes them vulnerable to climate changes, which can cause alterations in functions and structures of the ecosystem and loss of biodiversity (IPCC, 2019). In coastal environments, pH variations are influenced by biotic parameters such as respiration and photosynthesis, which are depending on a combination of biotic and abiotic factors. Over the year, seasonal changes and associated biological activities result in further variability in pH that the organisms in coastal areas are exposed to. These can lead to local adaptations and modulate species sensitivity to different stressors such as changes in pH and pCO_2 (IPCC, 2019; Vargas, et al., 2017). Species can evolve and adapt to local conditions and changes in the environment, which helps them survive and develop. This creates different niches and makes them more adapted to the conditions that they live in (Dorey el al., 2013). For instance in coastal environments, calcifying organisms can develop mechanisms to maintain homeostasis in low pH waters (Ventura et al., 2016).

Marine organisms closely interplay with the surrounding water. Species that experience significant environmental challenges must redistribute their energy to other processes and this may result in an energy reduction to support normal growth or reproduction (Thor & Dupont, 2015). It is important to understand specie sensitivity to environmental changes and local adaptations to predict future effects from ocean acidification and how different species can cope with it (Dorey et al., 2013). Interactions between species and biological processes can vary, for some organisms acidification and hypoxia can limit the habitable temperature range for organisms and aggravate their sensitivity to warming (IPCC, 2019). Organisms that are exposed to stress until the edge of their niche require more energy to maintain essential mechanisms such as reproduction and to uphold homeostasis. When exposed to unfavourable conditions for a longer period it can cause irreversible damage and death because of the arising unmanageable costs. Changes due to ocean acidification has already been seen in the marine ecosystems and are anticipated to cause significant alterations in species' niches (Dorey et al., 2013).

Sessile organisms such as barnacles and mussels live in rocky shore ecosystems. Evidence suggest that these rocky shores are at high risk of losing its biodiversity, ecosystem functions and structures because of changes like acidification. Recent studies on vulnerable rocky shore species such as mussels have demonstrated that food is a significant modulator of the effects of ocean acidification. Impacts of ocean acidification may hence be more pronounced in areas where available food is limiting and/or where the organism is exposed to other sources of stress. Moreover, ocean acidification also has the potential to change the quality and quantity of the food in a given location. Local effects from acidification and warming on rocky shores are anticipated to change the energy flows and reduce the energy to higher trophic levels in

the ecosystems and we might see alterations leading to a detritus-based food web. In specific ecosystems, sensitivity in organism groups can rise with a limitation of nutrients and available food. Their physiological ability to compensate for the extra energy costs associated with stress gets reduced. Other species interaction can also play a role. For example, changes can also modify the rules for competition between organisms and species with high ability to adapt to environmental changes may have an advantage (IPCC, 2019).

Nutrient composition changes

Due to rising levels of CO_2 in the atmosphere oceanic concentrations also rises. As a result, the available amount of carbon for primary producers in the ocean has increased. This has the potential to change the stoichiometry of primary producers and a higher carbon-to-nutrient ratio is anticipated as their nutrient composition follows the surrounding mediums concentration and composition. Elevated CO_2 concentrations can stimulate photosynthetic organisms to fixate more carbon and therefore result in a reduced nutrient content in relation to the amount of carbon, which determines the quality of the food for consumers (IPCC, 2019). Multiple drivers such as hypoxia, acidification and a reduction in food supplies and nutrients pose risks to marine ecosystems, which can be enhanced when combined with other stressors. Herbivores consumption and metabolic rates increases with rising temperature, although this does not result in a greater secondary production. In calcifying and non-calcifying species ocean acidification reduce secondary production (IPCC, 2019). This could cause a mismatch between herbivores nutrient requirement for somatic growth and phytoplankton stoichiometric composition. This might not be the case in all organisms as it is very species dependent (Rossoll, et al., 2012). Consumers needs to maintain carbon homeostasis and if there is an excess of carbon, they need to get rid of it somehow, which comes with a cost. This usually results in a reduction in growth and development (Schoo et al., 2013). This can have cascading impacts in the food web. For example, copepods can be indirectly impacted by ocean acidification through a reduced nutritional quality in algae (Rossoll, et al., 2012). Because some of the most productive ecosystems in the world are supported by this diatom-copepod relationship, ocean acidification could have devastating consequences for food webs in the oceans and result in cascade effects to higher trophic levels due to the changes in quality of essential nutrients in primary producers. However, our knowledge on the effects from ocean acidification is manly limited to single specie response not on food web interactions (Rossoll, et al., 2012).

Effects on marine organisms

It is expected that ocean acidification will decrease the calcification in bivalves. However, Thomsen et al. (2013) showed that in juvenile blue mussels (*Mytilus edulis*) the amount of available food is more crucial for their calcification and growth than elevated pCO_2. Benthic stages of *M. edulis* can tolerate high seawater pCO_2 when there are enough food resources. Thomsen et al. (2013) concluded that to predict species vulnerability to ocean acidification it is crucial to assess species interactions and the availability of energy in habitats. Ventura et al. (2016) confirmed the fact that declining seawater pH increases the mortality rate and the number of abnormal larvae in the blue mussels. Ventura et al. (2016) showed that exposure to low pH increased energy costs to maintain a skeleton. This increase does not affect their food intake or growth, but it probably effects their ability to store energy for later developmental stages. Moreover, they demonstrated that species sensitivity is not linked to a simple chemical threshold. Rather, a species specific tipping point is related to the natural variability in their habitat and its niche. For *M. edulis* in this experiment, their tipping point was identified to be 7.765 pH units and was close to the minimum pH in the species niche. Under milder acidification the pattern of larval development suggested a large individual variability in response to ocean acidification and a potential for further natural selection (Ventura et al., 2016).

Schoo et al. (2013) studied the copepod *Acartia tonsa* feeding on *Rhodomonas salina* that had been growing under different pCO_2 conditions relevant to IPCC scenarios. They saw that with an increased availability of CO_2 for the algae, the rate of development in *A. tonsa* declined. They also showed that there was an increase of unsaturated (essential) fatty acids in *R. salina* when grown under high carbon-to-nutrient ratios. It may then enhance the nutritional quality in *R. salina* as a food source and thus all the negative effects in the copepods could only be explained by the direct changes in composition of carbon. However in studies on other algae the opposite results have been demonstrated. Which yet again amplifies the importance of a high biodiversity and demonstrates that even species in the same taxonomic groups can have different or opposite effects in response to the same stressor (Schoo et al., 2013). Thor & Dupont (2015) revealed that in the calanoid copepod *Pseudocalanus acuspes* grown under different pCO_2 conditions a transgenerational effect was detected that alleviated the negative effect from exposure to ocean acidification. Even though they experienced a significant reduction in fecundity, the loss of fecundity and increased metabolic rates were not as high after multiple generations as compared to one generation (Thor & Dupont, 2015). With continued rising levels of available CO_2 for primary producers along with changes in the nutrient loading of coastal ecosystems, this could possibly change the trophic connections between herbivores and primary producers. A reduced growth rate in consumers can have dire consequences in higher trophic levels and trough out the entire food chain. The higher carbon availability and the following changes in nutritional composition can result in loss and replacement of some species due to shifts in competitive interactions between algae. All these changes might change the flow of matter and energy though the food web and alter the functions of ecosystems and communities (Schoo et al., 2013).

Sea urchins

One taxonomic group that has been pointed out as particularly sensitive to decreased pH in seawater caused by CO_2 is sea urchins, mainly in their early stages of development (Stumpp, et al., 2012). Their response to ocean acidification has been extensively studied (Jager et al., 2016). Sea urchins are a central component of benthic marine communities. For instance, there is a delicate balance between kelp forest productivity and sea urchin grazing pressure. Sea urchins are heavily dependent on successful recruitment for population sustainability and it is then critical to understand how ocean acidification can affect this process (Stumpp et al., 2011). The planktonic larvae stage is an important part of benthic marine organisms life cycle since adults have a reduced ability to move. Due to their mobility, planktonic larvae have a crucial and significant function for population distribution, genetic diversity and abundance. Environmental factors can affect larval survival, transport and settlement although this can be modulated by the larvae's mobility and ability to swim. Larval swimming is limited by biomechanical and physiological factors and larvae development and growth can be affected by stressors such as ocean acidification. Larvae ability to filter particles for food, maintaining mobility and swimming in still waters are affected by changes in orientation and length of the ciliated arms and research has shown that these parameters are sensitive to ocean acidification (Chan et al., 2015).

Studies on planktotrophic echinoid larvae have indicated that food limitations result in an increase in arm length. This increases the length of their ciliated bands and then their feeding efficiency. However, this comes at an energy cost leading to a delayed rudiment formation. There is also a trade-off between using ciliary bands for swimming and capturing food with ecological implications. Previous experiments have shown changes in larval morphology under ocean acidification associated with an increased rate of feeding in the green sea urchin (*Strongylocentrotus droebachiensis*) plutei. This was hypothesised to be a compensatory mechanism caused by a reduction in digestion efficiency (Stumpp, et al., 2013; Chan et al., 2015). Sewell et al. (2004) exposed larvae of the sea urchin *Evechinus chloroticus* to different levels of food and observed a developmental plasticity. Larvae kept at low and no food concentrations did not evolve past their four-arm stage of development (Sewell et al., 2004).

Dorey et al. (2013) investigated the impacts of pH on the green sea urchin (*S. droebachiensis*). They concluded that when exposed to lower pH, the larvae are highly plastic until a physiological tipping point at pH= 7.0, which is described as the lowest pH where normal growth, development and survival could occur. Although the displayed plasticity was associated with indirect and direct consequences for fitness, such as decreased growth and increased mortality. They also concluded that the *S. droebachiensis* larvae are plastic and robust in responses to pH variations in a broader range then what they experience today and possibly what they might experience in the future as well. Lowering of the pH increased mortality, respiration and decreased growth rate which results in allometric alterations in plutei morphology (Dorey et al., 2013). Chan et al. (2015) confirmed that in low pH green sea urchin larvae had a reduced growth rate and an overall change in shape. They collected data on swimming behaviour and showed the change in shape was adaptive to modify their ability

to swim. This was explained as a compensation for the extended pelagic larvae stage and to ensure settlement in the right habitat. These results suggest that the larval sea urchins are under strong selective pressure to maintain their high plasticity (Chan et al., 2015).

Acidification affects organisms metabolism and their energy budget. A shortage of energy can result in negative carry over effects in later developmental stages and a reduces success of juveniles (Dorey et al., 2013). Jager et al. (2016) used Dynamic Energy Budget (DEB) models to evaluate the effects of pH on green sea urchins larvae (*S. droebachiensis*). Using published data, they showed that ocean acidification primarily leads to higher maintenance costs for the sea urchin larvae. Working with DEB modelling Jager et al. (2016) calculated a tipping point at pH of 7.5, corresponding to the extreme of the present natural variability. This contributes to an interesting perspective in the potential relationship between species sensitivity to ocean acidification and the natural variability in pH. (Jageret al., 2016).

Echinus esculentus

E. esculentus also known as the edible sea urchin naturally occur in waters with temperatures around 4-18 °C, mainly in in coastal areas or areas with hard substrate in the North Sea and they usually live in depths of 8-1200 meters (Artfakta, 2016). Sexual maturity is dependent on the surrounding temperature but generally it occurs when they are 1-3 years old and have a diameter of at least 4 cm. Their growth rate depends on water temperature, food availability and age. They have a generation time of 1-2 years and reproduce annually and spawning mainly occurs in the spring and early summer usually before the seasonal rise in temperature and the spring bloom starts (Tyler-Walters, 2008; Naturvårdsverket, 2008). The planktotrophic larvae stage of development lasts for 1-2 months. *E. esculentus* are active suspension feeders and their typical food is barnacles, worms, macroalgae, detritus and bottom material (Tyler-Walters, 2008). Kain and Jones (1966) showed that *E. esculentus* is limiting *Laminaria hyperborean* populations. According to SLU Artdatabanken. (2020) *E. esculentus* are classified as viable/ least concern (LC) in Sweden redlist from year 2020. But according to IUCN it is classifies as near threatened (NT), although this has not been updated since 1996 (World Conservation Monitoring Centre, 1996). Due to the fact that sea urchins are well studied and have an important role in ecosystems it is important to investigate how they will respond to global changes such as ocean acidification.

Aim and hypothesis

The aim with this project is to test the ideas that food modulates the response to changes in pH.

The hypotheses are:

I) *Echinus esculentus* will have a negative physiological response to low pH lower than experienced today (e.g. pH<7.6)

II) Negative effect of decreased pH will be partly compensated by increased food.

Method

Experiment

This work is based on an experiment that was conducted at Kristineberg Marine Research Centre. The experiment was done to investigate how different levels of food affects *E. esculentus* response to acidification in their larval stage. The used sea urchins in this experiment were collected in Gullmar Fjord (Sweden) pH can vary between 8.7 to 7.6 throughout the years and monthly variations can reach 0.9 pH units.

The experiment started 21st of June 2017 and was performed by Sam Dupont and colleagues. Specimens of the sea urchin *E. esculentus* were collected and brought to the lab. Two females and one male sea urchins were used. Methods for spawning, fertilization and larvae culturing were following protocols from Dorey et al. (2013). In brief, spawning was induced by injecting KCl across the peristomial membrane. Eggs were collected in filtered seawater and sperm were collected dry and kept on ice until use. After fertilization, eggs were transferred to 5L culturing flasks filled with filtered seawater at a starting density of approximately 8 eggs mL^{-1}. The density in the aquariums was kept at this level (8 eggs mL^{-1}) which results in approximately 40 000 larvae. 24 different aquariums were used and maintained in a thermo-constant room (15 °C). Aquariums were randomized and the experiment lasted for 22 days.

Once they developed a functional stomach (day 4), larvae were fed daily with the microalgae *Rhodomonas spp*. To prevent changes in food concentration, the size and concentration of the algae were checked daily in every larval culture using a Coulter counter (Elzone 5380; Micrometrics) and adjusted when necessary. Alkalinity, salinity, seawater pH_{TS} and temperature were measured every second day and aquariums were also cleaned on a regular basis. pH was maintained by injection of pure CO_2 into the seawater and controlled in each culture using a pH-stat system (Aqua Medic, Bissendorf, Germany). All of the cultures were aerated continuously and mixed through air bubbling.

The 24 aquariums were divided into two separate pH treatments (8.2 vs 7.5) and four different food concentrations (0, 100, 1000 and 10000 cells mL^{-1}). Strains of microalgae were

provided by the Marine Algal Culture Centre of the University of Gothenburg (GUMACC). The carbonate chemistry is summarized in Table 1.

For each larval culture subsamples of 2x10 mL were collected daily for the first 12 days then every other day until day 22 (end of experiment). Each sample contained approximately 80 larvae and in aquariums with high mortality, the sampling volume was increased. Samples were fixed with a paraformaldehyde solution. Larvae were counted to evaluate mortality and pictures were taken which resulted in a total of 3500 pictures. These were used to document the impact of pH on growth and development.

		Measured			Calculated	
Target pH	Food (cells mL^{-1})	pH_T	Temperature (°C)	CO_2 (µatm)	Ωcalcite	Ωaragonite
High	0	8.05±0.01	12.5±0.07	401±15	3.38±0.08	2.15±0.05
	100	8.06±0.01	12.5±0.10	389±11	3.45±0.07	2.19±0.04
	1000	8.06±0.01	12.6±0.01	391±6	3.46±0.04	2.19±0.03
	10000	8.02±0.02	12.7±0.02	439±18	3.19±0.100	2.03±0.06
Low	0	7.43±0.05	12.7±0.05	1952±221	0.93±0.100	0.59±0.06
	100	7.44±0.03	12.6±0.03	1906±174	0.95±0.063	0.60±0.04
	1000	7.48±0.03	12.7±0.03	1909±98	1.03±0.061	0.66±0.04
	10000	7.50±0.09	12.3±0.09	1889±291	1.18±0.260	0.75±0.16

Table 1. Carbonate chemistry (average ± standard error of mean). CO_2, Ωcalcite and Ωaragonite were calculated using alkalinity of 2302 µmol kg^{-1} seawater and salinity of 32.

Picture analysis

Pictures taken during the experiment were analysed in the programme ImageJ and using the criteria's from Dorey et al. (2013). Nine different measurements were taken on each larva (Figure 1): body length, stomach size (vertical and horizontal), head rod length, postoral arm length and anterolateral arm length. Measurements were used to calculate growth rates, allometries and asymmetry. Analyses focussed on the first 14 days of the experiment.

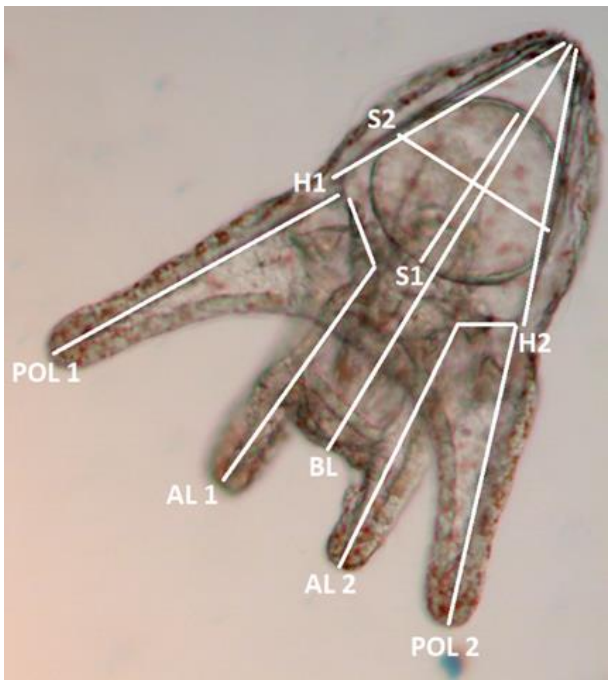


Figure 1. Morphometric measurements on *Echinus esculentus*: BL (body length), S1 (stomach vertical), S2 (stomach horizontal), H1 and H2 (head rod length), (head rod length), POL 1 and POL 2 (postoral arm length), AL 1 and AL 2 (anterolateral arm length)

Data analysis

The statistical analyses were carried out using Microsoft Excel and IBM's software for statistical analysis SPSS (Statistical Package for the Social Sciences). The applied level of significance was 5%. Statistical assumptions of homogeneity of variance and normality were tested using Leven's test and PP-plots. The used data for stomach volume was calculated using this equation $\frac{4}{3} \times \pi \left(\frac{AVERAGE\ S1{:}S2}{2}\right)^3$, where S1 and S2 are the two measurements for the stomach, displayed in Figure 1. This value was then used to calculate the relationship between the stomach volume and the body length. The equation used to calculate the asymmetry was $\frac{Min(POL1{:}POL2)}{Max(POL1{:}POL2)}$ and for the maximum values $Max(H1{:}H2)$. These values in were then used to calculate the relationship with body length. However, for POL asymmetry and stomach volume these relationships were not significant and the average of all the experiment was used. The relationship between parameters were tested using linear or logarithmic regressions and carried out in both Excel and SPSS. Relative mortality rate was estimated as the coefficient of the significant linear regressions between the relative density and time (days post fertilization). Growth rate was calculated as the coefficient of the significant logarithmic relationship between body length and time (days post fertilization). For allometries and asymmetry the significant linear relationship between the different measurements and the body length were calculated. When no significant relationship was observed, the mean values per aquarium was used for this parameter.

Food concentrations and pH effects were tested using a two-way ANOVA. This was performed for mortality, growth rate, allometries and asymmetry. To see if the required assumptions for an ANOVA was fulfilled, Levene's test have been performed and are presented in the Appendix. All of the processed data has not lived up to the required assumptions and therefore the data has been transformed for each of these groups to see if they can be used to perform an ANOVA (Geert van den Berg, 2021). Post hoc tests (Tukey HSD) were also carried out when significant effects caused by food was demonstrated. When data were failing the ANOVA requirements, they were not included in this thesis and will be analysed at a further stage using non-parametric tests.

Results

Mortality rate

The two-way ANOVA revealed that food and the interaction between food and pH has a significant effect on the mortality rate (Figure 2; Table 2). There was no significant effect of pH on the mortality rate.

	MR		
Source	*df*	*F*	*P*
Food	3	6,306	0,008*
pH	1	0,017	0,899
Food x pH	3	8,829	0,002*
Error	12		

*Table 2. Results from two-way ANOVA on mortality rate data. Data marked with * indicates a significant result.*

A post hoc test (Tukey HSD) demonstrated that the mortality rate was significantly lower at 100 cells mL^{-1} as compared to all of the other food concentrations (Table 3).

Tukey HSD		
		MR
Food	Food	*P*
0	100	0,021*
	1000	0,999
	10 000	0,997
100	0	0,021*
	1000	0,017*
	10 000	0,030*
1000	0	0,999
	100	0,017*
	10 000	0,986
10 000	0	0,997
	100	0,030*
	1 000	0,986

*Table 3. results from Tukey HSD post hoc test on the effect in mortality rate between the different amounts of food. Data marked with * indicates a significant result.*

A one-way ANOVA was performed to resolve the interaction between pH and food (Table 4). At 1000 cells mL^{-1} food level, the mortality rate was significantly lower at low pH as compared to high pH.

Food x pH			
	MR		
	df	F	P
0	1	5,623	0,098
100	1	1,875	0,264
1000	1	16,794	0,026*
10 000	1	1,252	0,345

*Table 4. Results from one-way ANOVA performed on the Mortality rate data divided by the different levels of food to assess where there was an effect caused by the interaction of food and pH. Data marked with * indicates a significant result.*

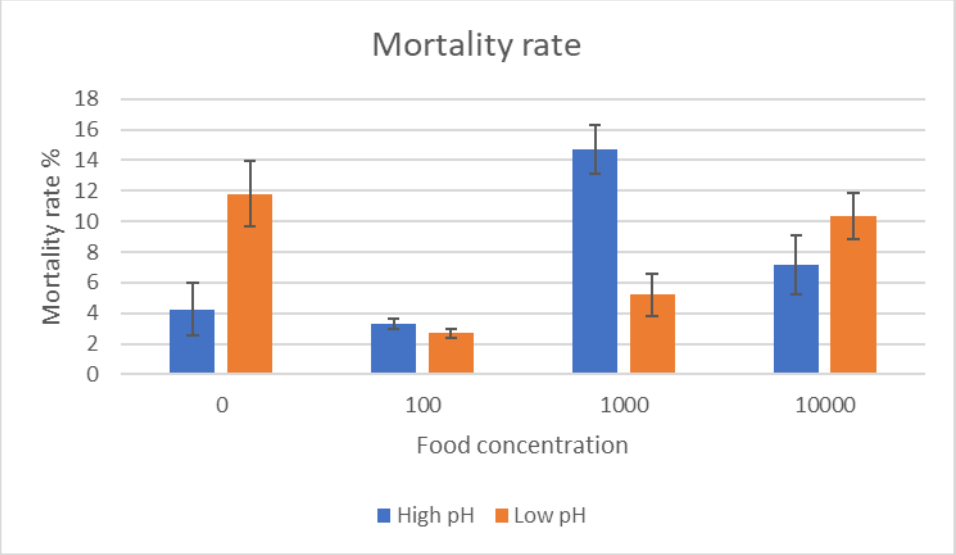


Figure 2. Mortality rates (in %) for each level of food (cells mL^{-1}) and pH. Right bars (blue) are for high pH and left bars (orange) is for low pH. Average with SD.

Growth rate

Only food had a significant effect on the growth rate (two-way ANOVA; Figure 3; Table 5)

	GR		
Source	*df*	*F*	*P*
Food	3	530,286	0,009*
pH	1	1,524	0,235
Food x pH	3	0,851	0,486
Error	16		

*Table 5. Results from two-way ANOVA performed on the data for growth rate. Data marked with * indicates a significant result.*

Post hoc test (Tukey HSD) revealed that the growth rate was higher at 10 000 cells mL^{-1} as compared to 0 and 1000 cells mL^{-1} (Table 6).

Tukey HSD		
		GR
Food	Food	*P*
0	100	0,484
	1000	0,790
	10 000	0,007*
100	0	0,484
	1000	0,951
	10 000	0,119
1000	0	0,790
	100	0,951
	10 000	0,045*
10 000	0	0,007*
	100	0,119
	1 000	0,045*

*Table 6. Post hoc test to evaluate which levels of food shows a significant effect on the growth rate. Data marked with * indicates a significant result.*

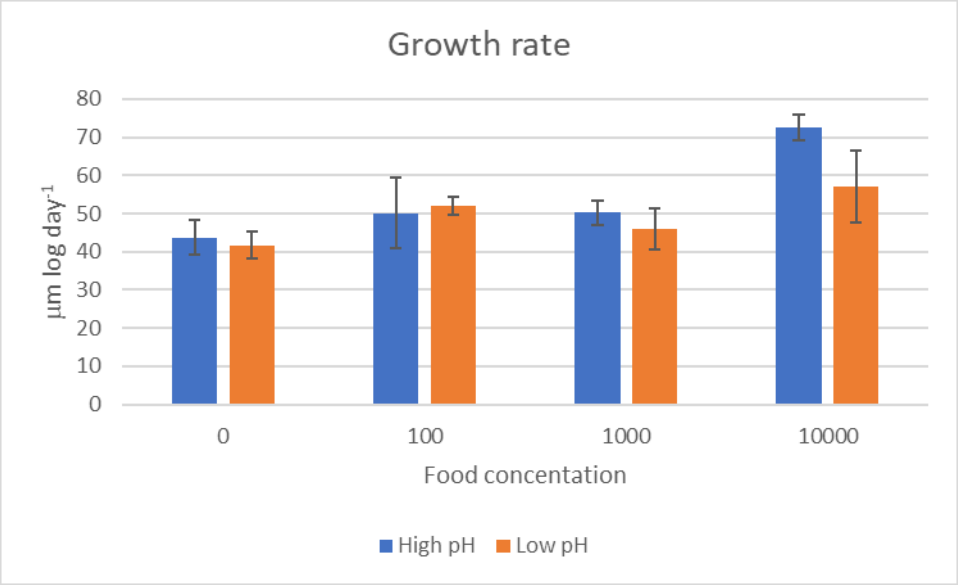


Figure 3. Growth rate in (µm log day^{-1}) for each level of food (cells ml^{-1}) and pH. Average and SD. Right bars (blue) are for high pH and left bars (orange) is for low pH.

Stomach volume

Food and pH had a significant effect on the stomach volume, but not their interaction (two-way ANOVA; Table 7; Figure 4). The stomach volume was significantly lower at low pH.

	SV_m		
Source	*df*	*F*	*P*
Food	3	5,900	0,007*
pH	1	6,242	0,024*
Food x pH	3	0,678	0,578
Error	16		

*Table 7. Results from the performed two-way ANOVA on the mean values for stomach volume. Data marked with * indicates a significant result.*

Larvae raised at 10 000 cells mL^{-1} had a larger stomach volume as compared to all of the other amounts of food (0, 100 and 1000 cells mL^{-1}; Table 8).

Tukey HSD		
		SV_m
Food	Food	*P*
0	100	0,995
	1000	0,922
	10 000	0,010*
100	0	0,995
	1000	0,978
	10 000	0,016*
1000	0	0,922
	100	0,978
	10 000	0,034*
10 000	0	0,010*
	100	0,016*
	1 000	0,034*

*Table 8. Results from post hoc test on stomach volume (mean values) to demonstrate where the significant effects between the various amounts of food are. Data marked with * indicates a significant result.*

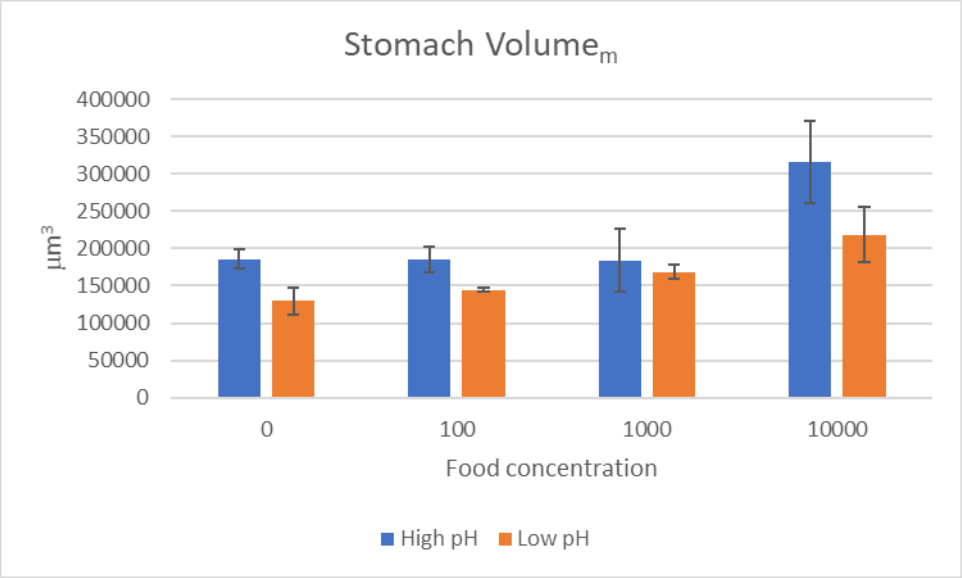


Figure 4. Stomach volume in µm³ for each level of food (cells mL^{-1}) and pH. Average and SD. Right bars (blue) are for high pH and left bars (orange) is for low pH.

Allometries

For the two skeletal rods, POL and AL there was no significant effects by food, pH or the interaction on their allometric relationship with body length (Table 9; Figure 5-6).

	Max POL			Max AL		
Source	*df*	*F*	*P*	*df*	*F*	*P*
Food	3	0,981	0,428	3	1,357	0,294
pH	1	2,573	0,130	1	3,727	0,073
Food x pH	3	0,330	0,804	3	1,797	0,191
Error	15			15		

Table 9. Displaying results from the two-way ANOVA carried out on the allometric relationship of the postoral arms (POL) and anterolateral arms (AL) with body length (BL).

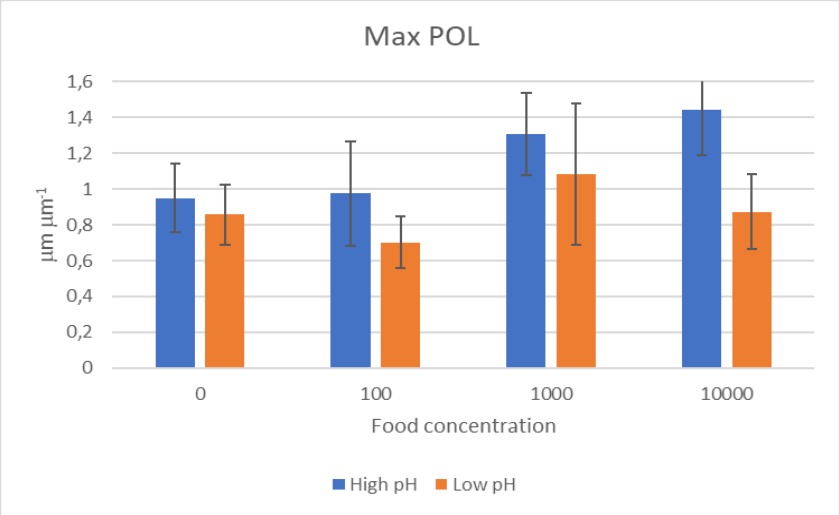


Figure 5. Allometric relationship in µm µm^{-1} between POL and BL for each level of food (cells mL^{-1}) and pH. Average and SD. Right bars (blue) are for high pH and left bars (orange) is for low pH.

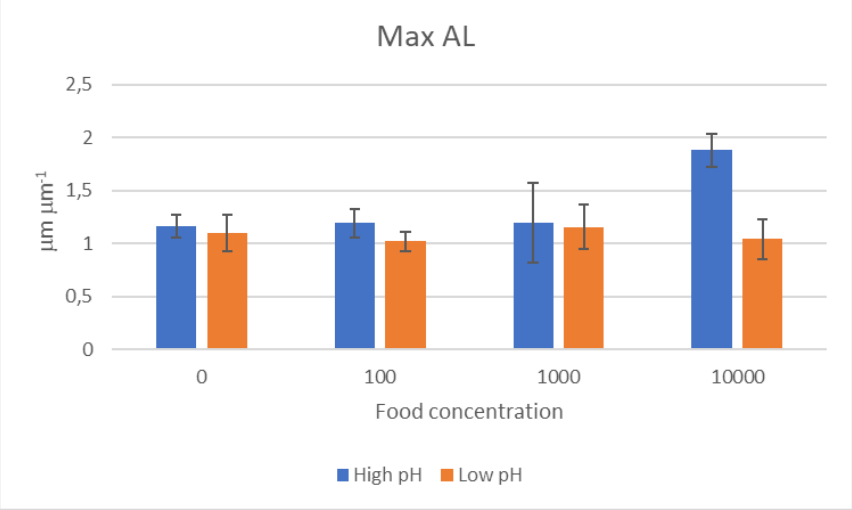


Figure 6. Allometric relationship between AL and BL for each level of food (cells mL^{-1}) and pH. Average and SD. Right bars (blue) are for high pH and left bars (orange) is for low pH.

Asymmetry

Only pH had a significant effect on the asymmetry index for POL (two-way ANOVA; Table 10; Figure 7). Larvae raised at low pH were more asymmetric than the ones raised at high pH.

	Asym POL_m		
Source	*df*	*F*	*P*
Food	3	1,296	0,310
pH	1	7,706	0,013*
Food x pH	3	0,322	0,809
Error	16		

*Table 10. Results from two-way ANOVA performed on the mean values for POL asymmetry. Data marked with * indicates a significant result.*

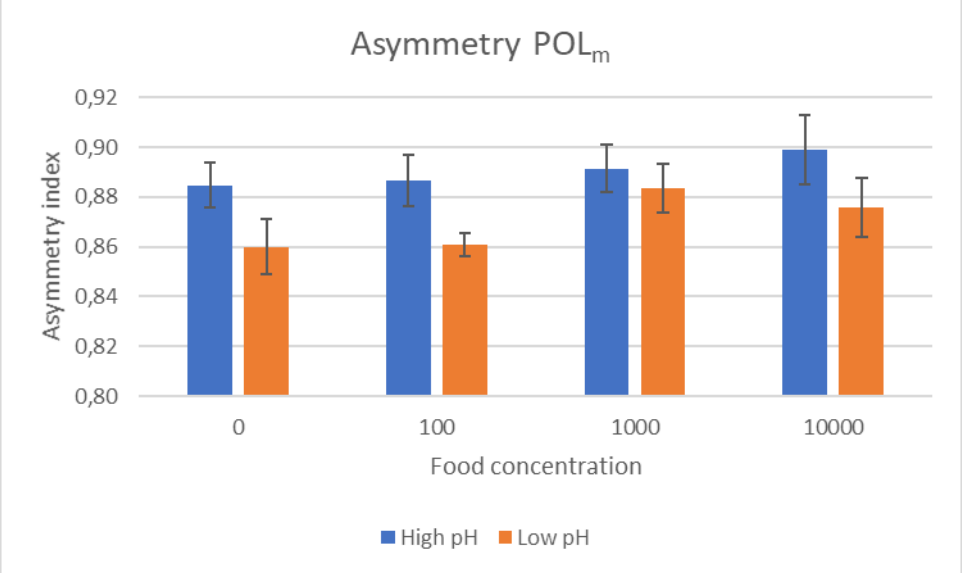


Figure 7. Asymmetry index for the postoral arms (POL) for each level of food (cells mL^{-1}) and pH. Average and SD. 1 = perfectly symmetric and 0 = completely asymmetric. Right bars (blue) are for high pH and left bars (orange) is for low pH.

Discussion

Mortality rate was significantly affected by food and the interaction between food and pH. Larvae that were fed 100 cells mL^{-1} had a lower mortality rate than other food levels. When it comes to the interaction between food and pH, the only significant effect was demonstrated at 1000 cells mL^{-1}, where an increased mortality was shown at high pH. These results suggest that 100 cells mL^{-1} is the optimal food level for this species. This could be explained by their spawning patterns since they normally spawn late in spring where these low amounts of food are observed (Tyler-Walters, 2008; Naturvårdsverket, 2008). 1000 cells mL^{-1} may in some cases be an overflow, but the results indicates that this is better than no or ten times as much food. These amounts of food are the concentrations that they could encounter in the wild even though 1000 cells mL^{-1} may be a bit higher than expected. Since it is clear that food is one of the main drivers in the sea urchins well-being it is important that the levels of food remain at current or higher levels. *E. esculentus* perform better in low food concentrations with no pH effect which is why changes in food availability or a reduced quality of food can lead to devastating consequences not just for the sea urchins but for the entire marine food web (Schoo et al., 2013).

It is not surprising that mortality rates were higher in absence of food. During the first days of development, sea urchins larvae are not feeding and are depending on the energy reserves in the eggs (Jager et al., 2016). In absence of food they can continue their development until exhaustion of these reserves but without food, energy limitation is eventually leading to stress and death (Dorey et al., 2013). Increased amount of food often leads to an increased metabolism (Thor & Dupont, 2015; IPCC, 2019). The observed interaction between pH and food could be explained by an antagonistic contribution of pH and food to the metabolism with pH partly mitigating the negative effect of increased food concentration. Similarly, pH has been shown to modulate metabolism in other organisms such as in the sea star *Crossaster papposus* and marine bivalves (Dupont et al., 2010; Ivanina, et al., 2020).

When food is available, larvae assimilate it and use the energy to increase their growth. Food had a significant effect on growth rates and stomach volume. Growth rate was higher in larvae raised at the highest amount of food as compared to two of the other treatments (0 and 1000 cells mL^{-1}). Larvae also had a larger stomach when raised at the highest food concentration. Sewell et al. (2004) demonstrated that food concentrations had a significant effect on the stomach volume in the experiment on *E. chloroticus*, especially between the treatments with high amount of food and no food as in this experiment. An increased stomach volume is therefore expected in higher concentrations of food. At the highest level of food (10 000 cells mL^{-1}) they also have the highest growth rate which is explained by them not being limited by food. Both Sewell et al. (2013) and McEdward. (1984) has described this relationship in their studies on echinoplutei larvae. In these experiments they have demonstrated that limited amounts of food reduce growth and the metabolism in sea urchin larvae. In higher concentrations of food their growth and stomach volume increase in relation to each other and the amount of food they receive (Sewell et al., 2004; McEdward, 1984).

In other experiments acidification has been demonstrated to affects organisms metabolism and energy budget, which is why low pH can result in a larger stomach volume since they compensate for these extra costs by increasing their feeding to obtain more energy (Stumpp, et al., 2013; Chan et al., 2015; Dorey et al., 2013). However, in the experiment on *E. esculentus* there was a reduced stomach volume in response to decreased pH. This could be explained by a decreased feeding rate or by an increased metabolism caused by the low pH which result in a higher digestion rate which has been demonstrate in other experiments such as in Chan et al. (2011). Although since pH mitigated the negative effects of food when it comes to the mortality the first explanation might be more probable. In Gonzalez-Bernat et al. (2013) study on the sand dollar *Arachnoides placenta* a reduced rate of feeding was seen as response to acidification. Since food is key to the health of the sea urchin larvae and a reduced pH could have a negative impact on their feeding this could affect their development and success later in life.

When exposed to low pH, larvae were significantly more asymmetric. Such asymmetry is an indication of stress in sea urchin larvae although it does not have a large impact on their fitness (Kurihara & Shirayama, 2004; Lenz et al., 2019). These effects have also been observed in other organisms such as corals and brittle star larvae in response to acidification (Foster et al., 2016; Dupont et al., 2008). These changes only demonstrate the challenges the larvae are experiencing due to the decreased pH. No significant effect on the allometries were observed which indicates that they do not change their morphology under the tested conditions. Previous experiment on other sea urchin species have demonstrated a change in larvae morphology under ocean acidification where they grew smaller in response to low pH (Chan et al., 2015; Dorey et al., 2013; Stumpp et al., 2011). Chan et al. (2015) described this as a possible adaptive change in *S. droebachiensis* response to reduced pH but in this experiment with *E. esculentus* we demonstrated that it is not. These results indicates that *E. esculentus* is more resilient to stress caused by decreased pH when it comes to their morphology then other sea urchin species.

Overall, pH has a mild effect on *Echinus*' larvae. While exposure to low pH leads to a small increase in asymmetry, it does not have any effect on allometries, growth rate and only have a mitigating effect on the mortality rate at 1000 cells mL^{-1} food levels. This suggests that the larvae have a high plasticity to changes in pH, at least in the range used in this experiment (8.2-7.5). In the Gullmar Fjord where the used sea urchins for this experiment were collected, they currently experience monthly pH fluctuations of 0.9 pH units with a minimum pH of 7.6 units. The tested scenario (7.5) is then deviating from the present range of natural variability. These results suggest that *Echinus* may be able to cope with decreased pH relevant in the context of ocean acidification.

Conclusion

Our result shows that the optimal food concentration for *E. esculentus* is quite low. At realistic and optimal food concentration, low pH relevant in the context of ocean acidification does not have a strong negative effect on this species of sea urchin larvae. While food modulate the response to low pH, our hypothesis of higher resilience to low pH by increased food concentration is not supported. Overall, increased food concentration has a negative effect (increased mortality) that is partly mitigated by low pH. They also seem to have developed a tolerance to the natural pH fluctuations and therefore as mentioned earlier might be able to cope with ocean acidification as long as food is available in favourable concentrations. Since this study only investigates the larvae stage of development longer studies might be needed do investigate what effects it will have on the species in the long run. This is only an experiment performed on a single-life history and possible carryover effects would be interesting to evaluate for a better understanding on the effects of ocean acidification in different food concentrations over a longer period of time and how it will affect *E. esculentus* future success. It would also be interesting to evaluate *E. esculentus* threshold value/ tipping point to further evaluate how they will cope in a more acidic environment and how it will affect them over generations and possibly do the same with the algae to see if *E. esculentus* food resources will change or remain stable.

References

Artfakta. (2016). *Ätlig sjöborre*. Retrieved March 25, 2021, from https://artfakta.se/naturvard/taxon/echinus-esculentus-217716

Chan, K., García, E., & Dupont, S. (2015). Acidification reduced growth rate but not swimming speed of larval sea urchins. *Scientific Reports, 5*.

Chan, K., Grünbaum, D., & O'Donnell, M. J. (2011). Effects of ocean-acidification-induced morphological changes on larval swimming and feeding. *The Journal of Experimental Biology, 214*(22), 3857–3867.

Dorey, N., Lancon, P., Thorndyke, M., & Dupont, S. (2013). Assessing physiological tipping point of sea urchin larvae exposed to a broad range of pH. *Global Change Biology, 19*(11), 3355-3367.

Dupont, S., Havenhand, J., Thorndyke, W., Peck, L., & Thorndyke, M. (2008). Near-future level of CO2-driven ocean acidification radically affects larval survival and development in the brittlestar Ophiothrix fragilis. *Marine Ecology Progress Series, 373*, 285–294.

Dupont, S., Lundve, B., & Thorndyke, M. (2010). Near Future Ocean Acidification Increases Growth Rate of the Lecithotrophic Larvae and Juveniles of the Sea Star Crossaster papposus. *Journal of Experimental Zoology Part B Molecular and Developmental Evolution, 314*(5), 382-389.

Foster, T., Falter, J. L., McCulloch, M. T., & Clode, P. L. (2016). Ocean acidification causes structural deformities in juvenile coral skeletons. *Science Advances, 2*(2).

Geert van den Berg, R. (2021). *SPSS tutorials*. Retrieved May 4, 2021, from https://www.spss-tutorials.com/spss-two-way-anova-basics-tutorial/

Gonzalez-Bernat, M. J., Lamare , M., Uthicke, S., & Byrne, M. (2013). Fertilisation, embryogenesis and larval development in the tropical intertidal sand dollar Arachnoides placenta in response to reduced seawater pH. *Marine Biology, 160*(8), 1927–1941.

IPCC. (2019). *IPCC Special Report on the Ocean and Cryosphere in a Changing Climate.* [H.-O. Pörtner, D.C. Roberts, V. Masson-Delmotte, P. Zhai, M. Tignor, E. Poloczanska, K. Mintenbeck, A. Alegría, M. Nicolai, A. Okem, J. Petzold, B. Rama, N.M. Weyer (eds.)].

Ivanina, A. V., Jarrett, A., Bell, T., Rimkevicius, T., Beniash, E., & Sokolova, I. M. (2020). Effects of seawater salinity and pH on cellular metabolism and enzyme activities in biomineralizing tissues of marine bivalves. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 248*.

Jager, T., Ravagnan, E., & Dupont, S. (2016). Near-future ocean acidification impacts maintenance costs in sea-urchin larvae: Identification of stress factors and tipping points using a DEB modelling approach. *Journal of Experimental Marine Biology and Ecology, 474*, 11-17.

Kain, & Jones. (1966). Algal colonization after removal of Echinus. *In Proceedings of the fifth internation sea-weed symposium, Halifax, Canada*, 139-140.

Kurihara, H., & Shirayama, Y. (2004). Effects of increased atmospheric CO2 on sea urchin early development. *Marine Ecology Progress Series, 274*, 161–169.

Lenz, B., Fogarty, N. D., & Figueiredo, J. (2019). Effects of ocean warming and acidification on fertilization success and early larval development in the green sea urchin Lytechinus variegatus. *Marine Pollution Bulletin, 141*, 70-78.

McEdward, L. R. (1984). Morphometric and metabolic analysis of the growth and form of an echinopluteus. *Journal of Experimental Marine Biology and Ecology, 82*(2-3), 259-287.

Naturvårdsverket. (2008). *Eutrophication of the Seas along Sweden's West Coast.* Swedish Environmental Protection Agency.

Rossoll, D., Bermúdez, R., Hauss, H., Schulz, K. G., Riebesell, U., Sommer, U., & Winder, M. (2012). Ocean Acidification-Induced Food Quality Deterioration Constrains Trophic Transfer. *PLOS ONE, 7*(4).

Sabine, C. L; Feely, R. A., Gruber, N; Key, R. M., Lee, K; Bullister, J. L., Wanninkhof, R; Wong, C S., Wallace, D. W. R., Tilbrook, B., Millero, F. J., Peng, T., Kozyr, A; Ono, T., & Rios, A. F. (2004). The Oceanic Sink for Anthropogenic CO2. *Science, 305*(5682), 367-371.

Schoo, K. L., Malzahn, A. M., Krause, E., & Boersma, M. (2013). Increased carbon dioxide availability alters phytoplankton stoichiometry and affects carbon cycling and growth of a marine planktonic herbivore. *Marine Biology, 160*(8), 2145–2155.

Sewell, M. A., Cameron, M. J., & McArdle, B. H. (2004). Developmental plasticity in larval development in the echinometrid sea urchin Evechinus chloroticus with varying food ration. *Journal of Experimental Marine Biology and Ecology, 309*(2), 219– 237.

SLU Artdatabanken. (2020). *Rödlista 2020.* Uppsala: SLU.

Stumpp, M. A., Wren, J. B., Thorndyke, M. C., & Dupont, S. T. (2011). CO2 induced seawater acidification impacts sea urchin larval development I: Elevated metabolic rates decrease scope for growth and induce developmental delay. *Comparative Biochemistry and Physiology, Part A, 160*, 331–340.

Stumpp, M., Hu, M., Casties, I., Saborowski, R., Bleich, M., Melzner, F., & Dupont, S. (2013). Digestion in sea urchin larvae impaired under ocean acidification. *Nature Climate Change, 3*, 1044–1049.

Stumpp, M; Hu, M., Melzner, F., Gutowska, M. A., Dorey, N., Himmerkus, N., Holtmann, W. C., Dupont, S. T., Thorndyke, M. C., & Bleich, M. (2012). Acidified seawater impacts sea urchin larvae pH regulatory systems relevant for calcification. *PNAS, 109*(44), 18192-18197.

Thomsen, J., Casties, I., Pansch, C., Körtzinger, A., & Melzner, F. (2013). Food availability outweighs ocean acidification effects in juvenile Mytilus edulis: laboratory and field experiments. *Global Change Biology, 19*, 1017–1027.

Thor, & Dupont. (2015). Transgenerational effects alleviate severe fecundity loss during ocean acidification in a ubiquitous planktonic copepod. *Global Change Biology, 21*(6), 2261-2271.

Tyler-Walters, H. (2008). *Edible sea urchin (Echinus esculentus)*. Retrieved March 25, 2021, from https://www.marlin.ac.uk/species/detail/1311

Vargas, C. A; Lagos, N. A., Lardies, M. A., Duarte, C., Manríquez, P. H., Aguilera, V. M., Broitman, B., Widdicombe, S., & Dupont, S. (2017). Species-specific responses to ocean acidification should account for local adaptation and adaptive plasticity. *Nature Ecology & Evolution, 1*(4), 84 -84.

Ventura, A., Schulz, S., & Dupont, S. (2016). Maintained larval growth in mussel larvae exposed to acidified undersaturated seawater. *Scientific Reports, 6*, 1-9.

World Conservation Monitoring Centre. (1996). *Echinus esculentus.* The IUCN Red List of Threatened Species 1996. Retrieved 03 25, 2021, from https://www.iucnredlist.org/species/7011/12821364

Appendix

Tables

Levene's test					
MR	GR	SV_m	Max POL	Max Al	Asym POL_m
P	P	P	P	P	P
0,082	0,080	0,140	0,433	0,358	0,858

Table 11. Results from performed Levene's test on mortality rate, growth rate, mean values for stomach volume, postoral arms maximum, anterolateral arms maximum and mean values for postoral arm asymmetry.

MR				
Aq	R^2	*F*	*P*	*Equation*
1	0,779	38,684	0*	y = -0,0792x + 1,2428
2	0,672	8,212	0,046*	y = -0,1666x + 1,3141
3	0,027	0,307	0,59	y = 0,0029x + 0,8931
4	0,826	23,709	0,005*	y = -0,1494x + 1,2649
5	0,663	17,729	0,002*	y = -0,0846x + 1,236
6	0,695	11,42	0,02*	y = -0,1272x + 1,1964
7	0,883	52,693	0*	y = -0,128x + 1,179
8	0,608	17,062	0,002*	y = -0,0343x + 1,0279
9	0,638	19,349	0,001*	y = -0,0436x + 0,7571
10	0,719	20,462	0,002*	y = -0,0973x + 1,2794
11	0,082	0,987	0,342	y = -0,0065x + 0,9139
12	0,548	13,314	0,004*	y = -0,0311x + 0,8796
13	0,788	40,809	0*	y = -0,0768x + 1,1348
14	0,254	3,737	0,079	y = -0,0203x + 0,9832
15	0,811	47,234	0*	y = -0,0833x + 1,2738
16	0,685	23,886	0*	y = -0,0336x + 1,0655
17	0,641	19,638	0,001*	y = -0,0278x + 0,9237
18	0,614	17,53	0,002*	y = -0,0634x + 1,1141
19	0,517	11,777	0,006*	y = -0,0369x + 1,0672
20	0,444	8,796	0,013*	y = -0,0216x + 0,8939
21	0,201	2,766	0,124	y = -0,014x + 0,8902
22	0,668	22,146	0,001*	y = -0,0214x + 0,969
23	0,851	63,055	0*	y = -0,0293x + 0,9795
24	0,76	25,33	0,001*	y = -0,1221x + 1,358

*Table 12. Results from linear regressions carried out on mortality rate data. Data shown with * indicates a significant result.*

GR				
Aq	R^2	*F*	*P*	*Equation*
1	0,487	115,62	0*	y = 38,713ln(x) + 123,13
2	0,633	100,14	0*	y = 51,147ln(x) + 109,4
3	0,790	481,17	0*	y = 60,624ln(x) + 117,26
4	0,493	56,42	0*	y = 39,604ln(x) + 106,01
5	0,646	178,98	0*	y = 42,385ln(x) + 108,46
6	0,670	138,16	0*	y = 54,985ln(x) + 117,59
7	0,545	79,02	0*	y = 48,471ln(x) + 102,78
8	0,856	823,45	0*	y = 79,345ln(x) + 99,975
9	0,691	285,62	0*	y = 56,376ln(x) + 92,284
10	0,838	454,94	0*	y = 68,506ln(x) + 107,25
11	0,481	128,02	0*	y = 47,207ln(x) + 125,58
12	0,791	462,48	0*	y = 54,723ln(x) + 104,58
13	0,554	154,34	0*	y = 36,918ln(x) + 125,31
14	0,573	184,82	0*	y = 44,432ln(x) + 137,97
15	0,864	708,19	0*	y = 69,709ln(x) + 110,68
16	0,743	399,65	0*	y = 42,556ln(x) + 117,42
17	0,607	197,74	0*	y = 47,362ln(x) + 96,824
18	0,329	56,36	0*	y = 34,57ln(x) + 137,81
19	0,284	54,73	0*	y = 31,691ln(x) + 132,6
20	0,638	243,65	0*	y = 53,987ln(x) + 90,037
21	0,757	430,32	0*	y = 74,494ln(x) + 81,551
22	0,572	182,13	0*	y = 49,494ln(x) + 113,53
23	0,664	272,32	0*	y = 58,001ln(x) + 104,07
24	0,573	111,58	0*	y = 54,109ln(x) + 93,315

*Table 13. Results from linear regressions performed on growth rate data. Data marked with * indicates a significant result.*

Max POL				
Aq	R^2	*F*	*P*	*Equation*
1	0,137	14,115	0*	y = 0,5548x + 19,838
2	0,731	59,915	0*	y = 1,586x - 193,75
3	0,09	9,691	0,002*	y = 0,6016x + 92,489
4	0,331	11,405	0,003*	y = 0,7849x - 41,976
5	0,143	9,151	0,004*	y = 0,5208x + 7,6252
6	0,073	2,893	0,097	y = 0,6325x + 32,897
7	0,333	13,949	0,001*	y = 0,6101x - 2,186
8	0,345	56,268	0*	y = 0,9836x - 23,223
9	0,805	383,594	0*	y = 1,857x - 221,2
10	0,317	26,935	0*	y = 1,4754x - 135,21
11	0,047	4,302	0,041*	y = 0,5679x + 45,329
12	0,062	5,831	0,018*	y = 0,4899x + 51,919
13	0,296	37,927	0*	y = 1,1725x - 96,523
14	0,209	24,251	0*	y = 1,0266x - 66,1000
15	0,583	113,455	0*	y = 1,8698x - 226,4
16	0,286	40,538	0*	y = 0,839x - 60,054
17	0,594	137,573	0*	y = 0,9765x - 53,356
18	0,542	83,905	0*	y = 1,1221x - 70,075
19	0,452	68,322	0*	y = 0,7772x - 30,464
20	0,407	63,211	0*	y = 0,6386x - 14,847
21	0,649	197,562	0*	y = 1,2469x - 80,815
22	0,395	69,266	0*	y = 1,1569x - 59,481
23	0,718	274,711	0*	y = 1,5433x - 110,96
24	0,823	237,741	0*	y = 0,8493x - 41,275

*Table 14. Results from linear regressions carried out on the maximum postoral arm data. Data shown with * indicates a significant result.*

Max AL				
Aq	R^2	*F*	*P*	*Equation*
1	0,409	61,01	0*	y = 0,9007x - 32,323
2	0,87	153,272	0*	y = 1,859x - 222,5
3	0,434	75,045	0*	y = 1,1572x - 49,476
4	0,219	3,639	0,079	y = 0,4886x + 19,606
5	0,433	39,642	0*	y = 0,7929x - 22,174
6	0,208	9,962	0,003*	y = 0,57x + 41,567
7	0,616	44,961	0*	y = 0,8938x - 42,74
8	0,653	199,444	0*	y = 1,6323x - 163,23
9	0,872	604,094	0*	y = 1,5645x - 155,56
10	0,592	82,683	0*	y = 1,8393x - 227,61
11	0,223	25,557	0*	y = 0,9457x - 19,379
12	0,471	80,019	0*	y = 1,176x - 80,528
13	0,485	82,787	0*	y = 1,307x - 111,68
14	0,302	40,147	0*	y = 1,1663x - 70,245
15	0,712	197,804	0*	y = 2,1707x - 294,7
16	0,552	108,627	0*	y = 1,0122x - 64,669
17	0,772	317,525	0*	y = 1,0171x - 54,776
18	0,746	196,677	0*	y = 1,2616x - 81,329
19	0,629	139,226	0*	y = 0,9803x - 44,184
20	0,749	269,132	0*	y = 0,8685x - 27,65
21	0,776	367,922	0*	y = 1,4079x - 104,1
22	0,637	184,391	0*	y = 1,2769x - 73,708
23	0,851	616,007	0*	y = 1,4388x - 94,836
24	0,901	453,045	0*	y = 0,9259x - 39,179

*Table 15. Results from linear regressions performed on anterolateral arms maximum data. Data marked with * indicates a significant result.*

Mean values				
Aq	pH	Food	Stomach Volume$_m$	Asym POL$_m$
11	**High**	0	211846,24	0,87
18	**High**	0	176669,14	0,89
22	**High**	0	168980,95	0,89
3	**High**	100	217496,89	0,87
19	**High**	100	177347,89	0,88
23	**High**	100	159892,22	0,91
2	**High**	1000	110421,50	0,90
6	**High**	1000	184168,04	0,90
14	**High**	1000	256956,15	0,87
8	**High**	10000	411856,79	0,87
10	**High**	10000	220904,58	0,92
15	**High**	10000	315076,49	0,90
4	**Low**	0	92519,22	0,87
7	**Low**	0	151284,91	0,84
13	**Low**	0	144373,82	0,88
12	**Low**	100	149077,80	0,86
17	**Low**	100	142208,15	0,87
20	**Low**	100	141273,52	0,86
1	**Low**	1000	180401,15	0,89
9	**Low**	1000	174321,12	0,90
16	**Low**	1000	151350,38	0,86
5	**Low**	10000	194509,65	0,86
24	**Low**	10000	169925,59	0,90
21	**Low**	10000	290228,09	0,87

Table 16. Mean values for stomach volume and postoral arm asymmetry calculated for each of the aquariums and organised in a table and then used to perform a two-way ANOVA for the two different parameters.