ZOOPLANKTON ASSEMBLAGE IN OPUNOHU AND COOK'S BAYS (MOOREA, FRENCH POLYNESIA) AND THE RESPONSE OF COPEPODS TO CHANGES IN SALINITY

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Abstract. As secondary producers, zooplankton species are crucial as a vehicle for energy transfer in marine ecosystems worldwide yet few studies have observed the zooplankton community in tropical island estuaries. This study aims to understand the distribution of the zooplankton community in two of the largest estuaries (Opunohu and Cook's Bays) in the volcanic tropical island of Moorea, French Polynesia. The results show that a few taxonomic groups, especially copepod, dominate the zooplankton composition. Moreover, the zooplankton community and diversity is highly variable at each bay and between bays. In the laboratory, copepod salinity response was examined. Copepods were placed at different salinity treatments for 16 hrs and dead copepods were counted. The salinity experiments show greater mortality of copepods at the extreme ends of salinity concentration, 40ppt and 0 ppt, suggesting that salinity is a major component influencing the abundance in these systems. Monitoring fluctuations in the zooplankton abundances will help understand changes in the marine food web as the result of climate change.

Key words: abundance; composition; plankton; veligers; tropical estuaries; Simpson's Diversity Index, copepods

INTRODUCTION

Estuaries, partially enclosed water systems with a river input and an open connection to the ocean (Pritchard 1967), are natural ecosystems with significant economic value. Estuaries provide nursery habitat for fish, birds and many other organisms (Pritchard 1967). In addition, primary and fish production has been shown to be higher in estuaries as compared to upwelling and oceanic systems (Roman et al. 2005). Estuaries can provide habitat and support a large number of organisms because of the high plankton production that occurs in these ecosystems.

Zooplankton are essential in transferring energy from phytoplankton and microzooplankton to higher trophic levels, such as fish larvae (Nybakken 1993). Copepoda, a subclass of zooplankton, are usually the dominant zooplankton in marine waters and play an important role in transferring primary productivity to larger animals in the food web (Kimmel 2011). Understanding the spatial and temporal distribution of zooplankton communities provides crucial information about local marine productivity. Changes in zooplankton abundances differ with currents, daily tide fluctuations, depth, season, temperature, and salinity (Roman et al. 2005). Because plankton move in the water column, it is often difficult to base composition on daytime samples (Harris 2007). As a result, to better understand fluctuation of zooplankton community it is important to consider spatial and temporal changes in the ambient environment.

Zooplankton research has been concentrated on reef and lagoon systems (Achuthankutty 1989) and few studies have examined zooplankton composition in large bays on tropical islands. In a tropical island estuary, land development is an important factor that affects the zooplankton community, but more important is the fluctuation of salinity as the result of freshwater from river run-off and rain activity (Avois-Jacquet et al. 2000). Understanding the effects of water physical variables to the zooplankton community will provide knowledge about the affects in the marine productivity.

The goals of this study were to: (1) understand the distribution and composition of the zooplankton community in a tropical estuary at the head of Opunohu and Cook's Bays (Moorea, French Polynesia); (2) determine how zooplankton abundances change vertically and along the bays; (3) investigate how these differences response to spatial and temporal water physical changes (e.g. salinity); and (4) examine the response of copepods when exposed to different salinity levels. I hypothesized that the composition and abundance of zooplankton was predicted to increase as the distance from the river increased in the estuary, as a result of water physical changes, and that extreme salinity levels decrease copepod activity and could result in higher mortality.

METHODS

Study Sites

The largest estuary in Moorea, French Polynesia, located in the north coast of the volcanic island is Opunohu Bay (Figure 1). Opunohu Bay is 3.5 km from the river mouth to the barrier reef and a 0.6km in width with an approximate area of 28.5 km^2 (Adjerand and Salvat 1996). The Tareu pass bridges the open ocean and the bay and the estuary is nearly 50 m deep near the pass (Adjerand and Salvat 1996). Opunohu Valley is one of the most pristine catchments of the island. It consists of small pineapple plantations, a few hundred inhabitants, and there is there is no major town settled on this bay.There is only one river (Opunohu River) that drains into the bay.

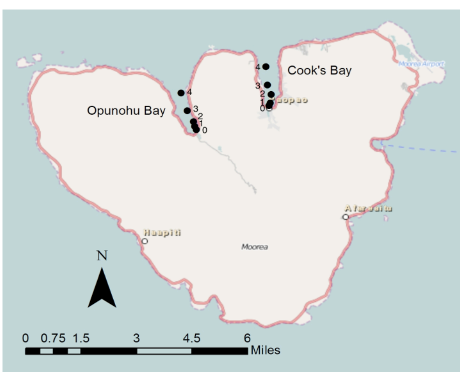

FIGURE 1. Opunohu Bay (left) and Cook's Bay (right) in Moorea, French Polynesia. Sites 0 were located right at the river mouth and sites increased as distance from the river increased.

Cook's Bay, also known as Pao Pao Bay, is the second largest estuary in Moorea with similar watershed catchments as Opunohu Bay. Cook's Bay is about 3 km from the river mouth to the barrier reef. In contrast to Opunohu Bay, Cook's Bay is more developed. A pineapple farm in the valley is close to Pao Pao River that passes through Pao Pao town, which is one of the biggest town in the island. Five sites where sampled in Opunohu and Cook's Bays in October and November 2012.

Sampling Methods

At each site, plankton tows were conducted at the surface and at the bottom twice per week between 9:00-14:00 hr. In a two-person kayak, a plankton net (0.3m diameter, 250µm mesh) was submerged and dragged. I slowly paddled for 2 min horizontally from the mouth of the bay. Each plankton tow roughly sampled 7068 m^3 of water. At site 0 from both bays, plankton tows were only conducted at the surface because the site is about 1 m deep. At site 1 and 2, bottom tows varied depending on the depth during that day. However, at site 3 and 4 bottom tows were only conducted at 20 m deep. A 4 kg weight was attached to the ring of the plankton net to help it sink to the desired depth. After each plankton tow was completed the net was brought to the surface and rinsed thoroughly before putting the samples in labeled containers. In addition, surface water samples were collected to measure salinity (ppt), temperature (°C), nitrogen (ppm) and pH.

The samples were stored in the refrigerator before being counted. All samples were mixed thoroughly and a 1mL sub-sample was extracted. The sub-sample was then placed in a Petri dish and viewed under a dissecting microscope for counting. All organisms were counted and identified under major groups found in *A Guide to Marine Coastal Plankton and Marine Invertebrate Larvae* (Smith and Johnson 1996). Voucher specimens of the zooplankton for each site and depth from Opunohu Bay are stored at the University of California Berkeley Museum of Paleontology.

To standardize sample counts, I calculated the specie abundance per m^3 (*A*, Equation 1) as follows:

$$A = \frac{(n * s)}{w} \qquad \text{Equation 1}$$

where *n* is the total count of a specie in the sample (individuals per mL), *s* is the volume of the sample (mL) and *w* is the sampled water volume (mL) (Perry 2003). A T-test was used to calculate if there was a significant differences in the zooplankton composition between the bays. In addition, a Discriminat Analysis (MANOVA) was performed to test for differences in the zooplankton community for the sites at each bay and between bays.

Furthermore, a One-way ANOVA was used to test significant differences of zooplankton densities for each site for the dominated taxonomic zooplankton found at each bay. The Simpson's Diversity Index (Equation 2) was calculated for each bay as follow:

$$D = \frac{\sum n(n-1)}{N(N-1)} \qquad \text{Equation 2}$$

where D is the calculated diversity index, n is the total number of organisms of a particular specie and N is the total number of organisms of all species. This index takes into account less present species and it measures the number of species present as well as the abundance of each zooplankton species. The value of D ranges between 0 and 1 and the smaller the value of D the higher the diversity (DeJong 1977). Diversity data is non parametric because its distribution is not known. Therefore, I used a Wilcoxon/Kruskal-Wallis test to test for significant difference of the diversity (D) index between Opunohu and Cook's Bays.

Salinity experiments with copepods

To test the response of copepods to different salinity levels, I collected copepods from site 0 from Cook's Bay because this area has high abundance of copepods. I tested the activity level of copepods under the following salinity treatments: control (9 ppt), 0 ppt, 5 ppt, 10ppt, 15ppt, 20ppt, 25ppt, 30ppt, 35ppt and 40ppt. To obtain the desire salinity treatments, saltwater from the Gump Station dock was collected and filtered. I then added salt and/or freshwater to reach each salinity treatment. In a Petri dish, 50 mL of each water concentration was placed and using a pipette about 5-20 copepods were transfer to each Petri dish. Treatments were observed under a dissecting microscope after 0.15 hr, 6 hrs and 16 hrs and live versus dead copepods were counted. Copepods were classified as being alive if mobile activity occurred within 10 sec and dead if no mobile activity occurred. Three trials were performed under similar temperatures.

ANOVA was used to test the relationship between copepod mortality at varied salinity levels after the end of the experiment (16 hrs). A Tukey-Kramer HSD test showed where the significance occurred between the treatments. All statistical analysis and graphs were performed in JMP.10 SAS Institute Inc. 2012.

RESULTS

Surface water physical variables were similar between both bays with fluctuations in salinity. The surface water temperature ranged from 26ºC to 30ºC for Opunohu and Cook's Bays. Salinity surface water levels varied from 2ppt to 35ppt and increased as the distance from the river mouth increased for both bays. Nitrate was not present in the samples and pH remained constant (pH=8).

Taxonomic composition

The zooplankton samples were dominated by two taxonomic groups: gastropod veligers and copepods, which together made-up approximately 85% of the plankton composition for each bay. Gastropods bivalves was the third highest zooplankton abundant group for both bays with a composition of 8.75% and 3.08% for Opunohu and Cook's Bays, respectively. Shrimp larvae made-up about 4% of the composition in Opunohu Bay while the composition doubled to 8% in Cook's Bay. Two suborders of pteropods were also found although Thecosomata species were more common in Cook's Bay than Opunohu Bay. There were also several types of plankton that occurred at <1% of the samples (Table 1).

Abundance comparison between the bays

The zooplankton composition and distribution was widely variable at each bay and between bays. In Opunohu Bay, site 2 was distinctly different in the zooplankton community compared to the other sites (Figure 2a). However, site 3 and 4 were more similar in the composition as were sites 0 and 1. A two-way ANOVA was used to test how sites (distance from river) and depth (surface and bottom) varied for the dominant zooplankton species at the bay (gastropods veligers, bivalves, copepods, and shrimp larvae). However, the test was not significant because of the low sample size. As a result, a one-way ANOVA was performed with the most abundant zooplankton for each site (pooled depth for each site), which showed no statistically significant differences for the site on Opunohu Bay (p-value > 0.05) (Table 2).

The zooplankton community in Cook's Bay was different than in Opunohu Bay. Sites 0 and 1 were distinctly different from sites 2, 3 and 4, which were much similar in the zooplankton community (Figure 2b).

TABLE 1. Percent composition of the sampled plankton for Opunohu and Cook's Bays. "Differences" show the p-value for the T-test.

Taxa	Opunohu	Cook's	Differences
Diatom: Bacillariophyceae	0	0.04	
Ciliophora (Ciliates): Tintinnid	0.03	0	
Foraminiferans	0	0.01	
Dianoflagellates: Ceratium	0	0.03	
Nemertea: Pilidium larvae	0.15	0.17	0.07
Annelida: Polychaetes	0.19	0.15	**0.04**
Arthropoda: Arachinida: Halacaridae	0	0.03	
Arthropoda: Crustacea			
Nauplius larvae	0.43	0.34	0.60
Copepoda	39.24	28.68	0.86
Mysid shrimp	4.74	8.11	0.41
Brachyura zoea larvae	0.03	0.03	0.33
Mollusca			
Gastropod veliger	45.4	56.64	0.53
Pelagic Gastropod (Pteropod)	0.58	2.49	**0.00**
Bivalve larvae	8.75	3.08	0.12
Bryzoa: Cyphonautes	0.14	0.02	0.86
Chaetognatha	0.04	0.12	0.12
Echinodermata			
Echinoid larvae	0.01	0	
Ophiuroidea	0.03	0.01	
Fish eggs/larvae	0.22	0.04	0.20
Total zooplankton abundance per m^3	2.9	4.0	0.63

* Note: bold p-values are statically significant

TABLE 2. One-Way ANOVA testing the difference between the zooplankton abundance (# per m^3) in Opunohu Bay for the 5 sampled sites.

Specie	DF	F-value	p-value
Gastropod veliger	4	0.67	0.62
Bivalve	4	0.54	0.71
Copepod	4	2.54	0.06
Shrimp larvae	4	1.34	0.28
Total zooplankton	4	0.13	0.97

* Note: bold p-values are statistically significant

TABLE 3. One-Way ANOVA testing the difference between the zooplankton abundance (# per m^3) in Cook's Bay for the 5 sampled sites.

Specie	DF	F-value	p-value
Gastropod veliger	4	1.47	0.23
Bivalve	4	1.80	0.15
Copepod	4	2.41	0.07
Shrimp larvae	4	0.57	0.69
Thecosomata	4	2.58	**0.05**
Total zooplankton	4	1.84	0.15

* Note: bold p-values are statistically significant

Furthermore, the abundances of the major dominant taxonomic groups (gastropods veligers, bivalves, copepods, pteropods, and shrimp larvae) were analyzed to test for differences between sites (Table 2). Two-Way ANOVA tests showed no significant difference in the zooplankton abundance between sites. However, Thecosomata species was the only species that was found statistically in higher abundance in site 1 of Cook's Bay (p-value = 0.05).

A comparison between Cook's Bay and Opunohu Bay show that both bays are distinctly different in the zooplankton composition (Figure 2c). Furthermore, Simpson's Diversity Indexes for both bays were similar with an average ± SE of 0.38 ± 0.02 for Opunohu Bay and 0.39 ± 0.03 for Cook's Bay. Wilcoxon/Kruskal-Wallis test showed no differences between the variance of the mean for the Simpson's Diversity Index for the bays (DF = 1, Chi-square = 0.75 and p-value = 0.39) (Figure 3).

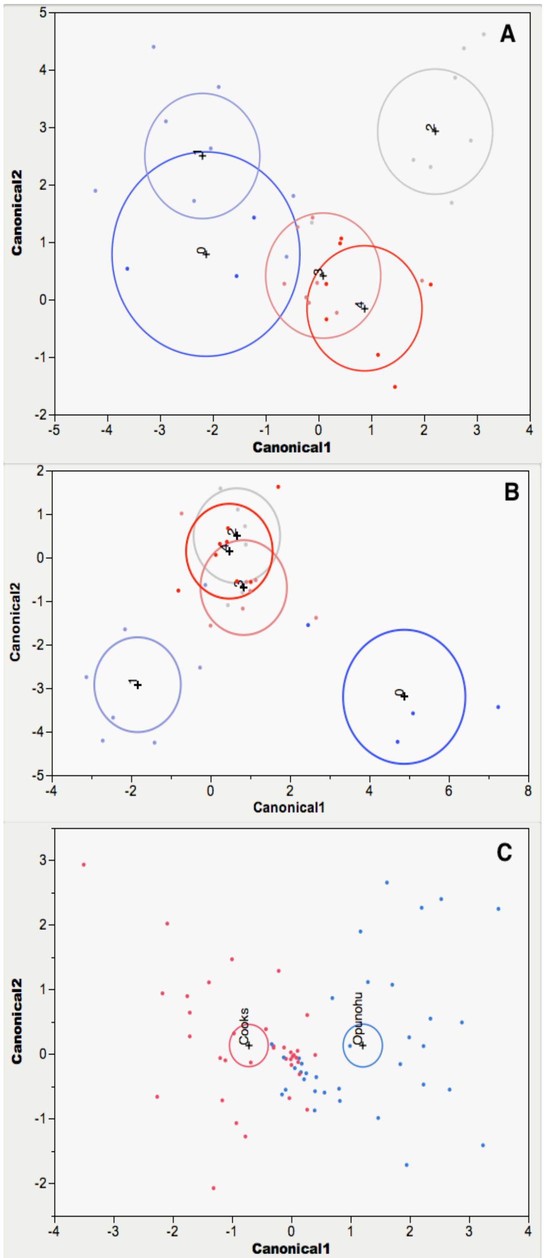

FIGURE 2. Discriminant Analysis MANOVA showing the zooplankton community composition for a) Opunohu Bay (percent-misclassified 51) b) Cook's Bay (percent-misclassified 55) and c) between Opunohu and Cook's Bays (percent-misclassified 12). The Canonical plots are constructed by a multivariable analysis (MANOVA), which compiles all the abundances for all the taxonomic groups into axis. The output is a Canonical Plot in 2-dimensions where the points represent each data point and the circles represent the 95% confidence intervals for a site. The smaller the distance between the confidence circles the more similar the sites.

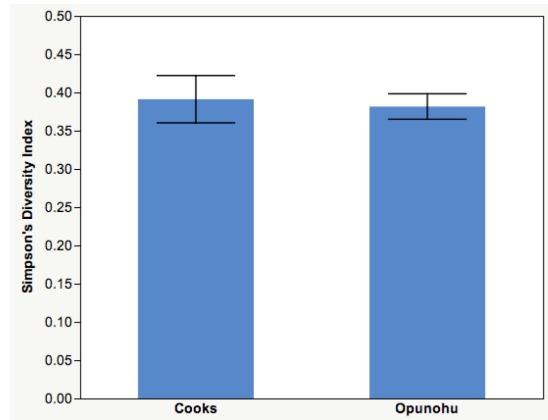

Figure 3. Simpson's Diversity Index for Cook's and Opunohu Bays. The average ± SE of the diversity index was 0.38 ± 0.02 for Opunohu Bay and 0.39 ± 0.03 for Cook's Bay.

Salinity experiments with copepods

The ANOVA test showed significant differences between the treatments for the number of copepods that died after 16 hrs for each salinity treatment (DF = 9, F-ratio = 0.07 and p-value 0.028). The Tukey-Kramer Test showed that the 0 ppt treatment was different from others. Low mortality occurred for 15 ppt and 9 ppt (control) (Figure 14).

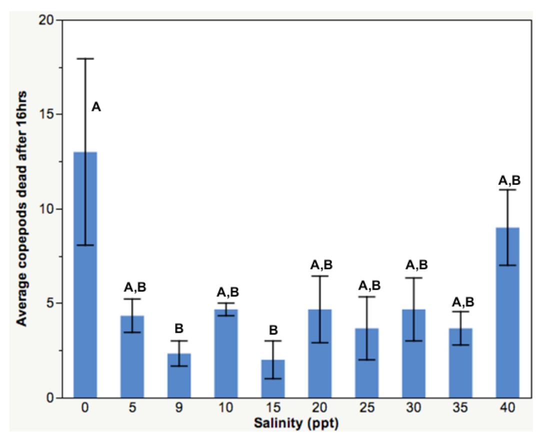

Figure 4. Average copepods dead after 16 hrs for each salinity treatment. ANOVA showed statistically different p-value = 0.028 between the treatments. Tukey-Kramer test showed where the differences occurred at 0, 9, and 15 ppt. Bars sharing the same letter are not significantly different.

DISCUSSION

Taxonomic composition

As in other tropical studies, the zooplankton assemblage was largely dominated by few species in both bays (Achuthankutty 1989). Up to 85% of the zooplankton composition was composed of gastropods veligers and copepods. Although other studies have found copepod species in higher abundances, in some cases compromising up to 70% of the composition (Kimmel 2011), this study found gastropod veligers in higher abundances than copepods. The differences in the zooplankton composition could be explained by the time and methods of sampling.

Since tides were not a variable that was focused in this study, the tidal effects were randomized during the sample collections. The diverse behavior of copepods and gastropod veligers could explain the differences in the zooplankton composition. Copepods experience a diel vertical migration where species are found in higher abundances in the daytime at the deep levels and are more randomly distributed in the water column during nighttime (Ma 2001). This observed behavior is an implication for the predator avoidance theory where copepods are avoiding surface waters during the day to reduce the risk of predations (Ma 2001). Moreover, gastropods veligers are diadromous species, meaning that a life stage is spend in marine environments. As a consequence, higher abundances could represent this veliger stage in marine waters in the bays of Moorea (Canepa 1996). Studies have showed that small copepods could be underestimated if used of net mesh size bigger than 200 μm (Turner 2004). The net use in this study was bigger than 200 μm so copepod abundance could have been underestimated.

Less present zooplankton species were also found. However, the sampling methods might have deteriorated some of the species. The most dominated species have calcified shells/bodies that helped in preservation of the organisms. formalin is the best chemical to preserve samples, but, in its absence I used ethanol, which may have deteriorated the plankton faster. Although samples were counted and identified within 24 hrs, deterioration of soft-bodied organisms is likely to occur within hours. For instance, the pteropod specie was difficult to identified because it soft-body deteriorated within hours and the only left part was the tube like shell that could be mistaken for the spine of a sea urchin (Appendix A).

Abundances comparison between the bays

Although not a clear zooplankton distribution patterns were observed in the two studied bays, the results showed that zooplankton composition is highly variable. High variability occurred specially at the sites closer to the river mouth. The wide range of salinity fluctuation at these sites could explained the distant zooplankton composition that was observed at both bays. Researchers suggest that the high variation in the zooplankton communities could also be the result of strong spatial variability (Avois-Jacquet et al. 2000). However, a study in these bays in Moorea by Canapeda 1996 suggests that higher zooplankton assemblage were found where salinity varies, which is usually closer to the river mouth. This study did found a difference in the zooplankton community at the sites closer to the river, although no clear trend was observed. This suggests that the less present species are highly contributing to the high variability in the zooplankton community throughout the bays.

The zooplankton community is different between the bays although the diversity indexes (*D*) were similar between bays. The diversity index (*D*) represents high diversity in the zooplankton composition for both bays. Adjeroud and Salvat 1996 also found a high diversity, using Shannon-Wiener Diversity Index, in the zooplankton assemblage for Opunohu Bay. Furthermore, the study found high diversity in the zooplankton species from the river-end to the middle of the bay, suggesting that zooplankton respond quickly to fluctuations of river-runoff. Between bays, Thecosomata species were significantly more abundant in Cook's Bay than in Opunohu Bay (Appendix B). The higher abundance of Thecosomata in Cook's Bay occurred at site 1 for one of the sampled days suggested that the plankton net might have collected a swarm of these species, which heavily influenced the distant zooplankton composition between the bays. In Opunohu Bay, copepods where also found closer to the river-end, although trend not significant, suggesting that these species can tolerate fluctuations in salinity.

The high variability in the zooplankton community throughout the bays is reflected by the changes in salinity and the effects of depth and water mixing. Future studies should continue to sample at multiple depths and should take into account water mixing through wave action and wind intensity. As suggested by Adjeroud and Salvat 1996, salinity is more important factor affecting the diversity, abundance and distribution of tropical estuaries than temperature or nutrient inputs such as nitrogen.

Salinity experiments with copepods

The results indicate that copepods in Moorea are able to tolerate a wide range of salinities. However, extreme salinity concentration (too low or high) might directly cause mortality for the organism. The indirect effects of copepods to extreme salinity levels are still being studied. Rosas et al 1999 found that the species of copepods (*Litopenaeus setiferus*) had a lower growth rate at 30 and 40 ppt than compared to natural conditions at 10 ppt. Although I was unable to identify the copepod species to genus, some copepods have a more difficult time regulating rapid changes in salinity levels. Future studies should examine direct (mortality rates) and indirect effects (growth rates and egg hatching success) of tropical copepods when exposed to changes in salinity.

CONCLUSION

Zooplankton communities play an important role in maintaining the diversity, production and health of reef systems and tropical estuaries. Therefore, understanding how the zooplankton assemblage is influenced by salinity fluctuations, as caused by run-off from rivers, rain discharge, and anthropogenic activity could provide insight about the overall health and production of these systems. It is crucial to monitor changes in plankton abundances and composition to better understand how these systems are being affected. In addition, monitoring plankton distribution and abundance could help understand changes in the marine trophic food web.

This study has established a foundation about the zooplankton assemblage for two of the major estuaries of Moorea. To continue the monitoring of the plankton community it is important to expand the timing and location of sample to better understand the spatial and temporal patterns throughout the year. In addition, many plankton organisms have yet to be identified to specie level and rare studies have examined biological traits (e.g. tolerance to salinity levels) and life history characteristics (e.g. distribution) of these tropical organisms. In order to predict changes in the zooplankton community caused by predictable or/and unpredictable disturbances (e.g. anthropogenic vs. storm activities) we first need to understand current trends in tropical ecosystems.

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APPENDIX

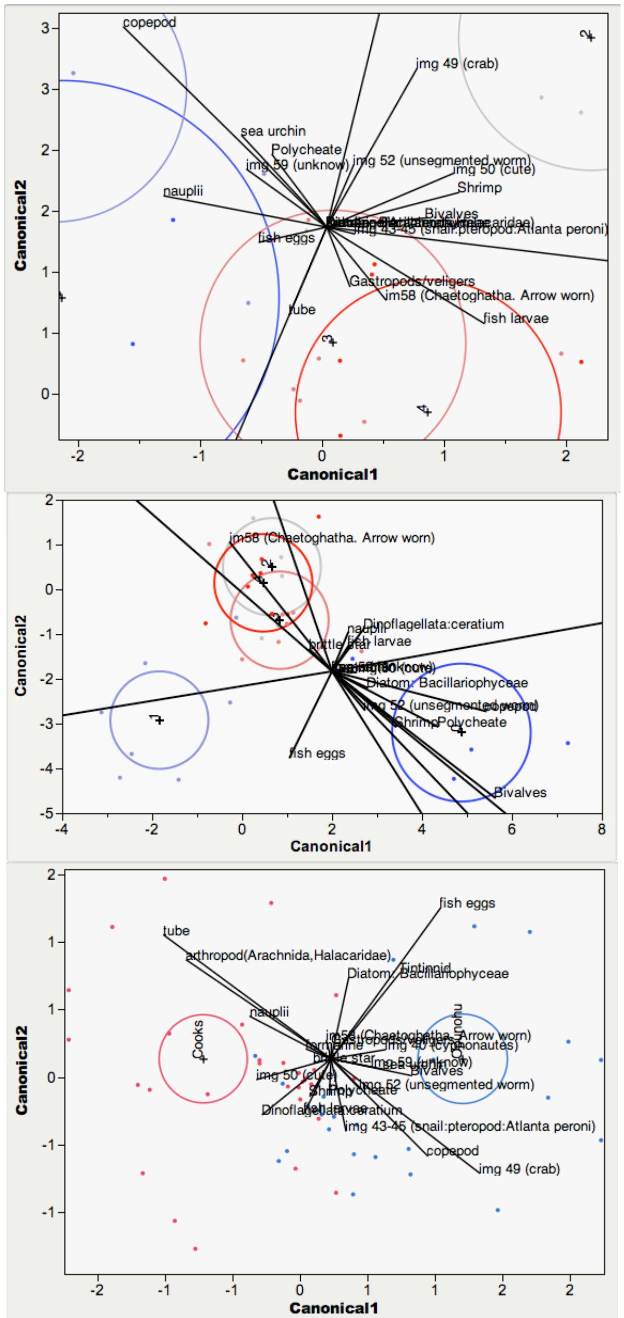


FIGURE A. Discriminant Analysis MANOVA showing the zooplankton community composition for a) Opunohu Bay (percent-misclassified 51) b) Cook's Bay (percent-misclassified 55) and c) between Opunohu and Cook's Bays (percent-misclassified 12). The biplot rays shows, which species are contributing to the difference in the composition between sites. The larger the arrow the more influence of the specie. In addition, the direction of the ray indicates the influence of the specie to each site

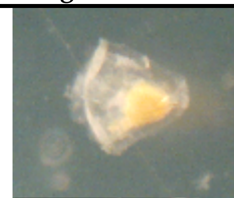
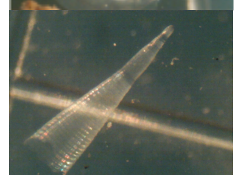
Taxa	Image
Bryzoa: Cyphonautes	
Mollusca: Pelagic Gastropod (Thecosomata)	 
Unknown	

FIGURE B. Selected plankton species that haven't been reported previously in Moorea.