Title

THE EFFECT OF LITOPENAEUS STYLIROSTRIS AQUACULTURE ON MACROALGAE GROWTH IN OPUNOHU BAY, MOOREA, FRENCH POLYNESIA

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Publication Date
2012-12-12

Undergraduate

THE EFFECT OF *LITOPENAEUS STYLIROSTRIS* AQUACULTURE ON MACROALGAE GROWTH IN OPUNOHU BAY, MOOREA, FRENCH POLYNESIA

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Abstract. Aquaculture has many effects on the surrounding landscape. In Mo'orea, the only aquaculture operation is a shrimp farm in Opunohu Bay. To test the effect of shrimp farm runoff into the bay, a macroalgae field survey was conducted at different distances from the Opunohu River, where the effluent outlets. Algae cover was used to indicate nutrient concentration. I found that there is no significant relationship between distance from the shrimp farm outlet and algae cover. The red algae *Acanthophora spicifera* is a cultivated human food source and also a highly invasive species in Hawaii. It is found in Opunohu Bay. To test the effect of shrimp farm runoff on the growth rate of *A. spicifera*, I grew the algae in a laboratory setting in shrimp farm water and in ocean water. I found there to be no significant difference between the growth rates in the two water mediums. Additionally, I found that algae cover increases as distance from shore increases, and that algae prefers rock substrate to sand substrate.

Key words: aquaculture; Litopenaeus stylirostris; Acanthophora spicifera; Mo'orea, French Polynesia; nitrogen runoff, algae

INTRODUCTION

Within historical record, global fisheries have decreased by a significant amount: the oceans can no longer meet the world market's demand for seafood (Pauly 2002, Burford 2003, Kaplan 2012, Jacquet 2007). Aquaculture, a 4000 year-old practice begun in China with carp ponds, has expanded worldwide to supply the market with what fisherman cannot (Rabanal 1988). But aquaculture does not come without its own effects. Manipulating a landscape to raise aquatic species changes the hydrological regimes of the watershed, increases the organic load that enters the water body, and may introduce non-native species to the area (Chua *et al* 1989, Senarath & Viscanathan 2001).

The expansion of shrimp monoculture farming in tropical and subtropical coastal lowlands to meet this seafood demand poses several challenging problems, one of which is concentrated wastewater runoff into marine ecosystems (Páez 2001, Huitric *et al* 2002). Diversifying monoculture shrimp farms into polyculture operations has become a popular idea to mitigate negative environmental impacts and increase the number of marketable outputs (Lin & Fong 2001, Paul & Vogl 2011, Islam *et al.* 2004). Polycultural systems aim to decrease the nutrient load in effluent by adding organisms that use the nitrogen before it reaches the adjacent water body (Jones 2001, Abreu 2009).

Acanthophora spicifera, a red algae native to Florida and the Caribbean, is a cultivated human food source high in nutrients (Kaliaperumal 1986, Lin & Fong 2008, Russel 1992). It is however, one of the most invasive algal species in Hawaii and poses threats to native species in the reef (Weijerman *et al.* 2008, Williams 2007). The potential for *A. spicifera* as a food source and as a formidable invasive warrants a study of how it grows in shrimp farm effluent.

In Mo'orea, French Polynesia, "Crevettes de Moorea", a saltwater shrimp (*Litopenaeus stylirostris)* farm, was constructed at the base of Opunohu Bay in 1979 (Patrois 2011). A 2008 study noted a nutrient gradient in the bay, with more nutrient-rich water at the base of Opunohu Bay and less toward the mouth of the bay (Lin & Fong). Increased levels of nitrogen nutrients in the bay could lead to eutrophication and decreased health of the ecosystem (Huerta-Aldaz 2012, P.T. Anh 2010, Peckol 1994).

The farm has a total of 11 ponds ranging from 1,300-3,800m^2 in area. It constantly pumps seawater from the bay to the ponds and discharges water into the Opunohu River, which then flows into the bay. Every 24 hours 5-15% of the pond water is cycled in this manner (Pamphlet 2012). Each pond contains a different age group of shrimp, ranging from zero to eight months. At the end of eight months, the entire pond is drained into the river, at which point the bay gets a murky brown-green (Dr. David Lin, pers. comm.). Opunohu Valley is a candidate for National Park recognition (Savy 2012). Given this possibility, it is further important to understand the effects of shrimp farm waste on algae growth in Opunohu Bay.

This study surveyed Opunohu Bay using macroalgae cover as a bioindicator to measure nitrogen concentrations at different distances from the Opunohu River farm outlet. It also compared the effects of shrimp farm effluent with open ocean water on the growth rate of *A. spicifera.* I expected that algae cover would increase as distance from Opunohu River decreased. I hypothesized that shrimp farm effluent is richer in nitrogen nutrients than open ocean water, and that algae will therefore grow faster in it.

METHODS

Field survey

The algae survey was conducted in mid November 2012 in Opunohu Bay, Mo'orea, French Polynesia. Opunohu Bay is located on the Northern coast of the island and is one of the two major bays. The bay is surrounded by volcanic mountain and 9.6km^2 of valley. Developments in the valley consist of a shrimp farm, an agricultural school, private homes, agriculture, and small lumber operations.

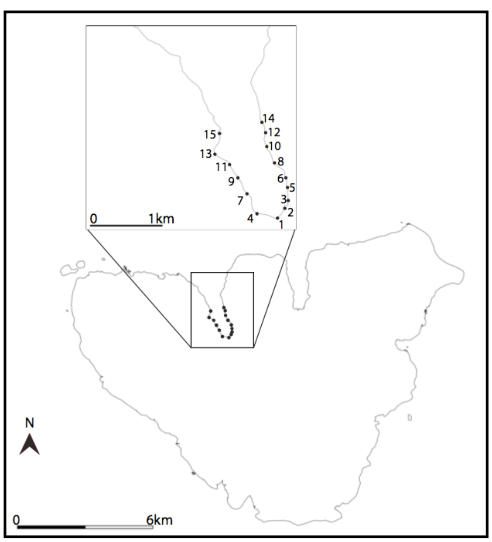

FIG. 1. Opunohu Bay field survey sample sites. The shrimp farm lies adjacent to the Opunohu River, which outlets near site 1 (A. Weiss).

Site	Distance (m)	Site	Distance (m)	Site	Distance (m)
1	100	6	680	11	1350
2	200	7	850	12	1580
3	300	8	980	13	1750
4	450	9	1150	14	1780
5	500	10	1180	15	2150

TABLE 1. Sample sites and distances in meters from the mouth of the Opunohu River.

The field study was performed at 15 different distances from the mouth of the Opunohu River (Table 1, FIG. 1). At each sample site, a 20-meter transect was run perpendicular to the shore, and a 1m^2 quadrat was used to assess percent cover of algae every four meters along the transect. Algae were identified in the field, and unknown species were taken to the lab for further identification. Algae percent cover was capped at 100%, even in the presence of epiphytic algae.

Lab experiment

The shrimp farm (-17.5156414,-149.84944) is comprised of 11 earthen ponds, though only 8 were in use at the time of the experiment (FIG. 2, Table 2). Shrimp production is on an approximate 8-month cycle, after which ponds are completely drained into the Opunohu River and shrimp cultivation begins again. The farm produces 15 metric tons of shrimp per year, sold to individual customers, restaurants, and local markets. Ponds 3 and 4 are input daily with an aquatic feed containing protein, fat, and fiber. All other ponds are additionally input with Vitamin A, Vitamin E, and Vitamin D3.

Samples of *A. spicifera* were grown for three days in 100mL cups of different types of water: water from each of the eight farm ponds and from the Gump Station ocean tap flow, which draws water from Cook's Bay

Pond(s)	Age
6	2 months
7&8	4 months
1-4	7 months
5	9 months

TABLE 2. The ages of shrimp in the ponds.

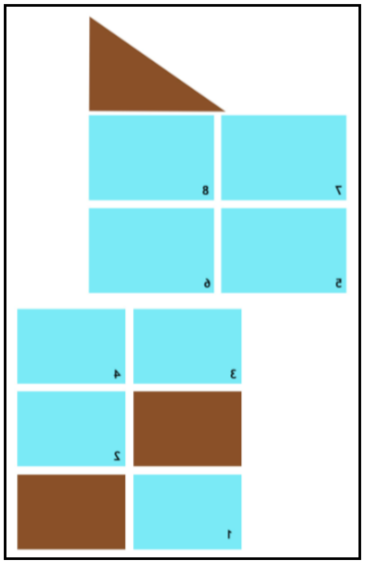

FIG. 2: The shrimp farm. Light ponds are active, dark ponds are not (G. Starrs).

(FIG. 3). Five cups of algae were grown for each type of water, summing to 45 total cups. Water from each of the shrimp ponds was diluted to 50% sample water, 50% ocean tap water to reach a final salinity of around 36ppt, the average salinity of the water from the Gump ocean tap. Water was changed every evening and cups were stirred every mid-day to prevent stagnation of water. The cups sat in a bath of flowing ocean tap water to regulate temperature. The samples were covered during times of rain to prevent fresh water dilution.

Initially, algae were collected from Site 1 in Opunohu Bay (FIG. 1), rinsed in fresh water, blotted dry with a towel, and weighed before being placed in the cups. After three days of growing outdoors, algal masses were

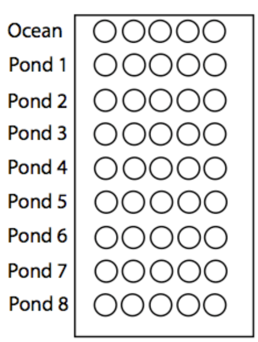

FIG. 3: Lab setup: five cups of *A. spicifera* per water type were grown (A. Weiss).

rinsed and reweighed. The experiment was performed four times from mid October to mid November. Percent growth rate was calculated using the following equation: $[(mass_{initial}-mass_{final})/mass_{initial}]x100$ (Lin & Fong 2008).

Statistical analysis

All tests were run in the statistical program JMP Version 10.0.

Field Survey: To test the relationship between the distance from the mouth of the Opunohu River and the algal cover at a site, I ran a linear regression. Non-normal distributions were transformed by taking the square root of the algal cover.

Lab Experiment: Initially, I ran an ANOVA to test if there was a difference in growth rate between any of the shrimp ponds. This is to test the effect of shrimp pond age on algae growth. Additionally, an ANOVA was run between the mean growth rate of algae in the shrimp farm and the ocean water. This tested the effect of shrimp farm water on the growth rate of algae.

RESULTS

Field survey

After running a linear regression, I found that the algal abundance along the bay does not indicate a nutrient gradient from the Opunohu River to the mouth of the bay (FIG. 4). As distance from the river increased, the 15 sites showed no significant decrease in algae

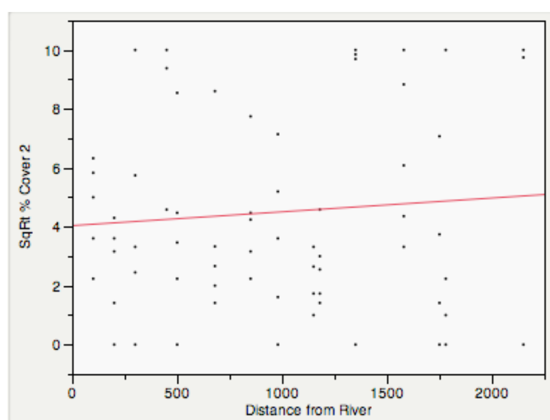

FIG. 4. Bivariate fit of the square root percent algae cover by distance from river. There is not a significant linear relationship between distance from the Opunohu River and algae cover. ($n=15$, $p=0.47$, $r^2=0.0070$, $DF=1$, F-ratio$=0.52$, $SE=0.00065$).

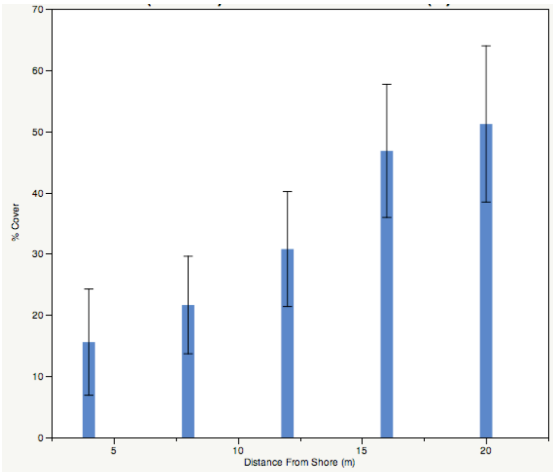

FIG. 5. Mean percent cover vs. Distance from shore in meters. Distance from shore and percent algae cover (n=15, p=0.0029, r^2=0.12, DF=1, SE=0.78, F-ratio=9.51).

cover (p=0.47, r^2=0.0070, DF=1, F-ratio=0.52, SE=0.00065).

In addition to the initial hypotheses, some results became apparent within the field survey. Within the 20-meter transect, I noticed an increase in percent algae cover as distance from shore increased (FIG. 5). A linear regression analysis showed a positive linear correlation between the distance from shore and the percent cover of algae (p=0.0029, r^2=0.12, DF=1, SE=0.78, F-ratio=9.51).

There was also a pattern between percent algae cover and type of substrate (FIG. 6). There were two types of substrate present within the 15 transects: rock substrate and sandy substrate. Using a 2-tailed t-test, I found that rock substrate showed significantly more algae cover than sandy did. (p<0.0001, DF=27.43, t-ratio=6.58, SE=9.19).

Lab experiment

In the lab, *A. spicifera* did not show significant difference in growth rate between ocean water and shrimp farm effluent (FIG. 7). A 2-tailed t-test yielded a p-value of 0.25 (DF=3.77, SE=6.83, t-ratio=1.35). Among the 8 ponds, there were four different ages (FIG. 8). An ANOVA showed there to be no significant difference between the growth rate of *A. spicifera* in the different-aged pond water (p=0.395, r^2=0.054 DF=3, F-ratio=1.01).

DISCUSSION

There was no increase in algae cover closer to the shrimp farm effluent outlet at the Opunohu River. There was a difference, however, in the amount of algae on different types of substrate. Algae showed more prolific growth on rock substrate than on sandy substrate. Additionally, percent coverage of algae increased at greater differences from the shore. This could be attributed to the decrease in the mild wave action farther from the shore, or with decreases in sun intensity as depth increases (Hanekom 2011, Christofoletti 2011).

The current field study was a snapshot of the algae community in Opunohu Bay at different distances from the Opunohu River. For further study, it would be beneficial to add a temporal component to the survey. As common practice, the shrimp farm drains entire ponds at 8-month intervals. An algal survey before and after such a large input of shrimp farm effluent could give better insight into the effects of the shrimp farm on Opunohu Bay. Additionally, surveying different types of organisms besides algae could help increase the scope of understanding of the effects of the shrimp farm on the bay.

In total, I identified 15 types of macroalgae in Opunohu Bay: *Amphiroa fragilissima, Hypnea spinella, Halophila ovalis, Ceramium subdichotomum, Cladophora patentiramea, A. spicifera, Halimeda sp, Padina pavonica,*

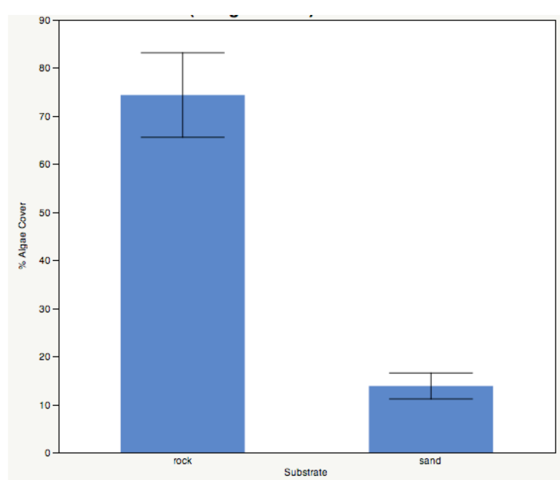

FIG. 6. Mean percent algae cover vs. Substrate. There is a significant difference between the type of substrate and the percent cover of algae (n=74, p<0.0001 , DF=27.43, t-ratio=-6.58, SE=9.19).

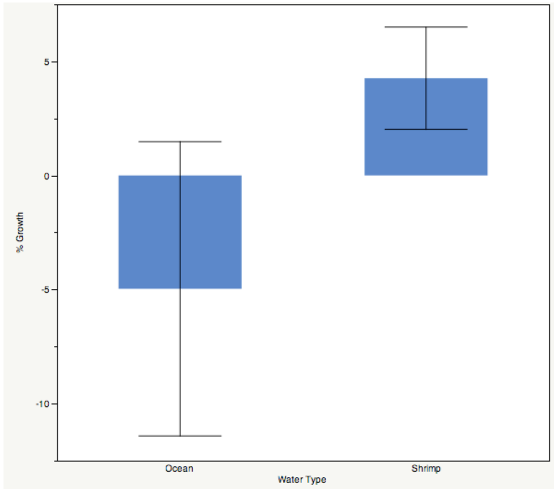

FIG. 7. Mean percent growth vs. Water type. No significant difference between ocean water and shrimp farm effluent in growth rate of *A. spicifera.* (n=61, p=0.25, DF=3.77, SE=6.83, t-ratio=1.35).

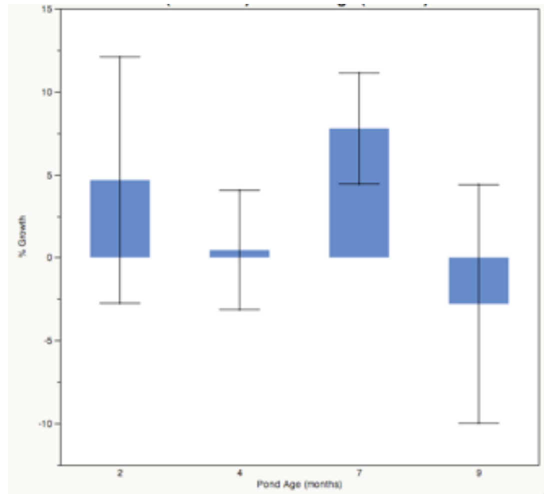

FIG. 8. Mean percent growth vs. Pond age in months. There is no significant difference between the different ponds in how they grow *A. spicifera* (n=57, p=0.395, r^2=0.054 DF=3, F-ratio=1.01).

Dictyosphaeria vershiysii, Dictyota bartayresiana, Turbinaria ornata, Actinotrichia fragilis, Galaxaura filamentosa, Jania micrarthrodia, Boodlea kaeneana. *H. spinella* was present the most and *D. vershiysii* was present the least, but I did not perform a community analysis. I suggest doing so at different sites along the bay to understand the conditions that different algae prefer so that they may be more informative bioindicators.

There were no statistically significant differences in the growth rates of *A. spicifera* between the shrimp farm effluent and ocean water medium. I had predicted that a higher concentration of nitrogen nutrients in the shrimp farm water would increase the growth rate of algae (Boyd 1992, Fox 2012). Although the shrimp-farm water was noticeably greener in color than the ocean water, nitrogen levels at neither the shrimp ponds nor the ocean water were detectable with the tools available. The nitrogen levels of the water before and after a day of growing algae were also undetectable. The green color of the shrimp water could have been the result of higher concentrations of photosynthetic microalgae that were already using nitrogen nutrients before the experimental macroalgae could utilize it (Huang 2012).

Instead of growth, some algae showed a decrease in wet mass within the three-day experiment. This could have been the result of UV-caused tissue deterioration (Britt 1996, Stapleton 1992, Strid 1994). *A. spicifera* collected for the experiment was found in turbid water at a depth of around 1 meter. In the lab, the algae were grown in shallow cups in comparatively clear water. The intense sunlight of the experimental set-up could have affected the growth of the typically fast-growing and resilient algae (Kaliaperumal 1986). Though *A. spicifera* is a critically invasive algae on the reefs of Hawaii, it does not show aggressive growth in Opunohu Bay, and shrimp farm effluent does not increase its growth (Dailer 2012, Weijerman 2008, Williams 2007). For future studies I suggest growing algae in deeper, more turbid water to mimic its environment in Opunohu Bay.

The world is witnessing an increase in consumption by humans, especially as developing countries continue their economic growth (Zhong 2010, Kaplan 2012). Demands for more expensive resources such as edible shrimp are only predicted to increase (van Been 2010, Field 2006). Before more tropical coastal areas develop their shorelines to supply this seafood demand, it is wise to understand the full implications of aquaculture systems. This study suggests that there should be further research in how aquaculture effluent affects the health of the aquatic and non-aquatic systems surrounding it.

ACKNOWLEDGMENTS

I thank Professors Jonathon Stillman, George Roderick, Vince Resh, and Brent Mishler for their guidance. GSIs Rosemary

Romero, Darcy Kato-Ernst, and Matthew Luskin for 24/7 support and amusement. Special thanks to my field buddies Jackie Hurley and Sergey Piterman, and to the Mo'orea Class of 2012 for being an especially cooperative group. Thanks to the hardworking staff of the Gump Station for fixing everything we broke. Thanks to the Ramsden fund, and to my parents for their unending support. And a very special thanks to Neetha Iyer, Vickie Ly, Genoa Starrs, and Amelia Weiss, whose formatting and technological prowess helped to create this document.

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