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Macroalgal bioindicators (growth, tissue N, $\delta^{15}N$) detect nutrient enrichment from shrimp farm effluent entering Opunohu Bay, Moorea, French Polynesia

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Abstract

Nutrient enrichment from shrimp aquaculture poses an increasing environmental threat due to the industry's projected rapid growth and unsustainable management practices. Traditional methods to monitor impacts emphasize water quality sampling; however, there are many advantages to bioindicators, especially in developing countries. We investigated the usefulness of three bioindicators—growth, tissue nitrogen content and nitrogen stable isotope signature ($\delta^{15}N$)—in the tropical red macroalga *Acanthophora spicifera*. Algae were collected, cultured, and deployed in a spatial array around the outflow from a shrimp farm in Moorea, French Polynesia, to detect nitrogenous wastes. All three parameters were highest adjacent to the shrimp farm indicating nutrient enrichment, and $\delta^{15}N$ values confirmed the shrimp farm as the dominant nutrient source (5.63–5.96‰). Isotope ratios proved the most sensitive indicator, as $\delta^{15}N$ signatures were detected at the most distant sites tested, confirming their usefulness in tracing nutrients and mapping the spatial extent of enrichment.

Keywords: Aquaculture; Effluent; Bioindicators; Nutrient enrichment; Stable isotopes; Macroalgae; Shrimp farming

1. Introduction

Shrimp aquaculture is a rapidly growing industry (FAO, 2006). Global production of shrimp has increased by more than 600% between 1984 and 2004 and is expected to continue this pattern through 2030 (FAO, 2006) due to the increasing global demand and high value of cultured shrimp (Rana et al., 1996). Poorly managed coastal shrimp farming has been cited for degrading nearshore water quality through nutrient enrichment (Boyd and Clay, 1998; Hargreaves, 1998). Shrimp pond effluent is generally greatly enriched in nitrogen (N) due to animal feces, added fertilizers and uneaten feed (Primavera, 1993), and it is common practice to release this enriched effluent directly into adjacent waters (Hargreaves, 1998; Naylor et al., 1998, 2000).

Nutrient enrichment from shrimp aquaculture is of particular concern due to (1) the projected rapid growth of the industry, (2) potential ecological impacts on adjacent nearshore ecosystems, and (3) possible implications for farm productivity.

Coastal shrimp farming predominantly occurs in warm, tropical regions and often among mangrove forests (Boyd and Clay, 1998; FAO, 2006). Many studies have shown tropical marine ecosystems to be highly susceptible to the impacts of excess nutrients (for a review, see Downing et al., 1999). For example, nutrient enrichment has been associated with worldwide declines in seagrass and coral reef ecosystems (McGlathery, 2001; Bellwood et al., 2004).

Nutrient enrichment could also affect the shrimp farms themselves through self-pollution (intake of their own or neighboring farms' waste effluents). Poor water quality

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may reduce farm productivity by diminishing shrimp growth and/or promoting shrimp disease outbreaks (Hargreaves, 1998; Lin, 1989).

To detect and monitor nutrient enrichment from shrimp farm effluent, researchers have traditionally employed water quality sampling techniques (Burford et al., 2003; Biao et al., 2004; Islam et al., 2004). Very few have used macroalgae as bioindicators (but see Jones et al., 2001; Costanzo et al., 2004), even though their use may be less expensive and labor-intensive than traditional water quality sampling. This factor is particularly important for long-term and large-scale environmental monitoring projects (Costanzo et al., 2001). Also, macroalgal bioindicators are able to reveal an *in situ* history of nutrient bioavailability in a particular ecosystem through their tissue nitrogen content (%N) and subsequent growth (Fong et al., 1994). They can even record non-steady nutrient flows or pulses, which are difficult to measure and often missed by direct grab sampling for water quality (Fong et al., 1998, 2004). These advantages aid in the study of temporal and spatial dynamics in the magnitude of nutrient supplies.

Stable isotopes have been used to trace nutrients in the water to sources characterized by their specific $\delta^{15}N$ signatures (Heaton, 1986; Peterson and Fry, 1987). For example, fertilizer-rich agricultural run-off typically features low $\delta^{15}N$ signatures close to 0‰, while human sewage produces $\delta^{15}N$ values from 6 to 10‰ (depending on the amount of denitrification and ammonification during treatment; Costanzo et al. 2001). Previous studies in Australia found shrimp farm effluent to produce a $\delta^{15}N$ signature of approximately 4.2–6‰ (Preston et al., 2000; Jones et al., 2001; Costanzo et al., 2004). Tropical oceanic water is typically low in $\delta^{15}N$—usually less than 3‰ (Costanzo et al. 2001). Differences among stable isotope signatures aid in relating nutrients in algal tissues back to their original sources. In addition, one recent study suggested $\delta^{15}N$ values in macroalgae may be particularly effective in tracing nutrient sources, as at least one species did not fractionate or select for specific nitrogen isotopes across a wide range of concentrations and isotope ratios (Cohen and Fong, 2005). Thus, isotope ratios in macroalgae may directly reflect what is available in the water.

The aim of this study was to test the effectiveness of macroalgal bioindicators and stable isotopes in assessing nutrient enrichment from shrimp farm waste effluents. Measures of macroalgal growth, tissue nitrogen content (%N) and nitrogen stable isotope signature ($\delta^{15}N$) were used to detect and quantify the spatial extent and magnitude of nutrient enrichment. Thalli of the tropical macroalga *Acanthophora spicifera* (Rhodophyta) were collected and cultured. Subsamples were deployed in a spatial gradient from the effluent outflow channel of a commercial shrimp farm, and responses were assessed after 3 days. Previous studies have shown *A. spicifera* tissues to absorb and store excess bioavailable nutrients and respond with growth (Fong et al., 2001).

2. Materials and methods

This study was conducted in Opunohu Bay on the island of Moorea, French Polynesia (Fig. 1) from late May to early June 2004 (late fall-winter). The northern two-thirds of the bay are characterized by fringing and barrier coral reefs. Periodically, strong winds blowing offshore from the south (the Tahitian *mara'amu* during the Austral winter season) create a mild current out of the bay for the upper 20 m of the water column (Wolanski and Delesalle, 1995). Tidal range in the bay is very limited-less than 0.2 m at spring tides. Thus, surface waters in Opunohu Bay generally followed a gentle northward flow during the course of our study.

Aquaculture Opunohu is a 2 ha intensive shrimp farm at the mouth of the Opunohu River in the southern end of the bay. This farm raises non-native western blue shrimp (*Penaeus stylirostris*) producing up to 800–1200 kg per month year round in thirteen shallow, aerated earthen ponds (800–3900 m^2 each). Commercial feed (40–43% protein) is added periodically. Fresh seawater is continuously pumped from Opunohu Bay into the shrimp ponds. Pond effluent drains through an earthen ditch that empties into the Opunohu River mouth and enters the bay. The only other developments in this valley are experimental lumber tree farms, an agricultural school and a small experimental freshwater shrimp farm.

Five sampling sites in Opunohu Bay were selected for their accessibility and varying distances from the shrimp farm effluent outlet (Fig. 1). The first site was positioned 10 m north of the effluent outlet—referred to as OPF. Next, sites ILL and WES were located 400 and 495 m away, respectively, on the western shore of Opunohu

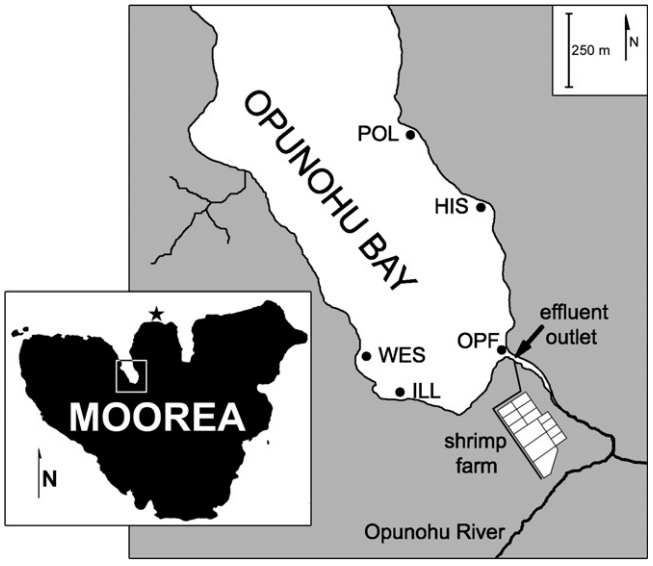

Fig. 1. Opunohu Bay with inset map of Moorea, French Polynesia. Five sampling sites are labeled in capital letters. On the inset map, a star indicates the collection source for the alga *Acanthophora spicifera*.

Bay. On the eastern shore were sites HIS and POL—515 and 800 m away, respectively.

The macroalga *A. spicifera* was selected for use as a bioindicator as it was (1) locally abundant and easy to collect, (2) native to all sampling sites, (3) a fast growing, nutrient opportunist (Fong et al., 2001), and (4) edible and used in Pacific and Asian cuisines (Payri et al., 2000). This coarsely branching, corticated red alga is commonly found on shallow coral rubble in local fringing reefs (Payri et al., 2000; Littler and Littler, 2003). Thalli were collected at 0.3–1.5 m depth from the fringing reef between Cook's and Opunohu Bays on Moorea away from any obvious nutrient sources (Fig. 1). Algae were cultured in an outdoor flow-through tank under mesh screen shade for a minimum of 2 days to subject tissues to uniform nutrient conditions (Fong et al., 2003). Algae were rinsed with seawater and epizoa, epiphytes and sediment were removed. Only healthy thalli were used. To ensure repeatable wet weights, thalli were placed in nylon stockings, spun for 30 s in a salad spinner to remove excess water and weighed.

Algal samples were sewn inside 15 × 15 cm (flat 2-dimensional area; squares) labeled mesh bags of fiberglass window screening to prevent herbivory from all but the smallest invertebrates (Fong et al., 1998). Four replicates were staked to the sediment in each corner of a 1 m^2 quadrat at 1 m depth. The only exception was site OPF where mesh bags were placed at 0.5 m depth due to increased turbidity.

Samples were collected after 3 days of deployment at the sampling sites, rinsed with seawater and re-weighed (final wet-weight). Growth was quantified by percent change in weight. Algae that showed obvious signs of deterioration were excluded from data analysis. All samples were rinsed with freshwater, oven-dried and ground with mortar and pestle before analysis at the UC Davis Stable Isotope Facility. To determine $\delta^{15}N$ and tissue N content (%N) in the algal samples, a Europa Hydra 20/20 isotope ratio mass spectrometer (IRMS) was used. The relative abundance of the heavier isotope, ^{15}N, to the more common ^{14}N was quantified by the term $\delta^{15}N$ as:

$$\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 10^3$$

where R is the ratio $^{15}N/^{14}N$ (Peterson and Fry, 1987).

This entire process was repeated 3 times over 2 weeks. During this time, the shrimp farm did not make any pond-emptying harvests, nor were there any abnormal weather conditions. Thus, we used experimental units deployed at different times as replicates. Since several algal samples were discarded due to deterioration or lost in the field, all sites yielded sample sizes of $n = 4$ with exception to site ILL with sample size $n = 8$.

Differences in mean algal growth, tissue N content (%N), and $\delta^{15}N$ among sampling sites were detected by one-way analysis of variance (ANOVA). Growth data were log-transformed to meet assumptions of normality and variance homogeneity. Transformations were not needed for $\delta^{15}N$ and %N. A Fisher's protected least significant difference (PLSD) *post hoc* test was used to determine significant differences in mean values among specific sampling sites. Differences were considered significant if $p < 0.05$.

3. Results

Growth rate of *A. spicifera* differed significantly among the 5 sampling sites (ANOVA, $p < 0.0001$) with growth at sites OPF and ILL 4–5 times greater than at the more distant sites (Fig. 2). Growth responded as a step function rather than a linear increase with distance, as mean growth was similar and highest in OPF and ILL while the other 3 sites were low and equal to each other.

Tissue nitrogen content (N as % dry wt) of *A. spicifera* differed significantly among the 5 sites (Fig. 3; ANOVA, $p < 0.0001$). Only algae from OPF had significantly elevated nitrogen content that was approximately twice as high as found at the remaining sites. All other sites were similar to each other (PLSD, $p > 0.05$).

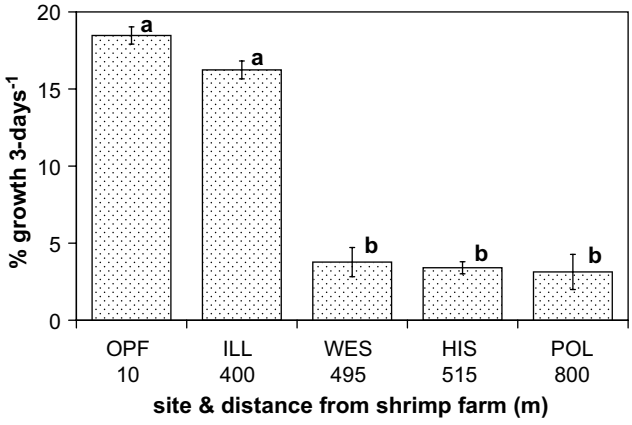

Fig. 2. Growth in *Acanthophora spicifera* after a 3 day deployment period. *Post hoc* associations are shown in lower-case letters. Means that share a letter are not significantly different. Error bars are ±1 SE.

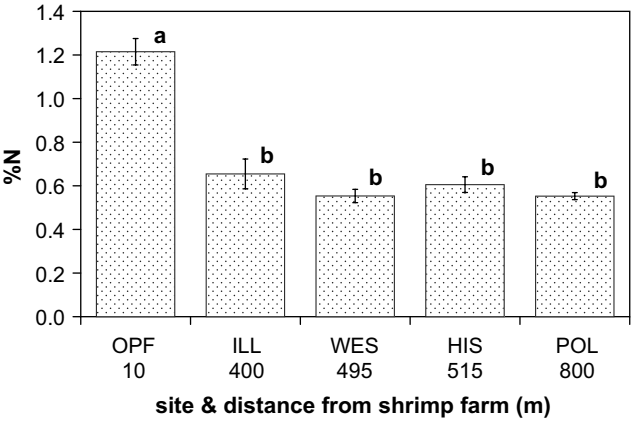

Fig. 3. Tissue nitrogen content (%N) in *Acanthophora spicifera* bioindicators. *Post hoc* associations are shown in lower-case letters. Means that share a letter are not significantly different. Error bars are ±1 SE.

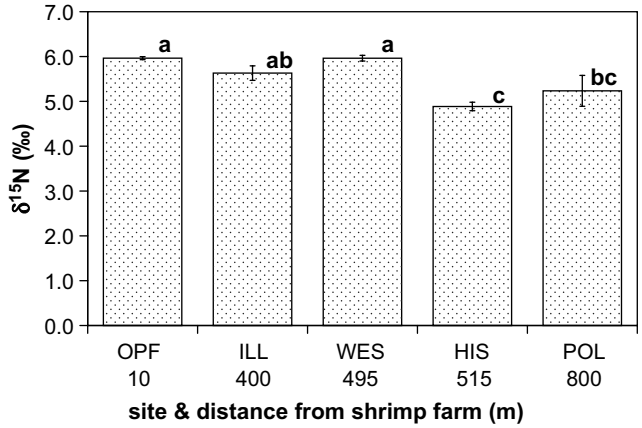

Fig. 4. Nitrogen stable isotope signature ($\delta^{15}N$) in *Acanthophora spicifera*. *Post hoc* associations are shown in lower-case letters. Means that share a letter are not significantly different. Error bars are ±1 SE.

Though differences were small, $\delta^{15}N$ values in tissues of *A. spicifera* varied significantly among sampling sites (Fig. 4; ANOVA, $p < 0.005$). The three sites closest to the shrimp farm (OPF, ILL and WES) had *A. spicifera* with the most elevated $\delta^{15}N$ signatures. The mean $\delta^{15}N$ from these sites range from 5.63‰ to 5.96‰. The more distant sites produced lower $\delta^{15}N$ signatures down to 4.89‰.

In the site closest to the effluent outlet, all three parameters (growth, %N and $\delta^{15}N$) indicated elevated nutrient supplies. However, only growth and tissue $\delta^{15}N$ were elevated in the next closest site (ILL). Elevated $\delta^{15}N$ values—similar to those observed nearest the shrimp farm—extended out to the three most distant sites up to 800 m away.

4. Discussion

Our study demonstrated the usefulness of measures of growth, tissue N content and $\delta^{15}N$ in macroalgae as bioindicators of nutrient enrichment from a commercial shrimp farm. Our results suggest that the most sensitive of these indicators for this species may be isotopic ratios, as this parameter could detect known effluent values at our most distant sampling sites. Decreasing $\delta^{15}N$ values with distance are likely due to greater mixing with oceanic sources of N at the northern sites, as open ocean water is typically low in ^{15}N (less than 3.0‰) in tropical seas (Costanzo et al., 2001). Our results compare with other studies of $\delta^{15}N$ signatures from shrimp farm effluent in Australia (Preston et al., 2000; Jones et al., 2001; Costanzo et al., 2004), where values ranged from 4.2‰ to 6‰ in the red alga *Catenella nipae*. This close-matching of $\delta^{15}N$ signatures suggests that the shrimp farm was the dominant nutrient source in Opunohu Bay rather than agricultural run-off (~0‰) or any sewage discharges (~10‰). Additionally, the point-source nature of the shrimp farm effluent, and the lack of other obvious inputs to the bay reduce the confounding effects of mixed nutrient sources (Costanzo et al., 2003). This study represents one of the few examples in the literature of nutrient enrichment solely from shrimp farm effluents (see also Costanzo et al., 2004). For such clean-catch cases, ^{15}N stable isotope methods may be ideal for studying nutrient enrichment from aquacultural wastes.

Although growth and tissue N content showed a much more localized response to the effluent, both parameters demonstrated an enrichment gradient that peaked at the southern end of Opunohu Bay near the shrimp farm. Other workers have found that growth and tissue N content, measured as concentration, were often confounded as tissue N content can be diluted during rapid growth (Fong et al., 2001, 2003). Although macroalgae often assimilate and store excess bioavailable nutrients in their tissues before responding with growth (Fong et al., 2001, 2003), storage over the longer-term only occurs when nutrient supply exceeds growth rate (Fujita, 1985; Fong et al., 1994, 2004). Macroalgae at intermediate-distance sampling sites likely used excess nitrogen primarily for growth, as nutrient supply was not high enough to merit storage. Thus, these sites yielded lower tissue N values in comparison to the site closest to the shrimp farm.

Our study suggests that nutrient enrichment from even a small commercial shrimp farm was significant on a local scale in a relatively pristine bay. Others have even found these impacts to be locally important in areas where aquaculture contributes only a small percentage of total anthropogenic N, compared to sewage and agricultural sources (Páez-Osuna et al., 1999). One solution to such local impacts may be found in polyculture systems (Sandifer and Hopkins, 1996; Troell et al., 1999; Nelson et al., 2001; Jones et al., 2002), where commercially valuable algae, with high nutrient requirements, are cultured in shrimp pond effluent. *A. spicifera* is edible, and therefore a possible candidate for polyculture. Sustainable shrimp aquaculture not only benefits the environment but the industry as well. Nutrient enrichment can degrade the quality of receiving waters adjacent to shrimp farms (Boyd and Clay, 1998; Naylor et al., 1998; Páez-Osuna, 2001), resulting in self-pollution (Costanzo et al., 2004) and outbreaks of shrimp disease (Lin, 1989). Thus, there is great urgency for more research on developing appropriate methods to monitor the spatial scale of nutrient enrichment associated with shrimp aquaculture and to mitigate its impacts.

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