Title

ZOOPLANKTON OF THE FRINGING REEF: SUBSTRATE PREFERENCE OF DEMERSAL ZOOPLANKTON, NON DEMERSAL ZOOPLANKTON IN THE FRINGING REEF ENVIRONMENT, AND THE EFFECTS OF THE LUNAR CYCLE ON ZOOPLANKTON ABUNDANCE

Author

kobervig, carolyn p

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CAROLYN P. KOBERVIG

Environmental Economics and Policy, University of California, Berkeley, California 94704 USA

Abstract. Zooplankton is an essential component of every coral reef system, not only because it is the base of many marine food chains, but also because it is an important stage in many marine animals' life cycles. While by definition plankton is free floating, zooplankton ha been known to move in predictable patterns. This includes a daily diel vertical migration towards the surface at night and back to the depths during the day and fluctuations in abundance over the lunar cycle, usually peaking around the full moon. This study aimed to look at the amount of control plankters have in choosing their horizontal position over the reef by looking at substrate preferences of demersal zooplankton in the fringing reef. It was found that zooplankton emerge in the largest numbers from branching coral followed by coral rubble and sand and in significantly lower numbers from smooth coral. This suggests that demersal zooplankton is able to select the substrate on which it seeks shelter during the day. Little evidence was found suggesting specific taxa prefer specific substrates. The study also compared plankton emerging from the substrate of the reef with those in the water above it. A zooplankton from the genus *Lucifer* was found to be dominant in the water column above the reef, but was not seeking shelter in the reef substrate during the day. Lastly, fluctuations in abundance were observed throughout the lunar cycle with a peak in numbers occurring 6-11 days after the full moon.

Key words: demersal, zooplankton, emergence traps, plankton tow, lunar cycle, Lucifer, *Mo'orea, French Polynesia, fringing reef*

INTRODUCTION

Plankton is an integral part of every coral reef ecosystem. Not only do both reef-building corals (Porter 1976) and many kinds of reef fish (Hobson 1973) rely on zooplankton as their main food source, but it is also one of the first stages in many reef animals' life cycles'. The abundance and distribution of zooplankton over a reef is important for many reasons. For example, the abundance of zooplankton is an indicator of food availability in the reef ecosystem (Gladfelter et al 1980). Also, because so many reef animals feed on zooplankton, the distribution and movement of this food source can directly affect the behavior of many other species in the community (Gladfelter et al 1980, Davis and Birdsong 1973).

Although by definition plankton live their lives floating in the water they are known to exercise their limited mobility in predictable patterns. For example, it is widely recognized that throughout a 24-hour period zooplankton make a vertical diel migration from the sea floor during the day to the surface at night (Forward 1988, Zaret 1976). More specifically, there is demersal plankton, which Alldredge and King (1977) define as plankton that hide within reef sediments during the day but emerge to swim freely over the reef at night. While it is clear that zooplankton actively control their vertical location it is still unclear to what extent they choose their horizontal location, or more specifically what substrate they are over. Previous studies using emergence traps over different substrates (Porter and Porter 1977, Alldredge and King 1977) have found that zooplankton emerge in greater numbers from coral, specifically branching coral, compared to sand and coral rubble. Higher numbers of specific kinds of zooplankton have also been observed emerging from specific substrates (Alldredge and King 1977). While this behavior has been observed on barrier reefs there is little knowledge of zooplankton substrate preference on shallow fringing reefs.

This project's study site is located on the northern side of Mo'orea, French Polynesian on the west side of Cooks Bay in the fringing reef environment. Constant recirculation of water flowing out of the bay through deep channels, and reentering through wave action over the barrier reef has been observed (Alldredge and King 2009) making the fringing reef in the lagoon a dynamic environment for those creatures floating in the water column. A wide range of types of zooplankton have been found in lagoon environments that are not found in surrounding open water tows including mysids, amphipods, cumaceans, polychaetes, crustacean larvae, and distinct species of copepods (Alldredge and King 1977).

Figure 1. Sampling site located in Cook's Bay on Mo'orea, French Polynesia

The goal of this study was to look at the extent to which zooplankton are really "free floating" by looking at substrate preference. By quantifying the amount and kinds of zooplankton hiding in each kind of fringing reef substrates including sand, coral rubble, branching coral, and smooth coral the extent to which plankton are actively selecting their horizontal location in their habitat can be inferred. This study aimed to answer four questions: (1) do zooplankton prefer to take shelter on a specific substrate (2) are there any taxonomical substrate preferences (3) is there a difference between the diversity of zooplankton emerging from the substrate and the diversity in the water and (4) does the amount of zooplankton fluctuate throughout the lunar cycle? The findings of previous studies suggest that branching coral holds the highest percentage of demersal plankton, which is what I expect to find in Mo'orea. I also expect to observe some fluctuation in plankton abundance over the lunar cycle that peaks around the full moon.

MATERIALS & METHODS

Samples of demersal plankton were collected from the fringing reef along the west side of Cook's Bay on the volcanic island of Mo'orea, French Polynesia over a period of five weeks in October-November 2009. Quantitative samples were collected using several techniques as described below.

Study site

The study area is a marine protected zone located in front of the Richard Gump Research Station on the northwestern flank of Cook's

Bay (Figure 1) (coordinates: -17.48° S, -149.83° W). This area is compromised of a sandy substrate with small coral heads dispersed throughout. Samples were taken in the fringing reef zone within 10-100 m from shore. Due to the fringing reef's relatively shallow water, traps were set at depths between 1.5 and 3.5 m.

Emergence traps

In order to capture demersal zooplankton from different substrates during the night, I used cone shaped mesh emergence traps. I constructed a total of four traps using insect collecting nets constructed of 250 μm mesh. The opening at the bottom of the cone is a circle 25 cm in diameter made of cloth piping covering a heavy metal chain 1 m in length and a rope attached to a buoy for relocating purposes. At the apex of the cone I attached a 400 ml collection jar using a hose clamp. I attached an inverted funnel to the mouth of the collection cup so that the opening was 4 mm at the smallest point to prevent captured plankters from escaping. A small buoy was duct taped to the top of each collection cup so that when submerged the emergence trap would stay fully open and erect reaching a total height of 70 cm (See Figure 2). In addition, on both the sand and rubble traps I inserted a stiff frame made of a wire coat hanger into the piping with the chain to ensure that the opening at the bottom did not close up due to wave action. The trap is a variation of the design used in Porter and Porter (1977) and Alldredge and King (1977).

I placed the traps out 2-3 times per week no more than 10 m away from each other over the respective substrates in the study area. After completing several trial runs setting and collecting the traps at different times, I decided that putting them out in the late afternoon and collecting them early the next morning was the best strategy to trap the highest number of plankton. I took care to avoid disturbing the trap sites due to the pressure from my snorkeling fins to mitigate sediment getting into the traps and the possibility of plankters being swept away do to the increase in water pressure over the substrate. I collected traps using snorkeling gear to dive down and gather the mesh as close to the substrate as possible to prevent anything from escaping. I then pulled up the net so that everything in it would drain to the collection cup and sealed it off to avoid anything from escaping while transferring the trap back to shore.

Figure 2. An example of an emergence trap over substrate.

In the lab I strained the samples individually using 250 μm mesh to concentrate the zooplankton. I then added the strained specimens to 2 ml of filtered seawater and 1 ml of 70% ethanol to fix the specimens. I placed each sample in a small petri dish 5.5 cm in diameter with a 2 mm grid on the bottom so that I could systematically count each plankter using a compound microscope. I identified the zooplanktons using *Coastal Marine Zooplankton: A Practical Manual for Students* by Christopher E. Todd, M.S. Laverack and Geoff Boxshall and categorized them into general taxonomic groups including but not limited to copepods, decapods, *Luciferidae*, annelids and hydrozoa.

Plankton tow

Each night that emergence traps were set I simultaneously conducted a plankton tow over both the reef and the lagoon between

22:00 and 24:00. I used a two-person kayak and a partner to do this. My partner would paddle from the front of the kayak for one minute while I held the plankton tow rope at surface level of the water allowing it to trail 3 m behind so that the tow was fully submerged but less than 1 m below the surface. Once the one-minute reef tow was complete I transferred the contents of the tow's collection jar into another jar. In order to prevent contamination I then thoroughly rinsed the tow before the second collection was done.

In the lab I strained the sample to concentrate the plankton using 250 μm mesh. I then added the strained specimens to 30 ml of strained seawater and 5 ml of 70% ethanol to fix the specimens. I then took a 3.5 ml sub sample by shaking up the sample cup and taking a random 3.5 ml sub sample with a pipette. I placed this sub sample into a small petri dish 5.5 cm in diameter with a 2 mm grid on the bottom so that I could systematically count each plankter using a compound microscope.

Statistical methods

First, to test the significance of the total number of zooplankton found over each substrate I used chi-squared tests to compare all substrates and each substrate individually. To look more specifically at this data and test the significance of the abundance of each taxon over each substrate I used an analysis of variance (ANOVA) test with a Tukey-Kramer HSD (honestly significant differences) test. In order to compare the composition of taxa in the emergence traps with that of the plankton tows I made a contingency table and did several chi-squared tests to look at each specific taxon. Lastly, to track the change in abundance of zooplankton over the lunar cycle I used an ANOVA with a Tukey-Kramer HSD test to look at the significance between four time spans over one lunar cycle. In order to correct for multiple comparisons I also did Bonferroni corrections for test with many comparisons. JMP 8 © software was used for all statistical analysis.

RESULTS

Abundance of fringing reef demersal zooplankton over different substrates

The amount of zooplankton caught with emergence traps over each of the four substrates varied widely. The total numbers of zooplankton captured in all 12 trapping events over each substrate are very dissimilar (Fig. 3). The most zooplankton was captured over branching coral with a total of 662 plankters. The next highest was sand with a total of 508 closely followed by coral rubble with 468. There was a large gap between the totals of each of these three substrates

Total Zooplankton Trapped Over Each Substrate

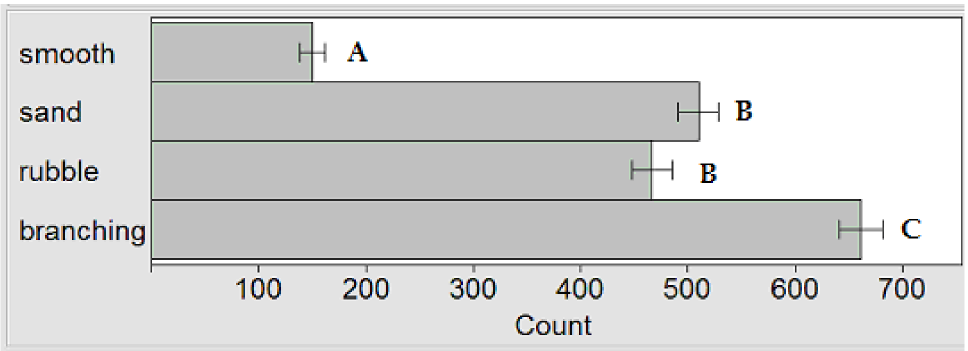

Figure 3. Total number of zooplankton caught in all 12 trapping events over each substrate. Error bars representing +/- one standard error are present for each substrate. Bars with different letters have significantly different chi-squared values.

Table 1. Results from χ^2 test between substrates with critical value and critical value with the Bonferroni correction. Values with * are statistically significant.

Substrates	DF	Critical value	Critical value with correction	χ^2 value
All	3	7.81	11.35	310.1*
Smooth/ Branching	1	3.84	6.64	322.8*
Smooth/ Sand	1	3.84	6.64	194.8*
Smooth/ Rubble	1	3.84	6.64	162.9*
Branching/ Sand	1	3.84	6.64	20.3*
Branching/ Rubble	1	3.84	6.64	33.7*
Sand/ Rubble	1	3.84	6.64	1.7

compared with the total captured over smooth coral, which consisted of only 150 plankters. These totals were compared using a chi-squared (χ^2) test and resulted in the values in Table 1. When all substrates were compared the χ^2 value was 310.1 with a critical value of 7.81. This value is extremely significant so each individual substrate was compared with the others. All comparisons besides that between coral rubble and sand were significant (see Table 1).

In order to correct for the fact that so many comparisons were done a Bonferroni correction was done. This increased the critical value to a level that corresponds to a P-value of 0.01 that the chi-squared value must exceed to indicate significance. The chi-squared values were high enough in these comparisons that this correction did not change any significances.

Table 2. Results from ANOVA tests comparing each zooplankton taxon with its abundance in the traps over each substrate. Values with * are statistically significant.

Taxon	DF	F-ratio	P-value
Copepods	3	3.1304	0.0387*
Decapods	3	1.1682	0.3366
Luciferidae	3	1.1065	0.3604
Annelids	3	1.4235	0.2534
Blue Copepods	3	0.8351	0.4843
Cirripidia	3	0.9528	0.4265
Mites	3	2.4870	0.0777
Snail Shells	3	1.9333	0.1434
Other	3	1.9442	0.1417
Macro	3	1.4348	0.2503

Substrate preferences of zooplankton taxa

The substrate preference of each taxon identified was also evaluated. Using an analysis of variance (ANOVA) test with a Tukey-Kramer HSD the significance of the abundance of each specific taxon over each substrate was tested. The percent of each taxon found emerging from each of the four substrates are a stark contrast (Fig 4). Results from statistical analysis (Table 2) show there were almost no significant differences in substrate preference for any taxon. One exception is copepods with a p-value of 0.039. The Tukey-Kramer HSD looked more closely at this significance to specify what substrates are significantly different. The difference lies between branching coral and smooth coral with a p-value of 0.03 indicating that copepods are significantly more likely to be found emerging from branching than smooth coral. Although copepods had the only

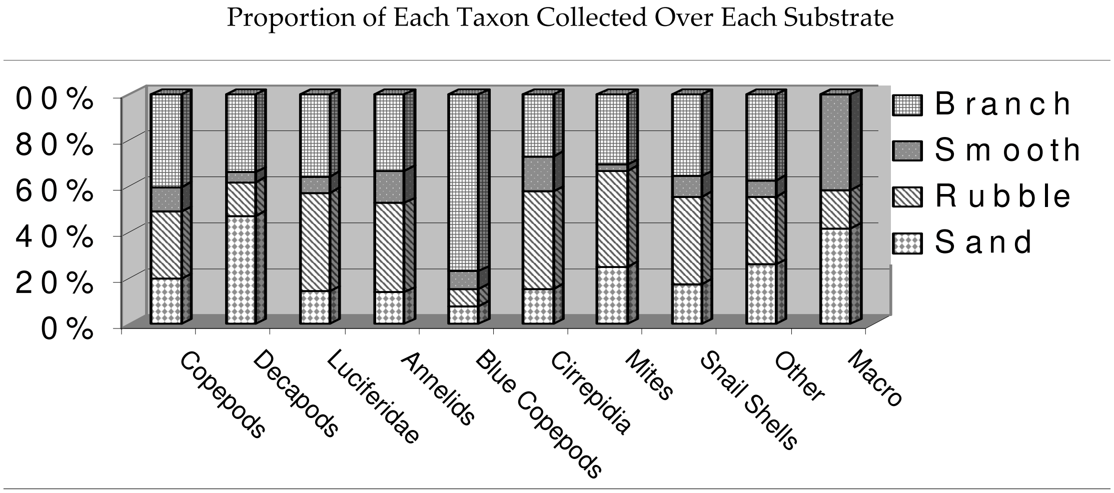

Figure 4. Each bar represents the frequency with which a specific taxon was found over each substrate. Substrate contributions to each taxon very widely from a large addition to blue copepods from branching coral to a minute addition to mite numbers from smooth coral.

significant difference, the ANOVA shows that mites had a nearly significant difference (p-value = 0.078), which the Tukey-Kramer HSD shows is between coral rubble and smooth coral. Although not statistically significant (p=0.056) this tests indicates there may be a trend that mites are more likely found emerging from coral rubble than smooth coral.

In order to correct for the large number of comparisons, I also did a Bonferroni correction taking the usual marker of statistical significance, 0.05, and dividing it by the number of comparisons, 10. This resulted in a new p-value, 0.005, that must be obtained to indicate statistical significance. When scrutinizing the data more thoroughly with this correction it appears that there are no statistical significances.

Demersal plankton vs. reef plankton

When comparing the composition of demersal zooplankton caught using emergence traps with the composition of those floating above the reef caught using tows, a large variation in taxonomic makeup was discovered. First, a contingency table was made to compare percentages of each taxon using each collection method. The percentages each taxon contributes to the total composition of each collection method have some obvious inequities (Fig 5). While both copepods and decapods are a large portion of the total makeup of each collection method it is obvious that the reef tow's primary contributor is *Luciferidae* (63%) (represented by

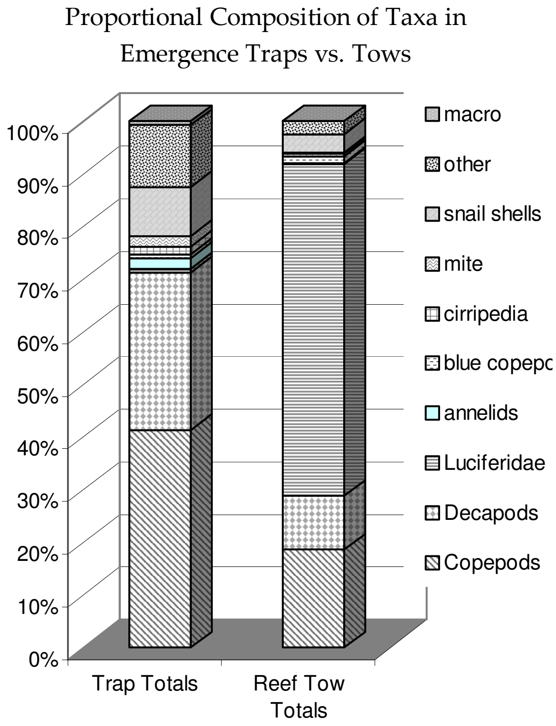

Figure 5. A visual representation of the percent each zooplankton taxon contributed to the makeup of each collection method.

horizontal stripes), which contributes only a tiny sliver (< 1%) to the composition of the trap total. Chi-squared tests were run to compare the significance of the difference between the percentages of each taxon making up the total sample of each collection method. The chi-squared values of copepods (χ^2=8.8), decapods (χ^2=9.9) and *Luciferidae* (χ^2=60.8) were all significant, exceeding the critical value of 3.81.

Effects of the lunar cycle

To track fluctuations in zooplankton abundance over the lunar cycle a total of 11 collection events were divided into four groups representing one full lunar cycle. Fig. 6 represents the average number of plankton caught on each night of collection within that group of days. A one-way ANOVA analysis was done to compare the means of each of the four groups, which indicated a significant difference (DF=3, F-ratio=3.7058, P-value=0.0226) between the four groups. By further analyzing this with a Tukey-Kramer HSD test it was found that the period 6-11 days after the full moon was significantly different from the period preceding it (P-value=0.02), 0-5 days after the full moon, and the period following it (P-value=0.045), 12-21 days after the full moon.

In order to compensate for the number of comparisons I did a Bonferroni correction on the p-value taking the classic indicator of significance and dividing it by the number of comparisons done, two, to get a new p-value that indicates statistical significance of 0.025. When the data is analyzed more carefully with this correction it appears that only the periods 0-5 days after the full moon and 6-11 days following the full moon are significantly different.

DISCUSSION

Abundance of fringing reef demersal zooplankton over different substrates

The abundance of zooplankton over each of the four substrates tested varied substantially, especially between branching coral and smooth coral. The differences in

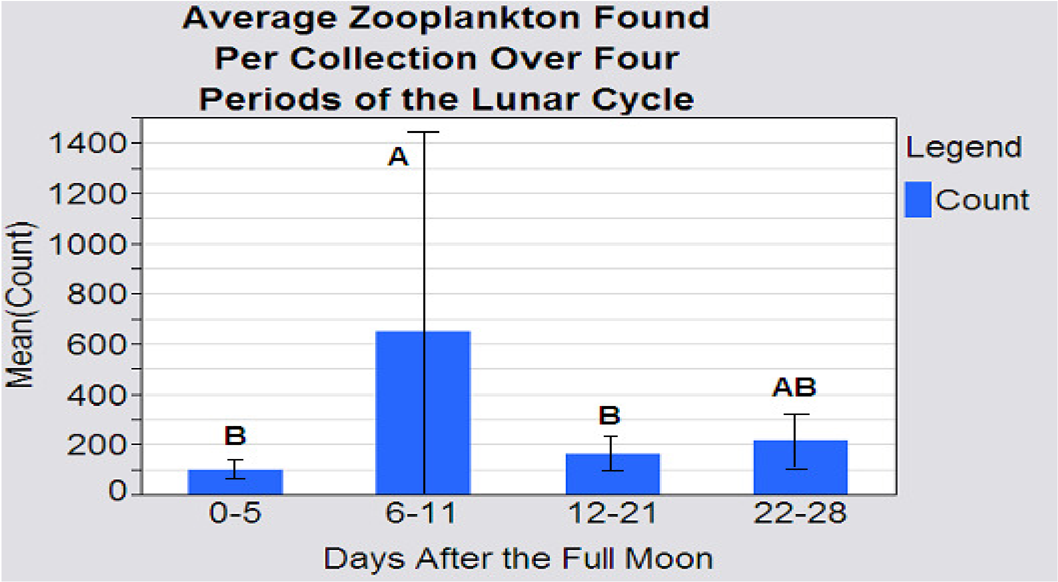

Figure 6. The average number of zooplankton collected on a night falling within each group of days following a full moon. Error bars are present to represent +/- one standard deviation. Bars with different letters (A or B) were found to be significantly different (ANOVA with Tukey-Kramer HSD) whereas bars that share a letter are not significantly different. On this scale the full moon is on day 0 and the new moon is on day 14.

total plankton emerging from each substrate is as follows: branching coral > coral rubble = sand > smooth coral. These differences are likely due to the fact that zooplankton hide in the interstices of the substrate and branching coral's 3-dimentionality makes it the most substantial provider of protected areas. On the other end of the spectrum smooth coral provides almost no interstices to take shelter in and is elevated above the sea floor making it more susceptible to pressure from currents. Coral rubble and sand lie in the middle ground between these two extremes providing more protection than smooth coral but not as much as branching coral. Alldredge and King (1977) and Porter and Porter (1977) also found a significantly higher abundance of plankton emerging from branching coral. The significant variation in plankton abundance over the sampled substrates suggests that plankters are in fact controlling their horizontal position within the reef environment. Alldredge and Kings' (1977) results also suggest that zooplankton behaviorally select preferred substrates to settle on during the day by either staying close to their preferred substrate during the night or actively seeking it out when returning from the water column.

Substrate preferences of zooplankton taxa

In regards to specific zooplankton taxa having substrate preferences almost no significant results were found. The one exception was that copepods prefer branching coral to smooth coral. This significance is not surprising due to the fact that copepods are highly mobile and therefore one of the most likely candidates to have the ability to choose the substrate they seek refuge in. Also, because branching coral offers so much shelter in comparison to smooth coral it is not surprising that they would select this substrate. Other research on demersal plankton by Alldredge and King (1977) found that Ostracods and nematodes preferred sand while copepods and a variety of other taxa preferred corals. Alldredge and King's (1977) study suggests that individual taxa do have specific preferences, which was generally not found in this study. This discrepancy could be a result of the relatively small scale of this study in comparison with that done by Alldredge or could be caused by spatial behavioral differences between Mo'orea and Lizard Island where Alldredge's study was conducted.

Demersal plankton vs. reef plankton

The composition of zooplankton in the water column over coral reefs is very complex. Previous studies have found that different size zooplankton tend to migrate different distances from the benthos (Alldredge and King 1985) and that only some plankton found over the reef at night are emerging from the reef substrates (Alldredge and King 1985). When I compared composition of zooplankton caught with emergence traps with those caught using plankton tows several significant differences between the two were discovered. First, the proportion of copepods and decapods found in the emergence traps is significantly greater than the proportion found in the tows. Previous studies have found that copepods tend to stay relatively close to the sea floor (Alldredge and King 1985) which would explain why there was a higher concentration of them in the traps which are a maximum of 70 cm above the substrate where as the tows collected plankton from just under the surface of the water. Another possible explanation is that the amount of decapods and copepods found in the traps is only significantly different from that of the tows due to the interference of the taxon *Luciferidae* in the counting process. When the amount of Luciferidae was very high it made it much more difficult to count the smaller zooplankton in the microscope. It is possible that the Luciferids in these counts obscured a portion of both copepods and decapods.

The other overwhelmingly significant difference between the two collection methods was the tremendous presence of *Luciferidae* in the tows and its almost complete absence from the traps. *Luciferidae* was caught almost exclusively with tows and made up 63% of zooplankton caught with this method, while it accounted for <1% of total zooplankton caught with emergence traps. This taxon was not caught consistently throughout the sampling period but sporadically in huge numbers. Swarms of *Luciferidae* could not only be seen with the naked eye but actually felt with the skin if swimming through a swarm. The presence of such an abundant and condensed amount of zooplankton over the reef has huge implications on food supply and planktivore behavior yet it is clear from their absence in the emergence traps that they do no rely on the reef substrate for protection. Large, seemingly sporadic swarms of *Luciferids* have been observed elsewhere in both the Pacific and Atlantic Oceans (Oishi and Masayuki 1997, Woodmansee 1966). Abundance of some *Lucifer* species has been found to significantly increase at night and during floodtides possibly as a mechanism for transport (Woodmansee 1966). It is unclear where the *Luciferidae* in Mo'orea are going during the day or what their distribution around the island is, but a planktologist that has previously done research on zooplankton of the barrier reef surrounding Mo'orea said that she only occasionally found *Luciferids* in her samples and was surprised to hear I had found them in such large numbers.

Effects of the lunar cycle

Changes in plankton abundance over the lunar cycle were observed when comparing four periods of one complete cycle. The period with the highest average number of plankton per collection was 6-11 days after the full moon, and was significantly higher than the period preceding and following it. Other studies around the world have also observed significant fluctuations in zooplankton abundance throughout the lunar cycle (Hernandez-Leon 1998, 2001, Gliwicz 1986, Jacoby and Greenwood 1989) and while some see a peak at the full moon others have observed peaks in plankton numbers elsewhere in the cycle. The mostly widely recognized pattern is a higher abundance during the full moon but the plankton of Mo'orea does not stick to this mold. This is likely due to the abundance of predators present in the fringing reef. An increase in light during the full moon would make plankters more susceptible to visual hunting planktivores and would likely discourage an increase in plankton activity. A simultaneous emergence of plankton would be beneficial for breeding among other reasons which is why it is likely that Mo'orea's plankton have shifted this behavior to occur when there is less light to prevent increased predation.

Conclusion

Although specific zooplankton taxa were not found to be actively choosing the substrate on which they settle, zooplankton of the fringing reef as a whole were found to prefer branching coral, coral rubble and sand to smooth coral with branching coral being the most preferred. This result implies that zooplankton do have enough mobility to actively choose their horizontal location in the fringing reef environment. Also, the difference between the demersal zooplankton and those found generally in the water column was dominated by the presence of a large amount of *Luciferids* in tow collections and almost none in trap collections. This sporadically swarming zooplankton has a large effect on the abundance of food over the reef yet does not depend on the reef for shelter. Lastly, zooplankton in the fringing reef of Mo'orea appear to emerge in significantly higher numbers between the full and new moon phases 6-11 days after the full moon. I suggest that further research be done on the presence of *Luciferidae* around Mo'orea. More specifically what their diel migration patter is like, if their presence fluctuates throughout the year, what determines where their swarms are and if they are present in the lagoon or barrier reef in as large of numbers as on the fringing reef.

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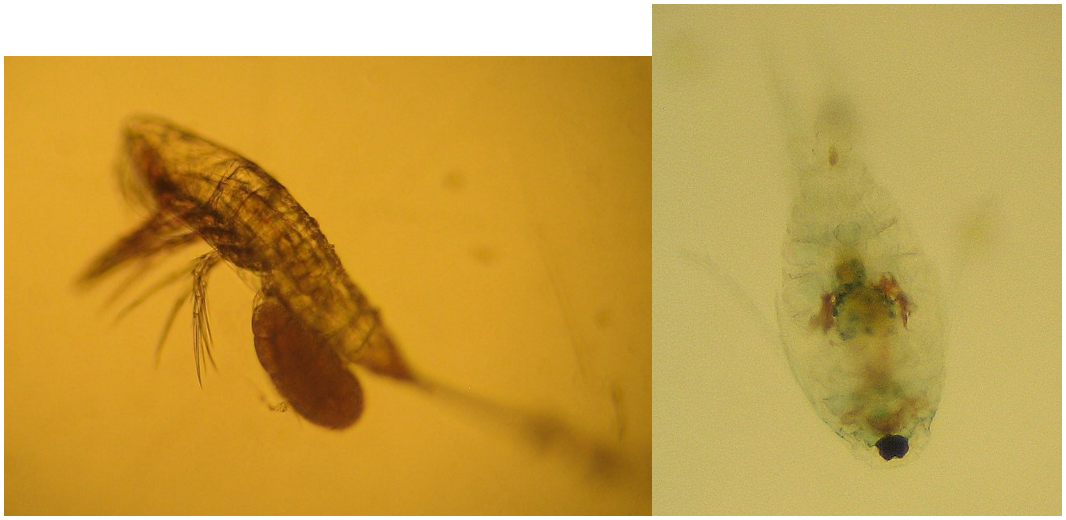
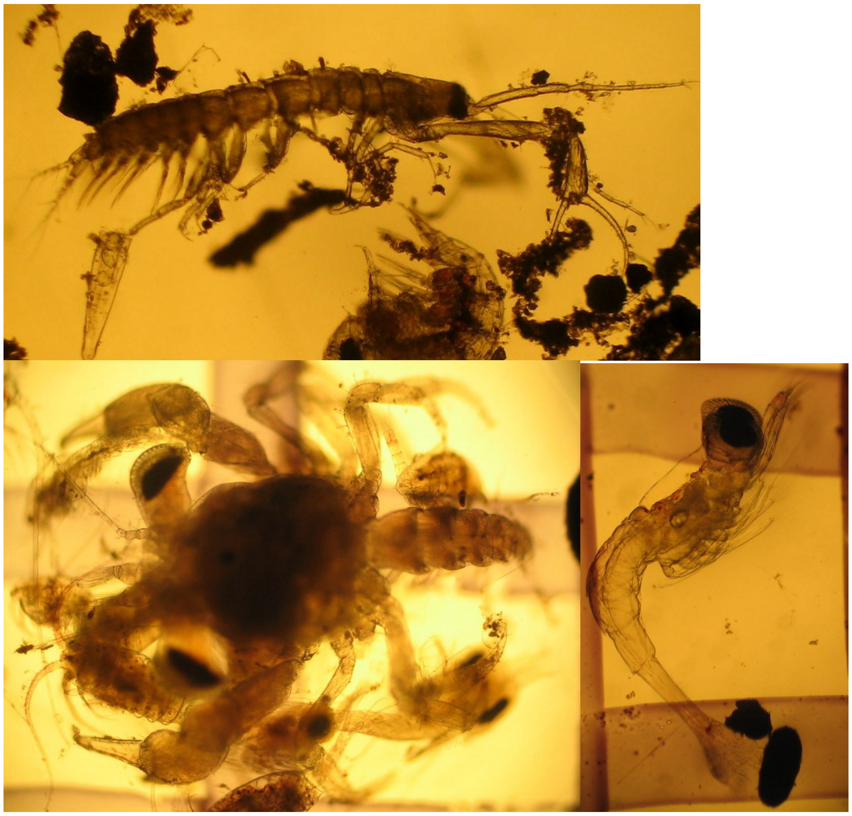
APPENDIX A

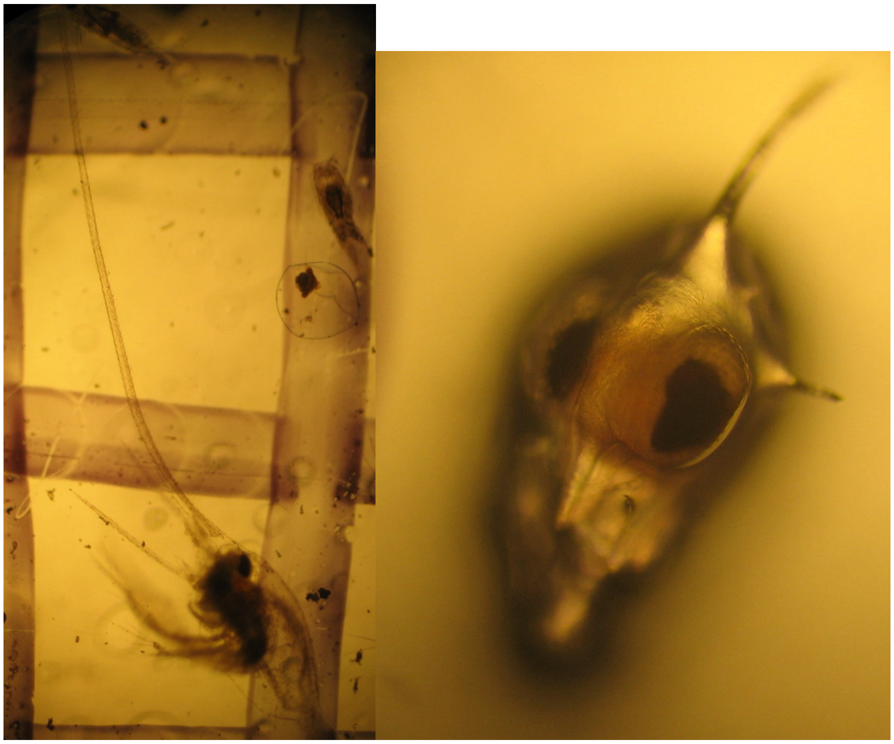
An example of plankters from each taxonomic group studied.

Copepods

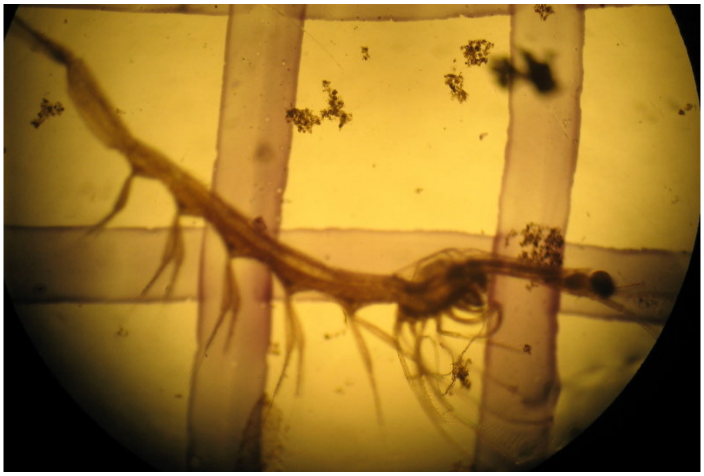


Decapods





Luciferidae

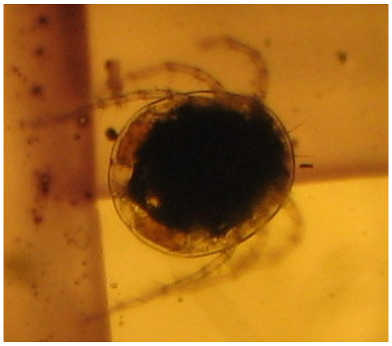


Annelids

Blue Copepods

Cirripedia

Mites



Snail Shells