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Impacts of ocean acidification on the hatching and early larval development of *Calanus pacificus, Calanus marshallae* and *Euphausia pacifica*

Melissa Prechtl, Oregon State University
Mentor, Bill Peterson with help from Leah Feinberg
HMSC REU program
prechtlm@onid.orst.edu

Abstract

The issue of ocean acidification is a growing concern that is a direct result of rising atmospheric CO_2 concentrations. The consequences of ocean acidification include a decrease in the ability of marine organisms to produce shells and skeletons composed of calcium carbonate and it may also disrupt physiological processes. Despite their importance to oceanic food webs, little research has been done on the impact of ocean acidification on zooplankton. With an expected drop in the pH of the world's oceans, it is important to explore the effects that ocean acidification will have on the foundation of the food web. We investigated the effects of ocean acidification on hatching and development of two copepod species, *Calanus pacificus* and *Calanus marshallae*, and one species of euphausiid, *Euphausia pacifica*. Euphausiids and copepods were collected at night at stations located 5, 10, 15, 20 and 25 nautical miles off the coast of Newport, Oregon, using the research vessel *Elahka*. Eggs were collected the following morning and placed into beakers with mesh bottoms to allow sea water to enter the beaker. The beakers were put into tanks of varying pH and samples were preserved after 3 and 8 days (*Calanus pacificus* was preserved after 5 and 8 days.).We found evidence that decreasing pH slows development for all three species under investigation. There were no significant differences in hatching success between the pH treatments, but a larger proportion of larval deformity was observed for all species in the lower pH treatments. However, the effects of pH on development are slight and more data is needed in order to determine the significance of these results. Further investigation of how decreasing pH affects internal development is needed in order to fully study the consequences of ocean acidification.

Introduction

Anthropogenic CO_2 and its affect on global climate has become a significant concern. The planet's CO_2 levels have fluctuated from 180 parts per million to 280 ppm for 50 million years (Kurihara et al., 2004). However, post industrial revolution atmospheric CO_2 levels have sky-rocketed. According to the National Oceanic and Atmospheric Administration, the average level of CO_2 in the atmosphere for May 2010 was 392.94 ppm. Around the world, recent changes in climate have altered many ecosystems (Parmesan and Yohe, 2003). Studying the effects of these changes is crucial in determining the extent of damage caused by an increase in the level of CO_2 in the atmosphere. The issue of ocean acidification is a growing concern that is a direct result of rising atmospheric CO_2 concentrations. It is estimated that as much as 50% of CO_2 released from human activities is absorbed by the ocean (Calvo et al., 2010). This absorbed CO_2 from the atmosphere is converted to carbonic acid (Dubi, 2007). The increase of carbonic acid causes a decrease in the concentration of carbonate ions available to marine organisms and lowers the pH of the ocean, causing ocean

acidification (Calvo et al., 2010). The decrease in concentration of carbonate ions decreases the ability of marine organisms to produce shells and skeletons composed of calcium carbonate. Ocean acidification may also disrupt physiological processes that are sensitive to pH such as reproduction and gas exchange (Calvo et al., 2010). This could have dire consequences for many biological species, including plankton which make up the base of oceanic food webs.

Previous studies analyzing the effects of CO_2 induced acidification have shown that CO_2-acidified seawater dramatically reduces hatching success in the copepod *Calanus finmarchicus* (Cook et al., 2007). Another study, involving the copepod species *Acartia stueuri* and *Acartia erythraea,* yielded similar results with regards to hatching success in CO_2 *acidified* water (Kurihara et al. 2004). This indicates that CO_2 acidified seawater may also have detrimental effects on the hatching of other planktonic species such as the krill species *Euphausia pacifica.* I studied the impact of decreased pH on the hatching and early development of *Calanus pacificus, C. marshallae* and *E. pacifica.*

E. pacifica is an important species of krill in the north Pacific that serves as a substantial food source for birds, whales, and many fish species such as Chinook and Coho salmon. Monitoring the health of *E. pacifica* populations is important for understanding marine food webs. Off the coast of Oregon, spawning of *E. pacifica* occurs between March and October (Feinberg and Peterson 2003). Peak spawning occurs between June and August. Any decrease in recruitment of *E. pacifica* or *Calanus spp.* caused by ocean acidification would have an impact on local fisheries such as salmon, hake, and halibut.

Materials and Methods

Euphausiids and copepods were collected at night at stations located 5, 10, 15, 20 and 25 nautical miles off the coast of Newport, Oregon, using the research vessel *Elahka.* Obliquely towed, 60cm diameter, 333 μm-mesh bongo nets with solid cod ends were used for the collection of zooplankton. The zooplankton captured were kept cool and in the dark while being transported to a cold room set at 10.5°C. Gravid *Euphausia pacifica* were sorted and placed individually into 1 L jars of filtered seawater. I checked for the release of eggs the following morning. Copepods were held overnight in 50ml jars of filtered seawater and eggs were collected for my experiment the next morning. The size of each experiment varied, depending on the amount of eggs collected. Eggs were picked with aid of a dissecting microscope using a pipette and placed into 250 ml beakers. The beakers had mesh bottoms (200 μm for euphausiids, 120μm for copepods.) to allow sea water to enter the beaker. Each beaker contained at least 30 eggs. The beakers were placed into pH controlled tanks with target pH levels of approximately 8.0, 7.8, 7.6, and 7.1. The temperature of each tank was ~8°C. The control group was placed in the ambient seawater tank with a pH level of ~8.0. All experiments were run for a maximum of eight days. This time period was chosen because the first three larval stages of copepods and euphausiids do not feed; rather they depend on lipid-reserves for their early development. Thus we did not add need to add any phytoplankton food to our experiments which greatly simplified our experimental procedures.

The pH tanks are managed using real-time pH metering and automated control of CO_2 injection into a water conditioning reservoir. CO_2 is introduced by passing seawater

through a gas-fluid membrane exchanger. The pH of the reservoir is maintained at 7.05. This acidified water is pumped into a series of header tanks where it is mixed with ambient seawater in different proportions to achieve the 4 different pH treatments. Temperature, pH, and dissolved oxygen are continuously monitored for each treatment. Water samples are drawn from each pH treatment twice per week and analyzed for dissolved inorganic carbon and total alkalinity for validation of the pH measurements (Tom Hurst personal communication).

Euphausiid larvae were observed through the Calyptopis 1 (C1) stage. The median developmental time for larvae to reach this stage in 8°C water is 8 days (Ross, 1981). The median duration of time spent in the egg stage for *E. pacifica* is 39 hours at 10°C (Feinberg et al., 2006) and should be slightly slower at 8°C. Once hatched, the median developmental time is less than 1 day for larvae to go through nauplius 1 (N1) and 2 (N2) stages in 8°C water (Ross, 1981). Larvae develop into the metanauplius (MN) stage from the N2 stage. The median developmental time for larvae to reach the MN stage is 2 days in 8°C water (Ross, 1981).

Table 1: Duration of Development for *E. pacifica* at 10°C (Feinberg et al., 2006)

Stage	Median time in days
Egg	1.6
N1	0.3
N2	1.1
Meta	3.0
C1	6.4

Calanus larvae were observed through the nauplius 3 (N3) stage. Both species share similar developmental rates. In 10°C water, the median developmental time for *C. marshallae* to reach the N3 stage is ~ 6.8 days (Peterson, 1986). The median duration of time spent in the egg stage for *C. marshallae* is 1.6 days in 10°C (Peterson, 1986). The median developmental time for *C. marshallae* larvae in the N1 stage is ~1 day in 10°C water (Peterson, 1986). Time spent in the N2 stage is also ~1 day 10°C water (Peterson, 1986). The experiments took place in 8°C water, which resulting in slightly slower development when compared to experiments performed in 10°C water.

Table 2: Duration of Development for *C. marshallae* species (Peterson, 1986)

Stage	Duration in days
Egg	1.6
N1	0.9
N2	0.9
N3	6.8

For all species in these experiments, eggs were expected to hatch by day 3 at 8°C, thus on day 3, half of the beakers from each treatment were removed. By this time all both copepods and euphausiids should be in the second naupliar (N2) stage. Eggs and nauplii were rinsed from the beakers into a petri dish using filtered sea water. Eggs and nauplii were counted with aid of a dissecting microscope and placed into a vial using a pipette. Samples were preserved in a 5% formalin solution in a fume hood. Copepod species were also stained pink with rose bengal to make the nauplii more visible. The remaining beakers were removed from the tanks on day 8. By this time, Euphausiids should have been in either the metanauplius or calyptopsis 1 stage. Copepod species should have been in the nauplius 3 stage. Samples were then preserved in a 5% formalin solution and rose bengal was added to copepod samples. When samples were analyzed, vials were emptied under a fume hood into a 120μm mesh filter and rinsed with sea water into a petri dish. I analyzed the hatching and developmental success of the samples by counting un-hatched eggs and recording the developmental stage of each individual nauplii.

Many of my experiments had multiple beakers (replicates) in tanks of the same pH. In order to combine the results from these replicate beakers, two-sample t-tests were performed to ensure there were no significant differences in development between the replicates. Since none of the beakers that were in tanks of the same pH had significant p-values (p-value > 0.05), data collected from these beakers were combine for analysis (Table 3).

Table 3: p-values of 2-sample T-tests on replicate beakers

C. pacificus Day 3	
pH	P-value
8.148	0.70
7.874	0.39
7.663	0.61
7.241	0.77

C.pacificus Day 8	
pH	P- value
8.148	0.36
7.874	0.37
7.663	0.89
7.241	0.80

C. marshallae Day 3	
pH	P-value
8.013	0.349

C. marshallae Day 8	
pH	P-value
8.013	0.349

C. marshallae Day 3	
pH	P-value
7.976	0.34
7.800	0.34

C. marshallae Day 8	
pH	P-value
7.976	0.17
7.800	1.0

C. marshallae Day 3

C. marshallae Day 8

pH	p-value
7.820	0.34
7.747	0.34
7.406	0.35
7.215	0.34

pH	P-value
7.820	0.17
7.747	0.17
7.406	0.10
7.215	0.16

E. pacifica Day 3

pH	P-value
7.827	0.23
7.738	0.18
7.409	0.26
7.199	0.35

E. pacifica Day 8

pH	P-value
7.827	0.21
7.738	0.28
7.409	0.85
7.199	0.47

Results

There was no significant difference in hatching success between the different pH groups for *Calanus pacificus, C.marshallae,* or *E. pacifica* (Table 4).

Table 4: Hatching Success in Percent

pH range	*C. pacificus*	*C. marshallae*[1]	*E. pacifica*
8.2-8.0	97.8	92.2	92.1
7.9-7.7	98.8	97.4	92.5
7.6-7.4	78.3	84.3	92.6
7.3-7.1	92.3	96.1	91.7

[1] Data from one experiment (16 July) was excluded from this table since on that date, a large proportion of eggs in each treatment did not hatch (see discussion).

One of my experiments involved the copepod species *Calanus pacificus.* This experiment took place in May. The average percent of *C. pacificus* nauplii in the N2 stage for the day 5 experiment increased slightly as pH decreased (Table 5). This trend was also seen in the day 8 experiment (Table 6). Deformities were seen in nauplii from the day 5 experiment in the two lowest pH levels (Table 5).

Table 5: *Calanus pacificus* developmental stage analysis at day 5 for May experiment

pH	Average % n1	Average % n2	Average % n3	Average % deformed nauplii
8.148	0	13.0	92.5	0
7.874	0	0	100	0
7.663	0	21.1	87.9	10.5
7.241	3.8	31.9	66.0	19.2

***Table 6: Calanus pacificus* developmental stage analysis at day 8 for May experiment**

pH	Average % n1	Average %n2	Average % n3	Average % deformed
8.148	0	0	100%	0
7.874	0	4.9%	95.1%	0
7.663	0	6.7%	93.3%	0
7.241	0	9.1%	90.9%	0

Four of my experiments involved the copepod species *Calanus marshallae.* The first experiment started on June 28th. There was a slight decrease in the percent of *C. marshallae* in the N2 stage for the day 3 experiment with a drop in pH level (Table 7). There was also a decrease in the percent of nauplii in the N3 stage in the lowest pH treatment for the experiment pulled on day 8 (Table 8).

***Table 7: Calanus marshallae* developmental stage analysis at day 3 for June experiment**

pH	Average % n1	Average % n2	Average % n3	Average % deformed nauplii
8.013	2.3	95.3	2.3	0
7.652	22.2	77.8	0	0
7.304	25	75	0	0

***Table 8: Calanus marshallae* developmental stage analysis at day 8 for June experiment**

pH	Average % n1	Average % n2	Average % n3	Average % deformed nauplii
8.013	0	0	100	0
7.652	0	0	100	0
7.304	20	0	80	0

The following *C. marshallae* experiment started on July 9th. The animals pulled on both day 3 and day 8 showed no significant differences in development between the pH treatments (Tables 9 and 10).

Table 9: *Calanus marshallae* developmental stage analysis at day 3 for July 9th experiment

pH	Average % n1	Average % n2	Average % n3	Average % deformed nauplii
7.976	6.9	93.1	0	0
7.800	0	100	0	0
7.504	7.9	92.1	0	5.3
7.248	1.7	98.3	0	3.3

***Table 10: Calanus marshallae* developmental stage analysis at day 8 for July 9th experiment**

pH	Average % n1	Average % n2	Average % n3	Average % deformed nauplii
7.976	0	3.4	96.6	0
7.800	0	0	100	0
7.248	0	0	100	0

The July 16th *C. marshallae* experiment did not show significant differences in development; however as noted above, there was unusually low hatching success and a high percentage of deformities were seen in all pH treatments (Tables 11 and 12).

***Table 11: Calanus marshallae* developmental stage analysis at day 3 for July 16th experiment**

pH	Average % n1	Average %n2	Average % n3	Average % deformed nauplii
7.820	31.6	68.4	0	1.4
7.747	50.0	50.0	0	1.0
7.406	35.7	64.3	0	4.3
7.215	26.3	73.7	0	2.9

Table 12: Calanus marshallae developmental stage analysis at day 8 for July 16th experiment

pH	Average % n1	Average % n2	Average %n3	Average % deformed nauplii
7.820	0	21.7	78.3	0
7.747	5.3	26.3	68.4	42.9
7.215	9.5	14.3	76.2	4.8

The final *C. marshallae* experiment took place on July 21st. The percentage of nauplii in the N2 stage of development dropped significantly in the lowest pH group (Table 13). However, animals pulled day 8 showed no significant differences in development (Table 14).

***Table 13: Calanus marshallae* developmental stage analysis at day 3 for July 21st experiment**

pH	Average % n1	Average % n2	Average %n3	Average % deformed nauplii
7.827	0	98.6	1.4	0
7.738	0	97.4	2.6	1.3
7.409	1.4	97.1	1.4	0
7.199	29.0	71.0	0	0

***Table 14: Calanus marshallae* developmental stage analysis at day 8 for July 21st experiment**

pH	Average % n1	Average % n2	Average % n3	Average % deformed nauplii
7.827	0	0	100	0
7.738	0	0	100	0
7.409	0	1.8	98.2	3.6
7.199	0	3.0	97.0	0

Euphausia pacifica was the study specimen for two of my experiments. The first experiment took place in June. Developmental rates were slowed in the lowest pH treatment for *E. pacifica* that were pulled from the experiment on day 3 (Table 15). The proportion of nauplii in the C1 stage of development pulled on day 8 decreased with pH, with none of the nauplii reaching this stage in the lowest pH treatment (Table 16).

Table 15: *Euphausia pacifica* developmental analysis day 3 June

pH	Average %n1	Average % n2	Average % metanauplii	Average % C1	Average % deformed nauplii
8.013	0	100	0	0	3.2
7.652	0	100	0	0	3.3
7.304	10.5	89.5	0	0	5.3

Table 16: *Euphausia pacifica* developmental analysis day 8 June

pH	Average %n1	Average % n2	Average % metanauplii	Average % C1	Average % deformed nauplii
8.013	0	0	76.3	23.7	0
7.652	0	0	82.6	17.4	0
7.304	5.6	0	94.4	0	0

The second experiment took place in July. There were no significant differences in development in nauplii between pH treatments for nauplii pulled on day 3 (Table 17). However, none of the nauplii in the lowest pH treatment pulled on day 8 developed through to the C1 stage (Table 18).

Table 17*: Euphausia pacifica* developmental stage analysis at day 3 for July experiment

pH	Average % n1	Average % n2	Average % metanauplius	Average % C 1	Average % deformed nauplii
7.827	0	100	0	0	3.8
7.738	0	100	0	0	0
7.409	0	100	0	0	0
7.199	0	100	0	0	5.6

Table 18: *Euphausia pacifica* developmental stage analysis at day 8 for July experiment

pH	Average % n1	Average %n2	Average % metanauplius	Average % C1	Average % deformed nauplii
7.827	2.5	0	75.0	22.5	5.0
7.738	0	0	77.4	22.6	0
7.409	0	0	67.4	32.6	11.6
7.199	0	0	100	0	3.5

Discussion

We hypothesized that hatching success would decrease with a decrease in pH. Despite seeing a decrease in hatching of the two copepod species in the second lowest pH group, percentage differences seen in all species between the varying pH levels are insignificant. A previous study involving the copepod species *Calanus finmarchicus*, showed a decrease in hatching success in acidified seawater (Cook, 2007). However, this experiment took place in seawater with a pH of 6.95. This extremely acidified seawater was chosen to investigate the impact of marine CO_2 storage/disposal. Low return rates of nauplii were a reoccurring issue in my experiments. All percentages were calculated based on the number of nauplii recovered and not the number of eggs originally placed into the beakers.

Our second hypothesis was that acidified seawater would slow developmental rates. Development of the copepod species *Calanus pacificus* was slowed as pH decreased. The experiments were confounded by low return rates of nauplii and lack of replicates. Nonetheless, *Calanus pacificus* is a species of copepod associated with El Niño events (Peterson and Keister, 2003). Warm currents off the coast of the Pacific Northwest are characteristic of El Niño events (Hamlet et al., 2010). Warm water holds less CO_2 (Fabry and Guinotte, 2008) and therefore, may be more resilient to changes in pH. This would leave warm water species of copepods less adapted to variability in pH and explain the sensitivity of *C. pacificus* to decreased pH seen in my experiments. Further research is needed in order to investigate this hypothesis.

There were four experiments involving the copepod species *Calanus marshallae,* although only three were used for analysis of effects of pH on developmental rates of nauplii (recall that results from the July 16th experiment were removed because of low hatching success in all pH treatments). The low egg hatching rates from the 16 July experiment may have been to due to the presence of a toxic diatom bloom. Previous studies have revealed that hatching and condition of nauplii of calanoid copepods can be adversely impacted by certain diatom blooms (Paffenföfer, 2002). This could have been a contributing factor to the low hatching success and high rates of deformities seen in nauplii of this experiment. Two out of the three *C. marshallae* experiments, pulled on day three, showed an increase in the percent of nauplii in the N2 stage of development in the lower pH treatments. This suggests that pH slowed development. However, there were no significant differences in development of these particular experiments in animals pulled on day eight. This suggests that although pH may slow development initially, animals are able to catch up by day 8. The July 9th experiment did not show differences in development of *C. marshallae* nauplii between the varying pH levels. *C. marshallae* are a cold water species of copepods. Living in a cold water environment may result in *C.*

marshallae being better adapted to lower pH treatments. For all four experiments, low return rates gave results that are statistically insignificant.

Two experiments investigated the impact of ocean acidification on the euphausiid species, *Euphasia pacifica*. By day three, nauplii should be in the N2 stage of development. Only in one of the experiments did the percent of N2 nauplii decrease with pH, making the results of my experiments inconclusive. By day 8, nauplii should be in either the metanauplius or calyptopis 1 stage of development. In the lowest pH treatment, nauplii did not develop past the metanauplius stage. This suggests that pH may have a slight impact on development of *E. pacifica*. More research is needed in order to make a comprehensive assessment of the effects of ocean acidification on *E. pacifica*.

Another aspect of my research involved assessing whether or not a decrease in pH would cause an increase in deformity. For all three species, deformities were consistently seen in the lower pH treatments. Deformities included nauplii that had developed entirely or partially in the egg without hatching and fused appendages of hatched nauplii. However, other factors besides pH could have contributed to observed deformities. These factors include diet and fertility of gravid females and exposure to certain diatom species (Paffenföfer, 2002). Each of these research topics are far beyond the scope of a summertime project. Longer term studies are needed in order to understand the impacts of ocean acidification on *C. pacificus, C. marshallae, E. pacifica* and other plankton species. This is important in determining the chronic effects of ocean acidification on all stages, including the feeding stages of development. In order to perform an experiment of this kind, a method for feeding nauplii in the tanks must be developed. Current methods need to be refined in order to improve return rate of larvae in the experiments. Improvements include additional floats in tanks to ensure beakers are always above the surface of water in treatments, increase in sample size and replicates, and overall enhanced care and attention. Despite seeing subtle changes in development between the different pH treatments, low sample sizes make results inconclusive. However, there is evidence suggesting that pH has an effect. Further research will determine the impact of this effect. It is important to oceanic food webs that the health of plankton populations continues to be monitored. Future research will push for enhancement of climate change legislation, increase public knowledge in the field of marine science, and help to improve the overall condition of our planet.

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