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The Effects of Pineapple Farm Runoff on Diatoms in Freshwater Streams of Moorea, French Polynesia

Author
Wong, Eileen A.

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THE EFFECTS OF PINEAPPLE FARM RUNOFF ON DIATOMS IN FRESHWATER STREAMS OF MOOREA, FRENCH POLYNESIA

EILEEN WONG

Environmental Science, Policy, and Management, University of California, Berkeley, 94720 USA
eileenw@berkeley.edu

Abstract. Pineapple farms dominate the agricultural landscape of Moorea, French Polynesia, with over 250 hectares of pineapple farmland.. Agricultural runoff has been well known to affect stream ecosystems, in particular, photosynthetic organisms such as diatoms. Few studies have looked specifically at environmental effects of pineapple farm runoff. This study looks at how 1) pineapple farms affect stream chemistry, 2) pineapple farms affect diatom assemblage, and 3) individual effects of herbicides (atrazine and diuron) and fertilizers on diatom populations. No significant differences in stream chemistry or diatom assemblages in farm affected and unaffected areas were observed. Herbicides and fertilizers did not have any significant effects on diatom species richness and abundance.

Key words: diatoms, pineapples, agriculture, freshwater streams, Moorea, French Polynesia

INTRODUCTION

The conversion of land into agricultural areas has had many negative effects on the environment, including soil nutrient loss, erosion, destruction of habitats, and accumulation of agricultural chemicals in water bodies. In particular, the effects of agricultural runoff, the leaching of surface water and chemicals from agricultural land, have long been studied. Previous studies have documented the presence of fertilizers and herbicides in streams near agricultural areas after application (Pfeuffer and Matson, 2001; Green et al., 1977). Fertilizers have been shown to increase nutrient load in water, causing quick and sometimes toxic eutrophication of surface waters (Silva et al., 2000; Csatho et al., 2007; Carpenter et al., 1998). Studies of herbicides in surface water have shown that they may negatively affect photosynthetic aquatic organisms, although the organisms may recover (Graymore et al., 2001; Gustavson 2003; Huber, 1993).

Diatoms, a fundamental component in many ecosystems, are sensitive to many biological, physical, and chemical changes in environment (Stoermer and Smol, 1999). In particular, studies have shown freshwater diatoms to be affected by agricultural runoff in continental streams (Winter and Duthie, 2000a; Lavoie et. al, 2004; Winter and Duthie, 2000b). Agricultural runoff is composed of many different types of fertilizers and herbicides. Added nutrients from agricultural fertilizers have been shown to increase diatom populations in some stream systems (Davies et al., 2006). However, the effect of herbicides on diatoms remains unclear (Legrand et al., 2006; Seguin, 2001; Leboulanger et al., 2001; Downing et al., 2004).

The island of Moorea in French Polynesia offers a unique setting in which to study the influence of pineapple farming on freshwater stream organisms. Although Moorea has several types of agriculture, including cattle pastures, pineapple farms, banana farms and papaya farms (personal observation), pineapple plantations are the most widespread and have the greatest potential biological impact, accounting for over 250 hectares of pineapple farms on the island (Coco Teraiharoa, personal communication). Although local government, farmers, and agricultural schools are currently working together to develop better and less invasive techniques for pineapple farming, Moorean pineapple farmers currently still use fertilizers, herbicides, and hormones to increase crop yield (Coco Teraiharoa, personal communication).

Although other studies have found agricultural runoff to affect freshwater stream ecosystems (Davies, 2006; Legrand et al., 2006; Winter and Duthie, 2000a), little information about pineapple farm runoff is currently available. This study explores the potential impact of herbicide and fertilizer use in pineapple agriculture on diatoms of nearby freshwater streams. I test the following hypotheses: 1) agricultural runoff from pineapple farms affects stream chemistry; 2) herbicide and fertilizer runoff affects freshwater diatom assemblages; and 3) herbicides and fertilizers have respective negative and positive effects on diatom population sizes. These hypotheses were tested in the field and under controlled lab conditions. In the field component, water and diatom samples were collected from streams upstream, adjacent to, and downstream from pineapple farms. In the experimental component, diatoms were treated with herbicides or fertilizers commonly used in pineapple farms to determine individual effects of these chemicals.

MATERIALS AND METHODS

Study sites

The study took place in two freshwater streams on Moorea, French Polynesia (Fig. 1). Stream sites were selected based on whether they were year-round freshwater streams and if they ran within 50 meters of at least one pineapple plantation. For all streams, the study sites were chosen to have minimal impact from non-agricultural sources. Any additional influences were recorded. All stream collections were conducted in October and November, at the beginning of the wet season in Moorea.

The Pao Pao Valley site is a branch of the Pao Pao River that runs alongside a 2 hectares pineapple farm, with no observed upstream influences.

The Opunohu Valley Co-op is 20 hectares large, and is shared for pineapple farming by several different farmers. A year-round stream runs alongside the pineapple farms. There are few other agricultural plants in the area.

Sampling and sample preparation

Each stream site consisted of three sub-sites; one upstream, one in the middle, and one downstream of the pineapple farming area. Upstream sub-sites were at least 5 meters from the upper edge of the pineapple farms, as close to that measurement as was accessible. Middle sub-sites were judged to be as close to the middle of the pineapple farm as possible. Downstream sub-sites were at least 5 meters from the downstream edge of the pineapple farms. At each sub-site, 4 samples were collected along a 15 meter transect, each collected at 3 meters, 6 meters, 12 meters, and 15 meters. Diatoms were collected by scraping a 2 centimeters by 2 centimeters square off the top of a rock retrieved from the middle of the stream into a vial of the stream water. All rocks sampled had a top surface area of at least 5 centimeters by 5 centimeters.

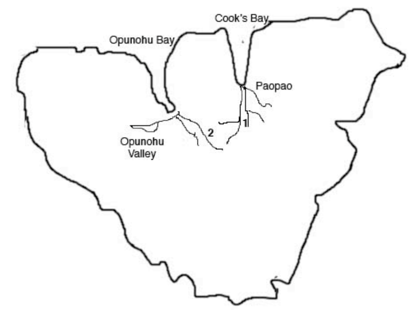

FIG 1. Locations of stream sampling sites on the island of Moorea, French Polynesia. Site 1 is in Pao Pao Valley and Site 2 is in Opunohu Valley. Both streams were adjacent to pineapple farms.

In addition, canopy cover, water temperature, stream width and depth, flow rate, and rock size were recorded at each sub-site. An additional cup of stream water was collected from where each rock was scraped and taken back to the laboratory for nitrogen, phosphorus, ammonia, and pH testing. Nitrogen levels were measured using the Lamotte Nitrogen-Nitrate testing kit (Chestertown, MD), while ammonia and phosphorus levels were measured using Sera aquarium testing kits (Heinsburg, Germany). The pH values were measured using a pH meter (YM Instrument Co. Ltd., Jiangyan, China). All water analyses were done within a week of collection date (Allen-Diaz et. al, 1998). Each diatom sample was mixed with a small amount of a 90% ethanol and Rose Bengal (Fisher Scientific, Waltham, MA) to preserve diatoms. After homogenizing, the samples were filtered through a 500 micrometer sieve, and 2 milliliters of hydrogen peroxide was added to each sample. The samples were heated in a drying oven at 75 degrees Celsius until each sample had approximately 2 milliliters of liquid left. The samples were homogenized and mounted with Permount (Fisher Scientific, Waltham, MA) on cleaned glass slides. They were then inspected under a microscope at 1000x magnification to identify and count diatom populations. For each slide a line in the middle of each slide was inspected in order to estimate total populations.

Experimental component

Fifteen rocks were collected randomly from the upstream portion of the Opunohu Valley Co-op stream study site. The rocks had a surface area of at least 5 centimeter by 5 centimeter and approximately the same size. Each rock's initial diatom population was determined by scraping of a 1 centimeter by 1 centimeter square with a razor blade and smearing evenly on a glass slide. A line across the middle of the slide was inspected by microscope under 1000x magnification for diatom identification and numbers. Three of these sub-samples were done for each rock. The rocks were then placed in individual plastic containers with 2 liters of unfiltered stream water, and divided into 3 treatment groups, with 5 rocks in each treatment group. The first group was treated with herbicides, using atrazine and diuron. The herbicides were mixed in 2 liters of unfiltered stream water in a ratio of 1 μg/L atrazine and 0.25 μg/L of diuron before addition to each container. The next five containers were treated with a fertilizer that included nitrogen, potassium, and phosphate. The fertilizer was mixed in 2 liters of unfiltered stream water in a ratio of 3.5 milligrams fertilizer to 1 liter of water before addition to each container. The last five containers were untreated controls that were filled with 2 liters of unfiltered stream water.

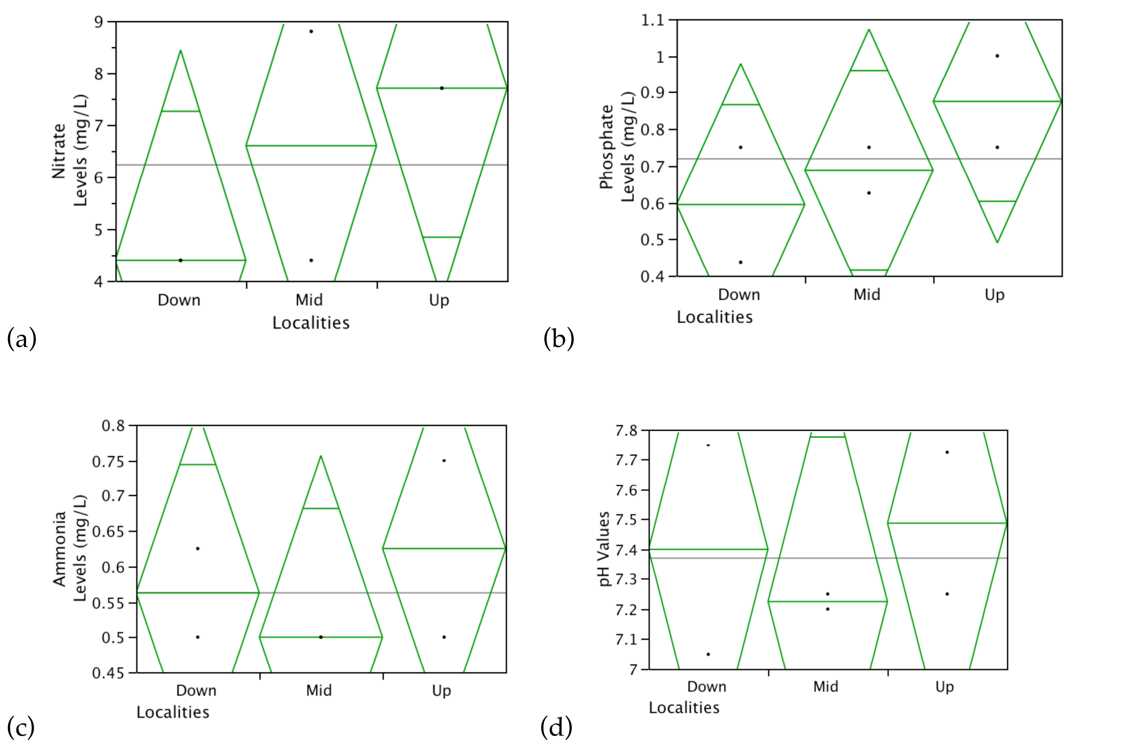

FIG. 2. Results of ANOVA tests of water analyses results by locality. (Up=upstream, Mid=midstream, D=downstream) (a) nitrate levels (mg/L), DF=2, F-ratio=1.75, p=0.3136; (b) phosphate levels (mg/L), DF=2, F-ratio=1.4, p-value=0.372; (c) ammonia levels (mg/L), DF=2, F-ratio=0.6, p-value=0.6037; (d) pH values, DF=2, F-ratio=0.2985, p=0.7617.

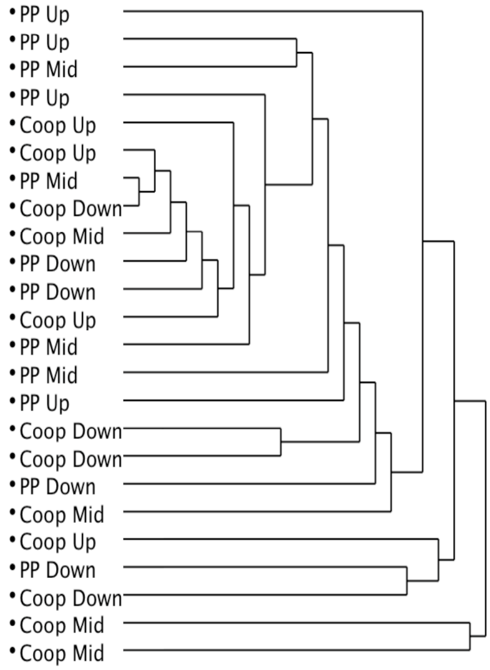

FIG. 3. Hierarchical cluster of diatom assemblages from upstream, midstream, and downstream of Pao Pao and Opunohu Valley streams. (PP=Pao Pao, Coop= Opunohu Valley)

The experiment was left to run for 27 hours. At the end of the experiment, two 1 centimeter by 1 centimeter squares were scraped off each rock with a razor blade and then smeared on a glass slide. A line across the middle of the slide was inspected by microscope under 1000x magnification for diatom identification and numbers.

Statistical methods

All statistics were conducted using JMP 7 Statistical Software. Differences in nitrate, phosphate, ammonia, and pH mean values between upstream, midstream, and downstream were examined by analysis of variance (ANOVA). Differences in diatom assemblages between the upstream, midstream, and downstream parts of the stream were analyzed with a hierarchical cluster. For the experimental component differences in mean before and after diatom abundance and species richness were examined by ANOVA and Tukey-Kramer tests.

RESULTS

Field results

Nitrate levels varied between 4.4 and 8.8 μg/L, with no statistical differences between the means of the upstream, midstream, and downstream samples (ANOVA, DF=2, F-ratio=1.75, p=0.3136) (Fig. 2a). Phosphate levels varied between 0.5 and 2 μg/L, also with no significant differences between the means of the different stages of the stream (ANOVA, DF=2, F-ratio=1.4, p=0.372) (Fig. 2b). Most samples had ammonia levels of 0.5 μg/L, with a range from 0 to 1 μg/L. Like the other nutrients, there were no significant differences in mean ammonia values (ANOVA, DF=2, F-ratio=0.6, p=0.6037) (Fig. 2c). The pH values from the upstream, midstream, and downstream were neutral, with a range from 6.8 to 7.9. The mean pH values of the three stages did not have any significant differences (ANOVA, DF=2, F-ratio=0.2985, p=0.7617) (Fig. 2d).

Diatom species composition and abundance widely varied across all the samples. No significant similarities were shown within upstream, midstream, or downstream samples, and no significant differences were shown between upstream, midstream, and downstream samples, as shown by the hierarchical cluster analysis (Fig. 3). Therefore, no particular area of the stream can be distinguished according to diatom assemblages. The small cluster in the middle of Figure 3 is most likely a result of particularly low numbers of diatoms in those samples.

Laboratory Results

A slight trend of decreasing diatom species richness after herbicide treatment as compared to the fertilizer or control treatment can be evident from a comparison of the species richness values from before and after treatment (Fig. 4). However, this trend has no statistical significance, as shown by ANOVA and Tukey-Kramer tests of changes in species richness (DF=2, F-ratio=1.9518, p=0.1846) (Fig. 5). Diatom abundance is variable, and the changes between the abundances before and after the

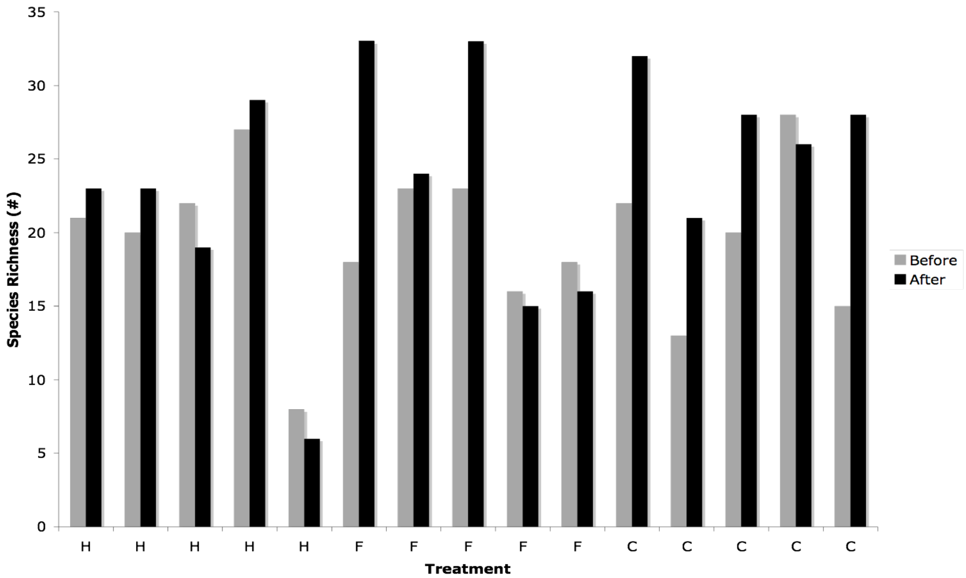

FIG. 4. Bar graph of delta species richness before and after treatment with herbicides, fertilizers, or control. (H=herbicides, F=fertilizers, C=control) Changes in species richness in herbicide treatment samples are noticeably less than changes in fertilizer or control treatments.

experiment are not significantly different (DF=2, F-ratio=0.446, p=0.6504) (Fig. 5).

DISCUSSION

The results of this study show that pineapple farms of Moorea seem to have little effect on their neighboring streams. Any agricultural runoff appears to have no significant effect on the nutrient and pH levels in the stream. In addition, diatom assemblages do not seem to be noticeably affected by pineapple farms.

These results do not agree with the findings of previous studies of agricultural runoff on diatoms. Rott et al. (1998) found a clear correlation between diatom species composition and organic pollution from farmlands in the Grand River in Ontario. Lavoie et al. (2004) examined diatom communities as bioindicators and found a significant difference in diatom communities in agriculture sites compared to control sites. A study done by Winter and Duthie (2000a) was even able to find a calibration modeling for the relation of diatom populations and total phosphorus and nitrogen in the streams.

There are several reasons that could explain the contrasting results of this study. One explanation is that the pineapple farms of Moorea do not have any agricultural runoff. Perhaps this study took place in the wrong season, before the next set of chemical treatments, causing the streams to appear to be unaffected by pineapple farms. In a study by Winter and Duthie (2000b), there were no consistent differences in diatom species number until seasonal variation was taken into account. Although the exact dates of treatment are unknown, Moorean pineapple farmers typically apply fertilizer treatments two to three times a year, and herbicide treatments one to three times a year, both typically after the rainy season (Coco Teraiharoa, personal communication). This study began and ended at the beginning of the wet season in Moorea.

Also, there is a possibility that other factors in the stream that were not tested in this study had an overwhelming effect on the nutrients and diatom levels, so that any differences caused by runoff were overcome. Some past studies have shown mixed conclusions about factors, such as phosphate versus atrazine levels, canceling each other's effects (Guasch et al., 2007, Guasch et al., 1998). Finally, the way that the diatoms were sampled may have caused some error in counting of populations. I only tested and sampled two streams on the island, each from a different valley. The small sample size may not have accounted for unfactored variations in the streams.

The results from the experimental study of the direct effects of herbicides and fertilizers on diatom populations were inconclusive. Although a slight trend in herbicides having decreased species richness compared to fertilizer and control treatments is evident, no significant results could be concluded. However, since the herbicide treatment only shows a slight decrease in species richness, and not species abundance it may be that only some species are affected by herbicide conditions. In the study by Leboulanger et al. in 2001, only a few species of diatoms were affected by herbicides.

Results from previous studies of herbicides on diatoms also show varied findings. Legrand (2006) found that increased amounts of atrazine and diuron herbicides caused decreased photosynthetic efficiency in diatoms. In contrast, a study by Downing et al. (2004) showed increases in diatom taxa and abundances after exposure to pesticides such as atrazine.

However, the lack of significant results in this experiment could be explained by the time limitations in the study. Although diatoms have a quick generation time, with doubling times possible from 0.3 to 5 days, the experiment may not have lasted long enough for the specific species of diatoms from Moorean freshwater streams (Cox, 1996). In addition, my sampling methods may have caused inaccuracies in species richness and abundance. Looking at only a small fraction of each slide may have caused some errors in counts of species richness, as some species may have been less common and thus not necessarily in the line of sampling.

To find more accurate, and perhaps more significant, results, larger sample sizes

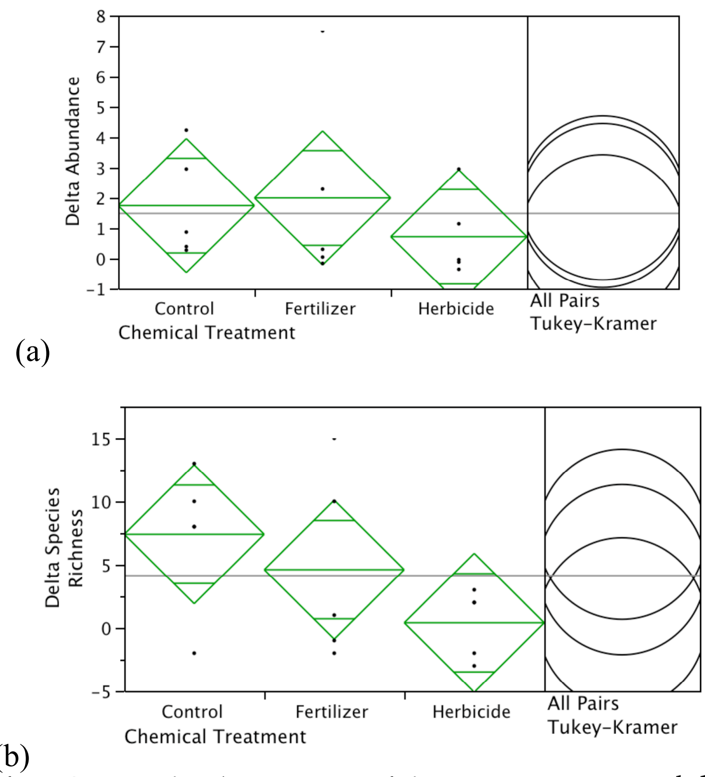

FIG. 5. Results of ANOVA and Tukey-Kramer of diatom experiment with herbicide, fertilizer, and control treatments. (a) Delta abundance of diatoms between the three treatments (DF=2, F-ratio=1.9518, p=0.1846); (b) Delta species richness of diatoms between the three treatments (DF=2, F-ratio=0.446, p=0.6504).

are needed. With more streams and better sampling techniques, more accurate counts of diatoms can be done, possibly leading to more significant data. Perhaps instead of looking at diatom abundances and species richness, measurements of chlorophyll a activity could be done, as in several other diatom studies. Future studies should also take into account seasonality and chemical application times, preferably looking at streams over an extended period. For future experiments, longer experiment times are necessary to ensure enough time for diatom population growth. Taking samples from experiment rocks more often would also provide more complete data on the effects of herbicides and fertilizers on diatoms. Future studies could include a field experiment to simulate effects of runoff on the stream.

CONCLUSIONS

The results from this study show that agricultural runoff does not affect stream chemistry, nor does it significantly affect diatom assemblages in freshwater streams. Any effects of herbicides and fertilizers on diatom species richness and abundance were statistically insignificant. However, a slight trend toward herbicides decreasing species richness compared to fertilizer and control treatments show that further and longer experiments are necessary. Even though results from this study show little effect of pineapple farms on freshwater stream diatoms, more studies during different seasons are needed for more complete conclusions.

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