

Effect of *Acartia* grazing on the size distribution of phytoplankton in Academy Bay, Galapagos

Jonathan Herzog

University of Washington

School of Oceanography

## NON-TECHNICAL SUMMARY

The goal of this experiment was to determine if *Acartia*, a species of zooplankton, fed based on the size of food available. *Acartia* which more specifically are a type of copepod, have demonstrated this in other experiments. This experiment consisted of gathering seawater from Academy Bay in the Galapagos Islands, filtering it to remove zooplankton, then incubating it for four set time periods. Each time period included three bottles with added *Acartia*, while three others served as the control. Once the incubations were finished, each bottle of water was filtered with three different size filters; chlorophyll and phaeopigment retained on the filters was then measured. These measurements were done because they informed me how much chlorophyll based life existed for each size range at each time interval and additionally how much had been consumed. Chlorophyll is a green pigment found in the part of plants that conducts photosynthesis; while phaeopigment is chlorophyll degraded after it is consumed. The data attained from this experiment were not conclusive to *Acartia* preying on specific size fractions of phytoplankton; rather they likely preyed upon smaller zooplankton. There has been prior research done indicating that this is a possibility.

## ACKNOWLEDGMENTS

I would like to thank:

The School of Oceanography at the University of Washington for having the creativity and patience to organize this trip to the Galapagos

The teaching team of Gabrielle Rocap, Llyd Wells, Seelye Martin, Mark Holmes, and Roy Carpenter for putting in the long hours, endless editing, and constant question answering to make this experience successful

Michelle Townsend for believing in me 100% as I worked myself through the admission process

Jennifer Nomura for sharing in my excitement, uneasiness, tension, confusion, and most importantly my laughter throughout this process

My family and friends who have listen to the stories, read the drafts, dealt with my absence and still wanted me around.

## ABSTRACT

Studies have shown that at many locations worldwide the copepod *Acartia* graze in a size selected manner. I attempted to determine if *Acartia*'s grazing rates and prey preference indeed vary with the size of the prey available in Academy Bay. To accomplish my research I collected water samples and *Acartia* from the *R/V Thomas G. Thompson* in Academy Bay, located off of the southern end of Santa Cruz in Ecuador's Galapagos Islands. These samples were filtered to remove zooplankton  $>330\ \mu\text{m}$ , then incubated for 12, 24, and 36 hr with ten *Acartia* added to each sample. Following each incubation period, chlorophyll size-fractionated with filters of  $10\ \mu\text{m}$ ,  $2\ \mu\text{m}$ , and  $0.7\ \mu\text{m}$ , was measured in *Acartia* treatments along with a sample incubated without zooplankton  $>330\ \mu\text{m}$  to determine whether *Acartia* affected the size distribution of phytoplankton. The data attained did not illustrate that *Acartia* affected the size distribution, or that they preferred a particular size of prey. Rather it opened up the possibility that *Acartia* in Academy Bay feed primarily on microzooplankton as opposed to phytoplankton.

## INTRODUCTION

Copepods are a type of crustacean found in every ocean of the world. A common member of this order is *Acartia* which can be found from the polar seas to tropical bays (Lee 1996; Figueroa and Hoeffel unpubl). Like the majority of zooplankton, *Acartia* feed primarily on phytoplankton. Most copepods tend to feed on larger sized phytoplankton when given a chance (Frost 1972). Copepods in the equatorial Pacific grazed primarily on diatoms (Roman and Gauzens 1997) as did the copepods collected during the winter-spring bloom in Narragansett Bay, Rhode Island (Durbin and Durbin 1992). The feeding behavior of *Acartia* may also depend on the size, type, and concentration of prey. More specifically, experiments on size-selective grazing of *Acartia* in various areas found that they retained larger (~25  $\mu\text{m}$ ) algal cells more efficiently than smaller (~5  $\mu\text{m}$ ) cells when presented with a variety of sizes (Støttrup and Jensen 1990).

The eastern equatorial Pacific is considered a High Nitrate Low Chlorophyll (HNLC) region because, though nutrients, especially nitrogen are plentiful, the chlorophyll levels are relatively low. This is believed to be because there is a lack of iron in the system, which commonly is the limiting nutrient for the growth of large phytoplankton cells (Martin et al. 1994); the smaller phytoplankton may be controlled by microzooplankton grazing (Banse 1995). Many iron enrichment experiments such as IronEx I and II have been done in the general region surrounding the Galapagos Islands, in the eastern equatorial Pacific. These experiments have suggested that iron-enriched environments support larger phytoplankton which, in turn, may eventually favor large zooplankton. For example, Rollwagen and Landry (2000; Fig. 2) showed that mesozooplankton (200-2000  $\mu\text{m}$ ) biomass increased substantially inside an iron-enriched

patch of ocean versus those outside the patch. Similarly, an iron enrichment experiment conducted in the Southern Ocean showed the largest increase in plankton-incorporated Fe was in the standing stock of plankton  $>5 \mu\text{m}$  from 1.9 pM to 12.9 pM in eukaryotic phytoplankton and 8.2 pM to 39.6 pM in autotrophic flagellates (Bowie et al. 2001; Fig. 3). These two experiments show that iron-enriched systems may support larger phytoplankton, which in turn may favor larger zooplankton.

Though the surrounding equatorial Pacific is a HNLC region, around the Galapagos, chlorophyll concentrations are high, presumably due to iron derived from the platform (Martin et al. 1994). Conditions may therefore be similar to those found in the iron-enrichments experiments mentioned previously. Moreover, the population breakdown of zooplankton in Academy Bay is over 80% *Acartia* (Figueroa and Hoeffel (unpubl.), a copepod known to prefer large ( $\sim 25 \mu\text{m}$ ) phytoplankton in other environments (Støttrup and Jensen 1990). Similarly, high densities of *Acartia* in Galapagos Bays were found to be as high as 19 individuals  $\text{l}^{-1}$  or 75% of the zooplankton population (Arcos 1981). Their numerical dominance makes *Acartia* a regionally pertinent model system with which to study size-selective feeding of zooplankton and its effect on phytoplankton size distribution.

Based on the findings of Støttrup and Jensen (1990), I expected that the *Acartia* collected in Academy Bay would size-selectively graze on phytoplankton  $>10 \mu\text{m}$  until those became limited, after which they would turn to smaller phytoplankton  $>2 \mu\text{m}$ . If I had been correct and the *Acartia* behaved as expected it would have been evidence that *Acartia* in tropical waters behave similarly to their colder water brethren. It may also have suggested that they thrive in Academy Bay in part because of the availability of large phytoplankton (the prevalence of which I measured); these in turn could be supported by iron otherwise less available off the platform.

## MATERIALS AND METHODS

To understand the effect of *Acartia* on chlorophyll size distribution in Santa Cruz's Academy Bay, incubations were conducted and size-fractionated chlorophyll determined during an oceanographic cruise January 12-20, 2006 around Ecuador's Galapagos Islands. The cruise began in Academy Bay which is located on the Southern end of Santa Cruz Island at 0° 45.70' S and 90° 17.70' W (Fig. 1). The sample collecting for this experiment was performed on the *R/V Thomas G. Thompson* while at anchor in Academy Bay. A 0.6-m-diameter net with 330  $\mu\text{m}$  mesh was deployed to collect the *Acartia* samples. To assure that enough *Acartia* were collected the net tow began at depth of  $\sim 19$  m, the bottom being  $\sim 21$  m, and was brought to the surface at  $5\text{ m min}^{-1}$ . Once shipboard, the zooplankton assemblage from the net tow was transferred to a larger container of seawater to reduce stress. *Acartia* were identified and picked using a dissecting microscope and turkey baster.

The CTD was used to collect water and measure the temperature. From the CTD, 25 L of water at 5 m depth were collected and immediately brought inside to avoid further photosynthesis. From the 25 l of seawater, 5 l was removed and fixed with formalin (final concentration 2%) for analysis of the copepod community. Of the 5 L, 100 ml were separated and examined under a dissecting microscope to count the number of *Acartia* as opposed to other zooplankton. This gave me an idea of the relative density of the population so I had something to compare to the population density presented by Figueroa and Hoeffel (unpubl.) All data and observations from the experiment were recorded in a spreadsheet and notebook.

The remaining 20 l of seawater was then strained with nitex of a 73  $\mu\text{m}$  mesh to remove any larger organisms, including large zooplankton. Once strained, the water was divided into 18,

1-L amber bottles in which the incubations occurred. To 9 of these bottles 10 adult *Acartia* were added. The bottles sat in the back of the lab to incubate at a temperature of ~21°C. This was as close a simulation as I could come temperature wise to what was measured in Academy Bay, which was 24°C. Every three hours these bottles were stirred and uncapped to prevent particles from settling and for air circulation. With the remaining strained water, three 1 L samples were sent through a 3-filter system to size fractionate the particles. The filters had mesh sizes of 10 µm, 2 µm, and 0.7 µm (GF/F) in that order. Once the samples were finished, they were sonicated and run through the fluorometer which was used to measure chlorophyll and phaeopigment of each size range.

The incubated samples were divided into 3 groups of 6 bottles each: one group was incubated for 12 hours, the second group for 24 hours, and the third group for 36 hours. Out of each group of 6 bottles 3 had *Acartia* present and 3 did not (Fig. 4). All of the incubated samples were then sent through the same 3-filter system (10 µm, 2 µm, GF/F) as above and ran through a fluorometer to test for chlorophyll and phaeopigment concentrations.

## RESULTS

Figueroa and Hoeffel (unpubl.) reported that the population breakdown of zooplankton in Academy Bay was over 80% *Acartia*. A net tow from depth to surface on January 12<sup>th</sup>, 2006 found that *Acartia* were 84% of the population of >330 µm zooplankton. The remaining 16% of the zooplankton were divided among at least seven other types of organisms: polychaete worms, arrow worms, shrimp larvae, oncaii, acrocalanaii, and euchaetas.

The phytoplankton data did not follow the trends expected based on the research conducted prior to this experiment. The size-fraction of 2-10  $\mu\text{m}$  showed a decrease in chlorophyll concentration over the time of the experiment of  $\sim 43\%$  for the bottles with *Acartia* (Fig. 5b), which was to be expected. However that decrease was not significantly different than the decrease for those bottles that were without *Acartia* which was  $\sim 48\%$  (Fig. 5b). The decrease for all of the bottles was based on an average attained from each triplicate. The actual amount that decrease over the time of the experiment for the bottles with *Acartia* was  $0.183 \mu\text{g l}^{-1}$  while it was  $0.261 \mu\text{g l}^{-1}$  for those without *Acartia* (Fig. 5b).

As far as the other size-fractions were concerned, the 10-73  $\mu\text{m}$  size showed almost no change of chlorophyll concentration over the 36 hours for any of the bottles whether they had *Acartia* or not (Fig. 5c). The chlorophyll concentration for any bottle for this filter size fell between  $0.091$  and  $0.013 \mu\text{g l}^{-1}$ . The chlorophyll concentrations for the  $0.7 \mu\text{m}$  filter with *Acartia* decreased in concentration almost identically to that of the 2-10  $\mu\text{m}$ . The average decrease of the triplicates from 12 hr to 36 hr was  $0.166 \mu\text{g l}^{-1}$  (Fig. 5a).

The phaeopigment data was similar to that of the chlorophyll in many ways. The size-fraction of 2-10  $\mu\text{m}$  showed a decrease in concentration over the time of the experiment for the bottles with *Acartia* which totaled  $0.145$ - $0.096 \mu\text{m l}^{-1}$  (Fig. 6b). That result was the opposite of what was expected, yet this too was not significantly different from the bottles without *Acartia* because the 36 hr was the only point where the standard error did not overlap. Again the 10-73  $\mu\text{m}$  filter had almost no change for any of the bottles over the length of the experiment and provided the most consistent data (Fig. 6c). The range of values for all of the bottles was between  $0.005$  and  $0.041 \mu\text{m l}^{-1}$ .

## DISCUSSION

The objective of this experiment was to gain a better understanding of copepod feeding behavior or more precisely the feeding behavior of *Acartia* in Academy Bay. Although the results were not what was expected they still provide information on the possible grazing habits of *Acartia* at this location. Since the chlorophyll data did not show a strong downward trend in concentration over time for bottles laden with *Acartia* there was something different occurring than *Acartia* grazing on phytoplankton. There are many reasons why the results came out as they did. The *Acartia* may have subsisted on something other than phytoplankton during the incubation, there may have been problems with the scientific procedure, or something completely unknown occurred.

There is evidence that *Acartia* will graze on microzooplankton if there is not enough algal food available. A study conducted in San Francisco Bay found that *Acartia* had a diverse diet including both heterotrophic and autotrophic prey (Rollwagen and Penry 2003). An experiment examining what *Acartia* would eat when provided with both diatoms and dinoflagellates found that 66 to 89% of their diet was heterotrophic dinoflagellates (Wiadnyana and Rassoulzadegan 1989). This behavior would have dynamic effects on this experiment.

If the *Acartia* were indeed preying on small zooplankton then having only a small decrease in chlorophyll could be reasoned. The *Acartia* would have fed on microzooplankton instead of the phytoplankton, thus leaving the phytoplankton population to decrease just slightly from grazing via the microzooplankton. This model however is not without problems. If the *Acartia* were feeding exclusively on microzooplankton then the data would likely show a sharper decrease in chlorophyll in the bottles without *Acartia* because the microzooplankton community

would not being depleted in those bottles. A more likely scenario is that *Acartia* grazed primarily on the microzooplankton but they also fed on some phytoplankton balancing out the chlorophyll resulting in similar data between bottles with and without *Acartia* (Fig. 5A, Fig. 5B, Fig. 7A).

Since phaeopigment is degraded chlorophyll and any of the above hypotheses are true, there should be some increase in phaeopigment even if it is slight. Instead there is a slight decrease for both the 0.7-2  $\mu\text{m}$ , and the 2-10  $\mu\text{m}$  (Fig. 6a, Fig. 6B). This could be due to the microzooplankton grazing on the phytoplankton and then being grazed upon themselves by the *Acartia*. Another reason for the decrease in phaeopigment could be that the microzooplankton grazing on the phytoplankton degraded the chlorophyll to a point where the fluorometer could not pick up the phaeopigment.

When designing an experiment there are countless variables that have to be considered and choices to be made. In retrospect, there are things that I would have done differently. There was insignificant decline in chlorophyll for bottles with *Acartia* over the length of the experiment compared to those without which could be attributed to the ideas suggested above or to scientific error. At the end of each 12 hr period when a new set of bottles was ready to be filtered there was zero checks to see if the *Acartia* were still alive. Precautions were taken to make sure they received oxygen by opening the bottles and letting gas exchange occur but never were they actually examined. If they had indeed died then the fact that the bottles with and without *Acartia* were so similar would be a logical outcome. Another choice that had to be made was how many *Acartia* should be deposited into each bottle. Ten was chosen because it was a reasonable number for 1 L bottles based on prior experiments (Figueroa, D., pers.comm., 2006). Since this experiment had never been conducted in Academy Bay before the grazing rate and/or

metabolism of these *Acartia* could have been much smaller and ten was not enough individuals to show significant changes. Additionally there could have been a mix of these two factors wherein ten healthy *Acartia* should have been just barely sufficient to see clear results but even small mortality rates resulted in undetectable changes.

## CONCLUSIONS

The data I collected was not very conclusive. The possible trends that could be extrapolated all have their faults. If the *Acartia* were still in good health at the end of the experiment then it would be reasonable to assume that the *Acartia* in Academy Bay do not feed primarily on phytoplankton. If the mortality rate of *Acartia* was high then the data has far less implications.

## REFERENCES

- Arcos, F. 1981. A dense patch of *Acartia* Levequei (Copepoda Calanoida) in upwelled equatorial undercurrent water around the Galapagos Islands, p. 527-532. *In* F.A. Richards [ed.], Coastal upwelling. Academic.
- Bowie, A., M. Maldonado, R.D. Frew, P. Croot, E.P. Achterberg, R. Fauzi, C. Mantoura, P.J. Worsfold, C.S. Law, and P.W. Boyd. 2001. The fate of added iron during a mesoscale fertilization experiment in the Southern Ocean. *Deep-Sea Res Pt* **48**: 2703-2743.
- Durbin, A.G. and A.E. Durbin. 1992. Body size and egg production in the marine copepod *Acartia* hudsonica during a winter-spring diatom bloom in Narragansett Bay. *Limnol and Oceanogr* **68**:342-360.
- Figueroa, D. and K. Hoefel. 2005. The distribution and community structure of zooplankton in the Galapagos Islands: preliminary report for Galapagos National Park. 9 pp.
- Frost, B. 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. *Limnol and Oceanogr.* **17**:805-815.
- Lee, W.C. 1996. Distribution and species composition of mesozooplankton in the Bering Sea during summer 1994 and 1995. *Ocean Research* **18**:37-47

Rollwagen Bollens, G.C. and M.R. Landry. 2000. Biological response to iron fertilization in the eastern equatorial Pacific (IronEx II). I. Microplankton community abundances and biomass. *Mar. Ecol. Prog. Ser.* **201**:43-56.

Rollwagen Bollens, G.C. and D.L. Penry. 2003. Feeding dynamics of *Acartia* spp. Copepods in a large, temperate estuary (San Francisco Bay, CA). *Mar. Ecol. Prog. Ser.* **257**:139-158.

Roman, R. and A. Gauzens. 1997. Copepod grazing in the equatorial Pacific. *Limnol and Oceanogr.* **42**:623-634.

Støttrup, J. and J. Jensen. 1990. Influence of algal diet on feeding and egg production of the calanoid copepod *Acartia tonsa* Dana. *J. Exp. Mar. Biol. Ecol.* **141**:87-105.

Wiadnyana, N.N. and F. Rassoulzadegan. 1989. Selective feeding of *Acartia clausi* and *Centropages typicus*. *Mar. Ecol. Prog. Ser.* **53**:37-45.

## FIGURE CAPTIONS

Fig. 1. Map of Galapagos archipelago modified from the following website

([http://www.galapagosislandsdiscover.com/galapagos\\_%20map\\_large.htm](http://www.galapagosislandsdiscover.com/galapagos_%20map_large.htm)). The star indicates location where samples collected.

Fig. 2. Mesozooplankton (202–2000  $\mu\text{m}$ ) abundance from mixed-layer samples collected outside and inside the IronEx II patch (Rollwagen and Landry. 2000).

Fig. 3. Biogenic Fe (a) standing stocks and steady-state uptake rates for the producer pool, partitioned into four different plankton classes, namely eukaryotic picoplankton (0.2–2  $\mu\text{m}$ ), eukaryotic phytoplankton (>5  $\mu\text{m}$ ), autotrophic flagellates and heterotrophic bacteria. Three periods during SOIREE (Southern Ocean Iron RElease Experiment) and a control were identified: start (days 1–3), middle (day 5), end (days 12–13) and the mean biogenic Fe pool for all out-patch stations (Bowie et al. 2001).

Fig. 4. Schematic of experimental design, including the number of bottles with and without *Acartia* at each time point.

Fig. 5. Measured chlorophyll for each incubation period (0, 12, 24, 36hrs) with and without *Acartia*. Error bars represent the standard error calculated of triplicate bottles for each point (time, presence of *Acartia*). A) chlorophyll measurements for 0.7-2  $\mu\text{m}$ . B) chlorophyll measurements for 2-10  $\mu\text{m}$ . C) chlorophyll measurements for 10-73  $\mu\text{m}$ .

Fig. 6. Measured chlorophyll for each incubation period (0, 12, 24, 36hrs) with and without *Acartia*. Error bars represent the standard error calculated of triplicate bottles for each point (time, presence of *Acartia*). A) phaeopigment measurements for 0.7-2  $\mu\text{m}$ . B) phaeopigment measurements for 2-10  $\mu\text{m}$ . C) phaeopigment measurements for 10-73  $\mu\text{m}$ .

Fig. 7. Total chlorophyll (A) and phaeopigment (B) by percent for all size fractions for each time interval of the experiment.



Figure 2

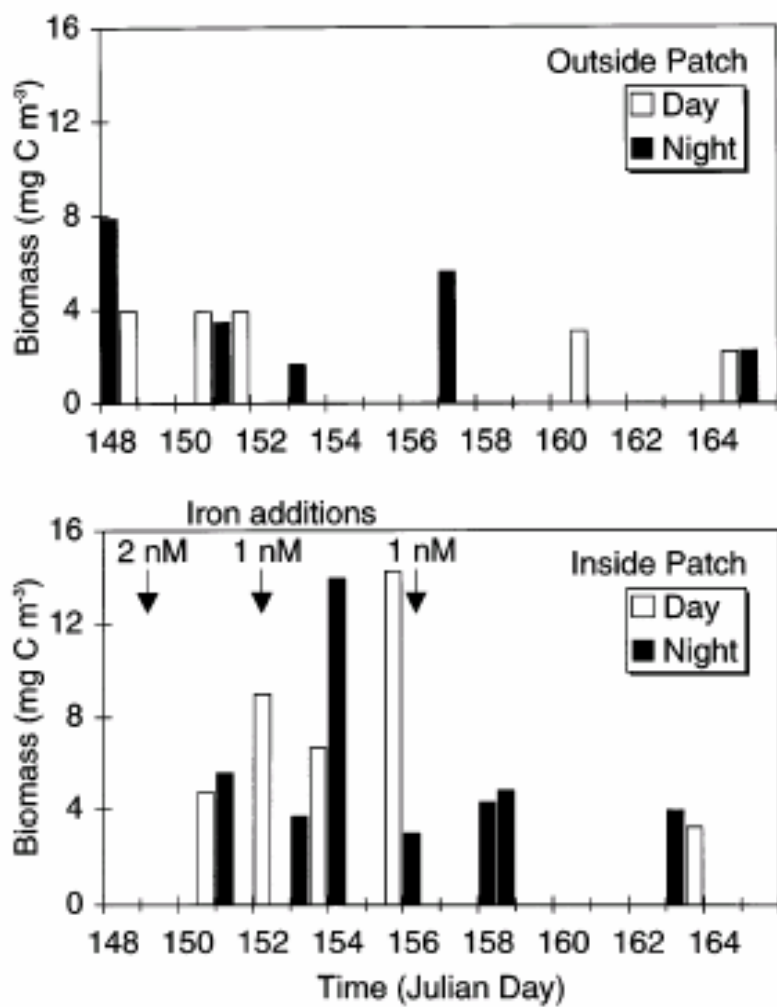


Figure 3

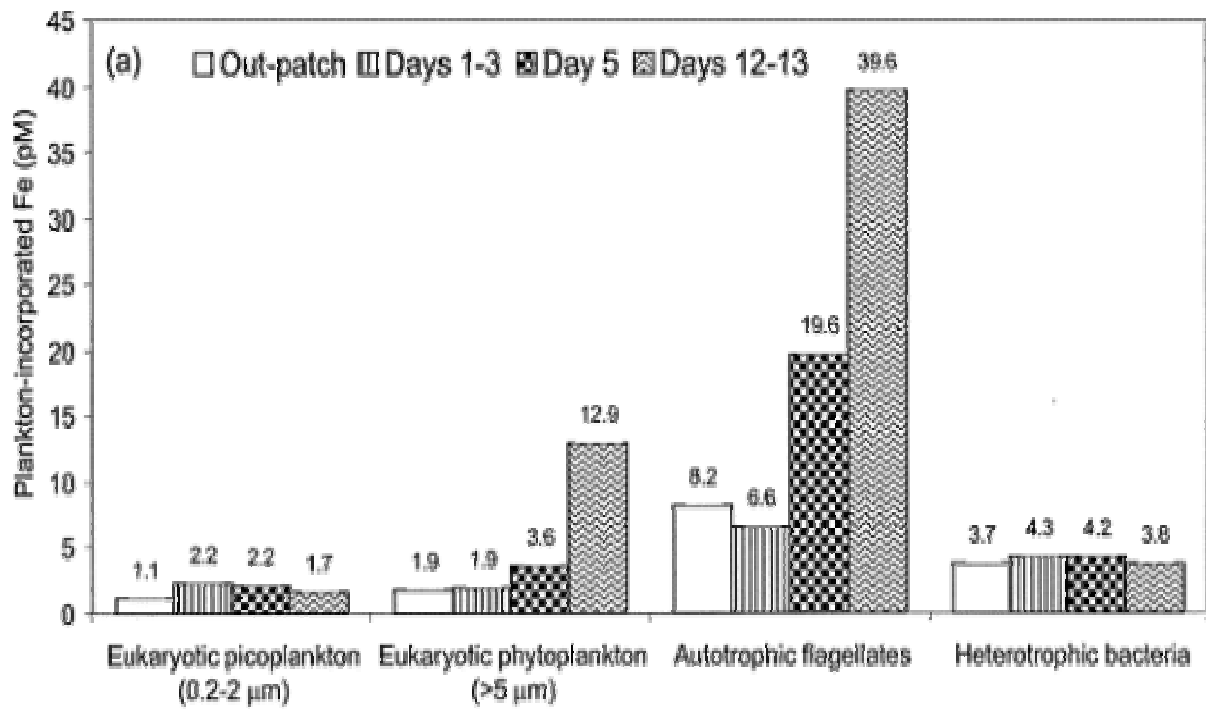
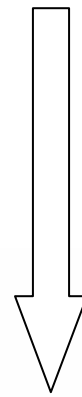
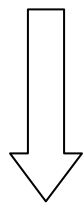


Figure 4

Schematic of Experiment

Seawater filtered through  
73  $\mu\text{m}$  nitex

Seawater filtered through 73  $\mu\text{m}$  nitex  
with 10 *Acartia* per bottle



Time filtered

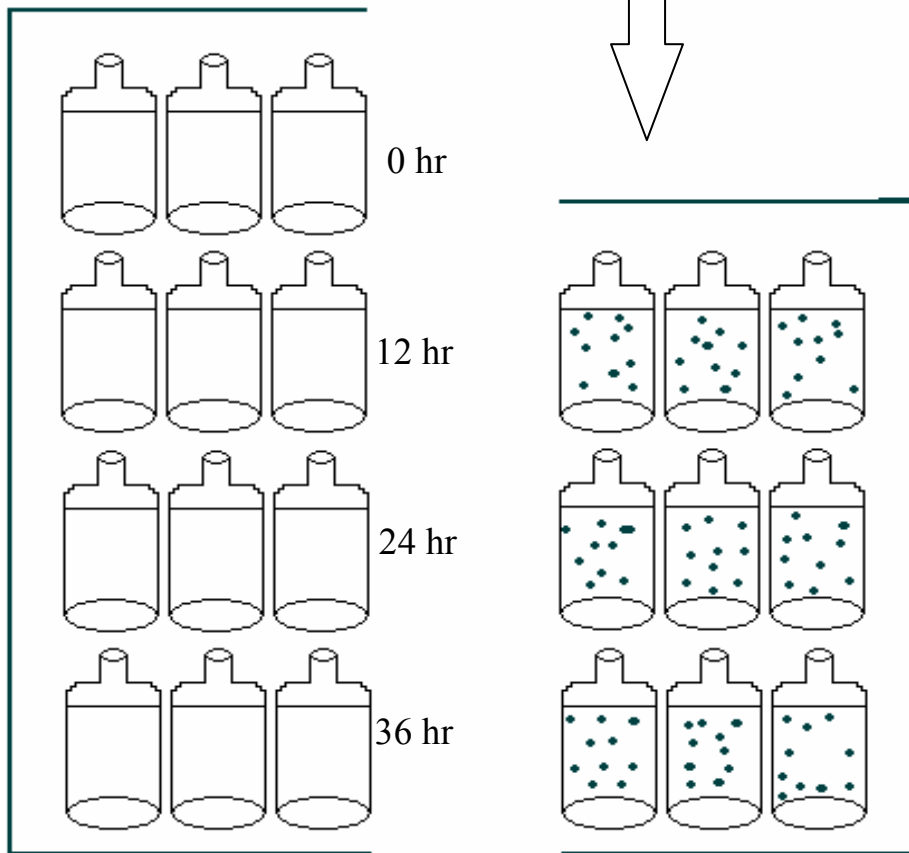
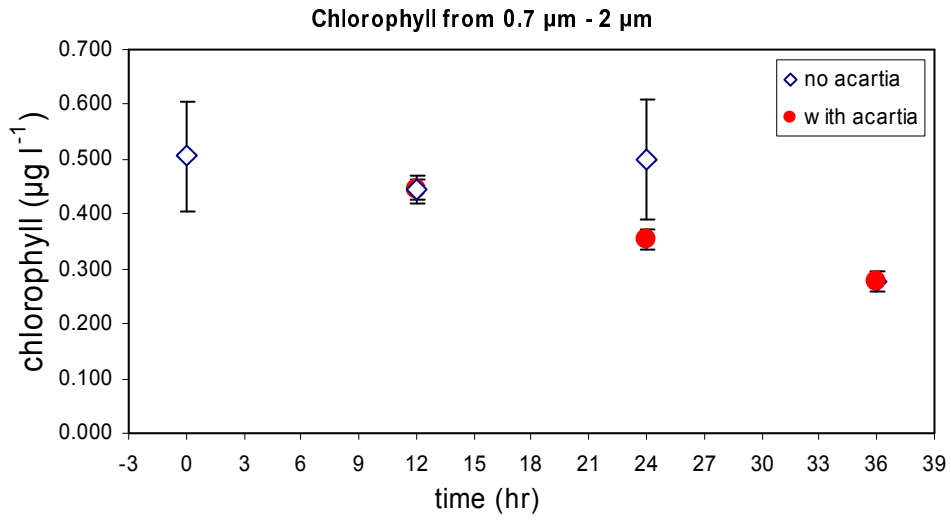
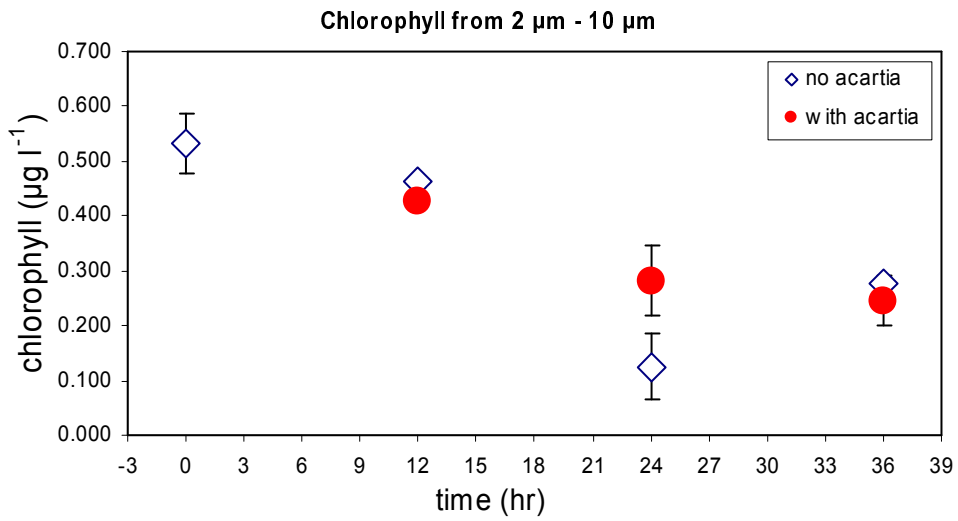


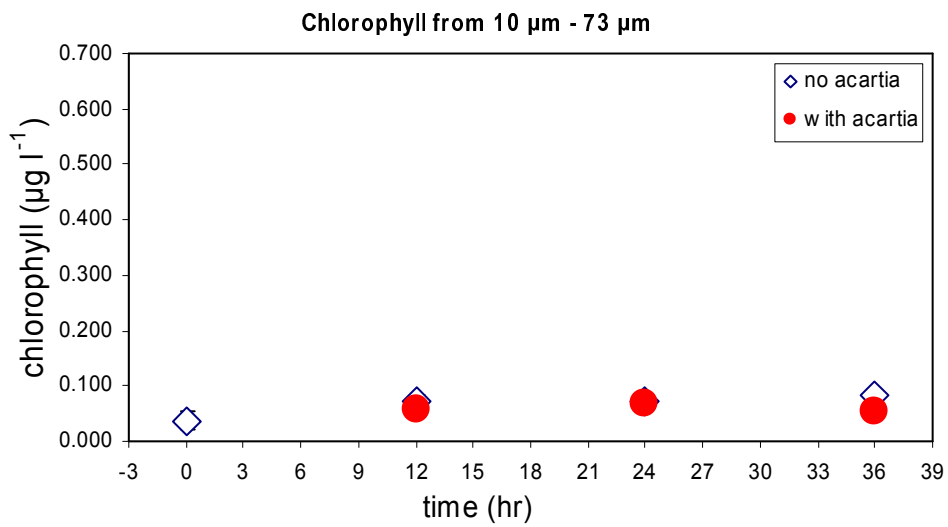
Figure 5



A)

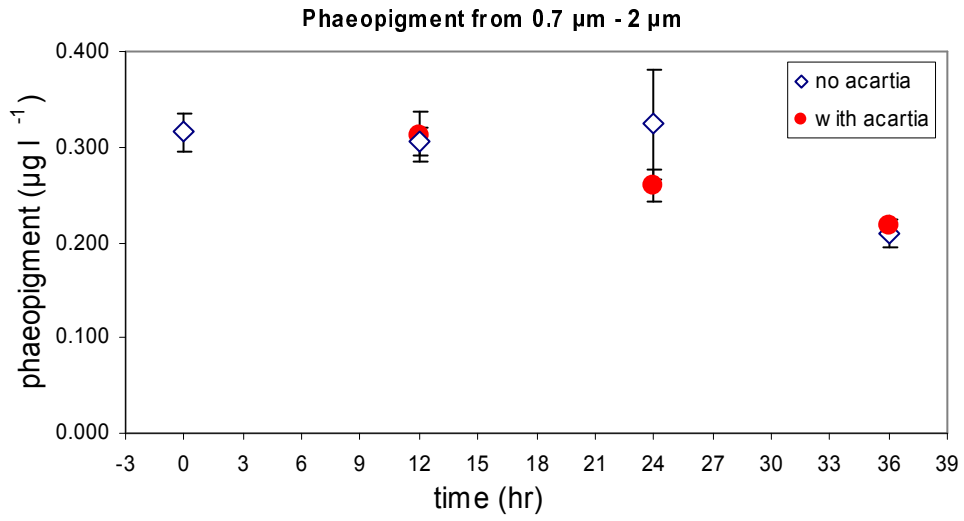


B)

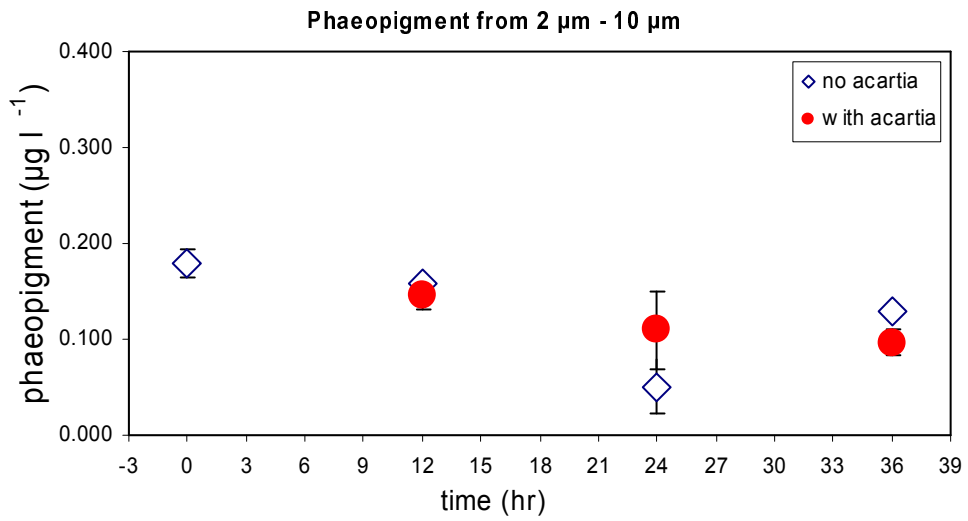


C)

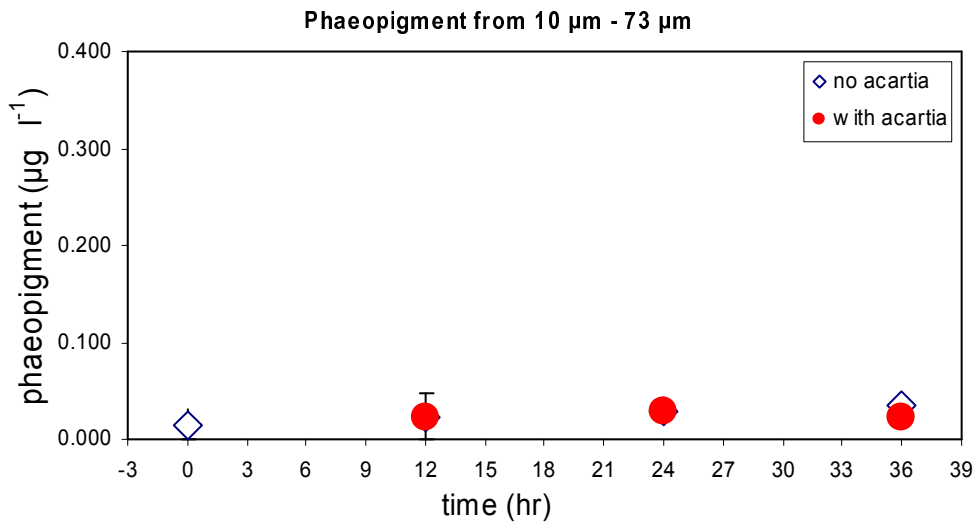
Figure 6



A)

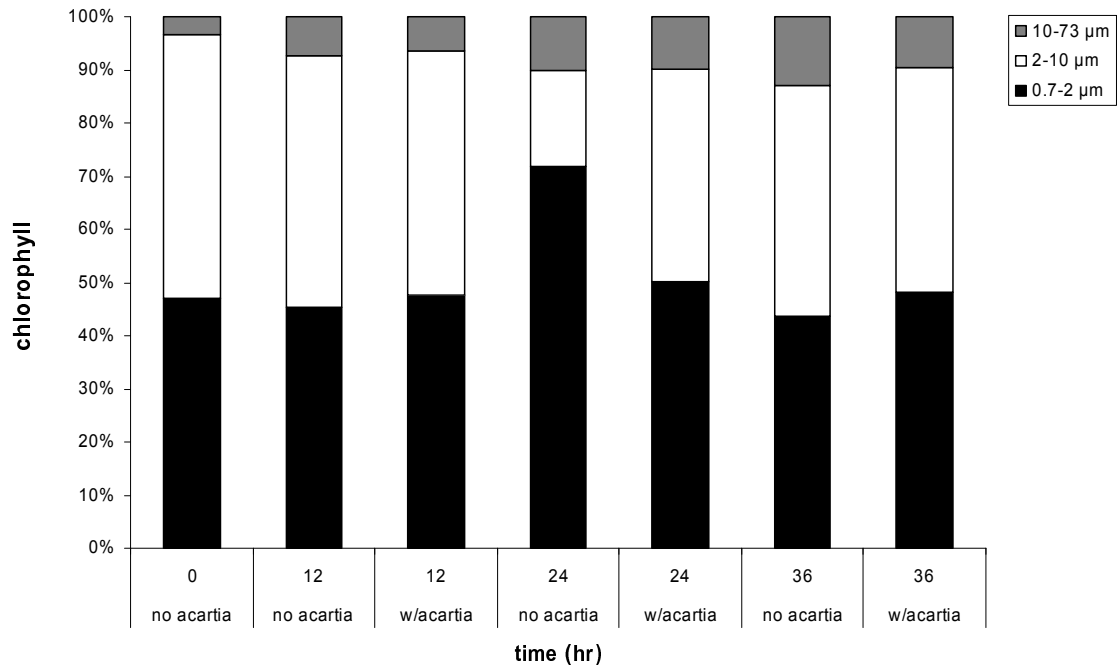


B)

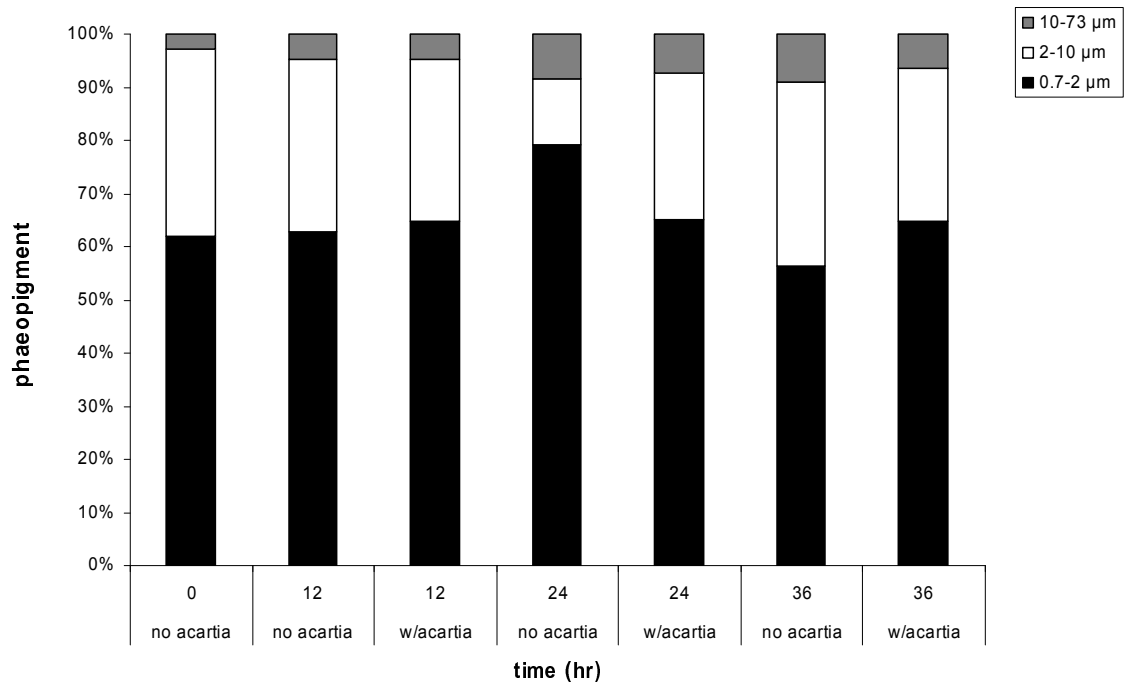


C)

Figure 7



A)



B)