Assessment of the Ecological Impacts of Two Shrimp Farms in Southern Belize

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Abstract

This study assessed the ecological impacts of the Belize Aquaculture Ltd (BAL) and Aquamar shrimp farms in southern Belize. Water and *in situ* periphyton samples (measuring nitrogen content, $\delta^{15}N$, carbon/nitrogen ratios, and growth) indicated that shrimp farm effluent was influencing receiving waterways. At BAL, periphyton sampling showed significant elevation of nitrogen content, $\delta^{15}N$, and growth within the group of sites below the effluent release creeks. Compared to other BAL sampling sites, nitrate, phosphate, and TSS were the highest and DO the lowest at sample sites in Santa Maria Creek, the major effluent release point at BAL. Seagrass distribution decreased significantly in the middle portion of Placencia Lagoon between 2003 and 2007, coinciding with increased nutrient loading from BAL and adjacent development in the area. At Aquamar, sampling showed significantly higher $\delta^{15}N$ and significantly lower carbon/nitrogen ratios within the group of sites closest to the effluent source (<2500 m). Compared to other Aquamar sampling sites, Nitrate and TSS were highest and DO lowest in Plantation Creek, the major effluent release point at Aquamar. The suite of parameters used in this study helped elucidate the magnitude and extent of the influence of shrimp farm effluent on the two receiving waterways. The results of this study also revealed the strengths and weaknesses of this novel periphyton bioindicator protocol as an effluent monitoring tool.

Introduction

World aquaculture production has increased by 8.8% annually since 1970, spurred by the increasing demands of a growing world population and stagnant or declining global seafood stocks (FAO, 2007). Shrimp is the world's most valuable seafood commodity, with an export industry worth $10.9 billion in 2007 (FAO, 2009). About half of this production is from aquaculture, which grew six fold from 1984 to 2004 (FAO, 2006). Often an economically important industry for developing countries, shrimp farming has been heavily criticized for adversely impacting coastal environments. Primary environmental problems associated with shrimp farming include eutrophication of receiving waters, destruction of coastal habitat, disease transfer, non-native species introduction, and net protein loss in feed conversion (Boyd and Clay, 1998, Naylor et al., 1998; Naylor et al., 2000; Paéz-Osuna, 2001; Primavera, 2006).

Of great concern to many critics are the impacts of effluent from shrimp farms on receiving waterways (Costa-Pierce, 1996; Sansanyuth et al., 1996; Hargreaves, 1998; Naylor et al., 1998; Boyd, 2003). Shrimp feed is high in nitrogen (N) and retention by shrimp is often less than 25% of N inputs (Briggs and Funge, 1994; Boyd and Tucker, 1998; Burford et al., 2003a). Nitrogen remaining in the ponds is generally flushed into surrounding waters, either periodically to alleviate shrimp stress, or at harvest. Excess N can cause eutrophication of receiving waterways, potentially resulting in water chemistry alteration, harmful algal blooms, increased turbidity, low dissolved oxygen, and altered food chains (Ryther and Dunstan, 1971; Paerl, 1988; Kennish, 1992; Nixon, 1995; Smith et al., 1999). Eutrophication resulting from multiple nutrient sources has been implicated in reducing seagrass abundance (Duarte, 1995; Burkholder et al., 2007; McGlathery et al., 2007; Wazniak et al., 2007; Fox et al., 2008). In some circumstances, shrimp effluent has negatively impacted shrimp farms themselves by polluting intake water, affecting crop growth, or promoting disease (Pruder, 1992; Phillips et al., 1993; Hargreaves, 1998).

While the damage caused by shrimp farm effluent can be serious, it is often difficult to measure the extent of its impacts. Research using traditional water sampling techniques to measure effluent has been the norm (Samocha and Lawrence, 1997; Funge-Smith and Briggs, 1998; Islam et al., 2004), but is often seen as inadequate (Jones et al., 2001; Boyd, 2003; Costanzo et al., 2004; Lin and Fong, 2008). A traditional water quality sampling protocol, where periodic samples are taken for analysis, gives only a snapshot of impacts. Such a protocol will

often miss inconsistent nutrient flows and pulses (Wolanski et al., 2000; Fong et al., 2004) typical of shrimp farm effluent timed to farm releases, tides, or weather events (Burford et al., 2003a). In attempts to assess bioavailable N that may be missed in traditional water sampling, some researchers have begun using *in situ* flora or fauna as a bioindicator, particularly deployed macroalgae (Costanzo et al., 2001; Jones et al., 2001; Lin and Fong, 2008). Algae and other organisms can indicate the fate of biologically available N via tissue percent nitrogen (%N) and the carbon to nitrogen ratio (C/N), with a greater %N (Fong et al., 1994; Naldi and Wheeler, 1999; Lin and Fong, 2008) and lower C/N ratios (Hillebrand and Kahlert, 2001; Nelson et al., 2001; Jiménez and Niell, 2003) generally associated with more available nitrogen.

Given the interest in identifying the sources of extraneous nitrogen, many researchers use the unique isotopic signatures of source N as a tracer in the biotic and abiotic environment (Rau et al., 1981; Heaton, 1986; Peterson and Fry, 1987). For example, inorganic fertilizer produced using the Haber–Bosch process has a $\delta^{15}N$ value (ratio of ^{15}N to ^{14}N) close to 0, while fishmeal-based feeds, flocculated particles in shrimp ponds, wastewater effluent, and organic fertilizers tend to have elevated $\delta^{15}N$ values due to biological reprocessing (Costanzo et al., 2001; Fry, 2006; Fertig et al., 2009). An elevated $\delta^{15}N$ signal of source material can often be detected in the tissues of organisms (McClelland and Valiela, 1998; Jones et al., 2001; Cohen and Fong, 2005; Lin and Fong, 2008), providing an indicator of the source of N in the environment. Assessing biological indicators for $\delta^{15}N$ is often superior to direct $\delta^{15}N$ measurements of the water column or sediment (Tucker et al., 1999), as these measurements reduce geographic and temporal variability (Fertig et al., 2009). Measuring *in situ* %N, C/N, $\delta^{15}N$, and maximum growth of organisms that assimilate N quickly can supplement traditional water sampling to give a more comprehensive picture of the temporal and spatial impacts attributed to specific sources.

A review of the relevant literature suggests that this is the first study to use *in situ* periphyton to assess shrimp farm effluent impacts. Compared to some macroalgae techniques (Jones et al., 2001; Costanzo et al., 2004; Lin and Fong, 2008), growing periphyton *in situ* may enlarge the range of possible sampling locations given that it does not require finding a suitable species, a "pristine" harvest site for that species, or incubation in a flow-through tank.

The aim of this study is to assess the ecological impacts of two shrimp farms using traditional sampling techniques and *in situ* periphyton as an N bioindicator. The two farms were selected based on similar total production levels as well as varied production techniques and

effluent release areas. While comparisons between farms are made, the primary focuses of this study are to 1) assess the impacts of each farm on its effluent receiving waterways; and 2) assess periphyton parameters to evaluate the usefulness of each metric in an effluent monitoring protocol. Given the intense interest in shrimp farm monitoring expressed worldwide by producers, consumers, retailers, governments, and NGOs, collecting off-farm data and testing new sampling methods are critically important.

Study Area

This study was conducted in Stann Creek District, Belize between 16°25' and 16°40' N latitude and 88°20' and 88°26' W longitude. The hydrology of this coastal area is influenced by freshwater drainage from the Maya Mountains to the west and by the Caribbean Sea to the east. The Mesoamerican Barrier Reef is adjacent to the study area and provides shoreline protection from wave action. Salinity in the study area ranges from 1 ppt in the creeks to 35 ppt near the mouth of the estuaries. Tides are microtidal and mixed semidiurnal with fluctuations measuring from 12 to 45 cm (PASCO, 2002). The mean temperature is 25.3 ºC with a mean summer temperature of 27 ºC (Boyd and Clay, 2002). Average rainfall is 2203 mm (Boyd and Clay, 2002) with a distinctive wet season occurring from July to October, during which freshwater discharge exceeds dry season discharge by a factor of five to nine (Heyman and Kjerfve, 1999). Lagoon bottom sediments are relatively uniform mud or fine sand with some areas covered in mangrove detritus.

Shallow (1.5 m average depth), mangrove-fringed lagoons characterize this system (Ariola, 2003). Seagrass covers much of this area, with *Thalassia, Halophila*, and *Haladule* meadows and macroalgae *Chara spp.* being prevalent in some areas. Smith and Mackie (2005) found that grazers and the major fishery species in the area derive most of their carbon from seagrass and epiphytes rather than phytoplankton. The study region is home to numerous threatened and endangered species (e.g., West Indian manatee (*Trichechus manatus*), jabiru stork (*Jabiru mycteria)*, hawksbill turtle (*Eretmochelys imbricata),* and Morelet's crocodile (*Crocodylus moreletii)*, provides forage areas for dolphins and sharks, and serves as a nursery ground for reef and other fishes.

Human settlements and industries have a major impact on the study region's landscape. Inland population centers in the study region's watershed include Mango Creek, Santa Rosa, San Roman, Maya Mopan, Georgetown, Independence, Big Creek, Riversdale, South Stann Creek, Bella Vista, and San Juan, with a cumulative estimated population of over 5,000 in 2000 (Belize Central

Statistics Office, 2000). Major industries for this region are banana, citrus, shrimp farming, and, to a lesser extent, coffee production. Big Creek, adjacent to the southern study sites, is the second largest deep-water port in Belize and serves as a major shipping port for commodities originating from southern Belize. The villages of Maya Beach, Seine Bight, and Placencia are located on Placencia Peninsula with an estimated population of over 1,300 in 2000 (Belize Central Statistics Office, 2000). This area has experienced considerable increases in development and tourism over the last decade.

Since the establishment of the country's first shrimp farm in 1983, the shrimp farming industry in Belize has experienced rapid expansion followed by contraction as prices fell with increased global production. There are currently 11 farms operating in Belize, down from a maximum of 19 in 2003 (Linda Thornton, Aquamar, August 2008, pers. comm.). In 2007, production totaled over 5,400 metric tons (Mt) ($21 million US) with a production area of 12 km^2 (Smith, 2008). This was down from over 8,000 Mt ($34 million US) and 21 km^2 in 2006 due to the closure of Lady Nova Shrimp Farm (Smith, 2008). While a diversity of farming techniques and stocking densities exist in Belize, there has been a marked trend towards higher intensity production systems over time. Business failure has been linked to low stocking density in Belize, though this may oversimplify the situation given that solvency of intensive farms may be linked to the capital reserves of owners. Of the 11 active farms, 8 are located in the study region. This study focuses on the two most productive shrimp farms in southern Belize: Belize Aquaculture Ltd. and Aquamar.

Belize Aquaculture Ltd

Belize Aquaculture Ltd. (BAL) is a 73 ha super-intensive shrimp farm with a processing plant and hatchery facilities. The farm currently has 51 ponds in operation and produces 2-3 crops year round. Ponds are stocked at 135 postlarvae (PL)/m^2, are 2.3 m deep in the center, and 1.6 ha in area. For growout, shrimp are fed 25 % protein feed supplied by the Archer Daniels Midland Company (Decatur, Illinois). Molasses is added to ponds to stimulate heterotrophic bacteria communities (flocculent) that provide more stable water quality and an extra food source for shrimp (Burford et al., 2003b; 2004). In order to prevent erosion, ponds are lined with plastic and drainage canals have buffer strips. BAL claims that their water management has "zero exchange" at this time, meaning there was no water discharge except during harvest. In the past, BAL had a primarily closed, recirculating system that did not release effluent directly

into the environment even at harvest (see Boyd and Clay, 2002). In 2007-2008 BAL did not recirculate their water and discharged directly into Santa Maria and Hensley's creeks. BAL has been seen as a model farm for production and environmental best management practices, as evidenced by a 2002 report by Boyd and Clay, participation in World Wildlife Fund's (WWF) incipient certification program, and their status as the first farm to take part in the Wegmans Food Markets Inc. shrimp purchaser standards program.

BAL is located on Santa Maria Creek near the western shore of Placencia Lagoon. Placencia Lagoon is semi-enclosed, 3.4 km at its widest extent, 20 km long, and covers 30 km^2 in area (Ariola, 2003). The middle and upper lagoons are heavily influenced by rainfall and subsequent watershed drainage and have low water exchange with the sea. Santa Maria Creek, which flows into Placencia Lagoon approximately 13 km from the lagoon's mouth, is the primary conduit by which effluent is released from the farm. Effluent flows 4 km before entering Placencia Lagoon proper; 2.5 km of that stretch is mangrove lined. A portion of Santa Maria Creek has been channelized to improve drainage of BAL operations (David Aguilar, BAL, July 2008, pers. comm.). The watershed for Santa Maria Creek is 247 km^2 with approximately 12.7 km^2 permanently inundated and 32.7 km^2 prone to flooding (Ariola and Morgan, 2000). Effluent is occasionally directed down Hensley's Creek, which enters the lagoon 1.5 km south of Santa Maria Creek (David Aguilar, BAL, July 2008, pers. comm.) .

Aquamar

Aquamar is a 405 ha farm with a processing plant and hatchery facilities. There are 83 operating ponds with four categories of stocking density ranging from extensive to super-intensive. During the course of this study, extensive earthen ponds, averaging 7.7 ha in area, were stocked at 8-10 PL/m^2; semi-intensive earthen ponds, averaging 6.4 ha in area, were stocked at 11-21 PL/m^2; intensive earthen ponds, averaging 5.4 ha in area, were stocked at 41-71 PL/m^2; and plastic lined intensive to super-intensive ponds, averaging 1.5 ha in area, were stocked at 61-162 PL/m^2. Aquamar used 25% protein feed for semi-intensive and 35% protein feed for intensive and super-intensive ponds from the Areca Corporation (Guatemala City, Guatemala). Molasses was added at 67 kg ha^{-1} day^{-1} in lined intensive and super-intensive ponds to promote microbial production. Within the study period close to 20 million shrimp were produced in 36 ponds, with water discharged into Plantation Creek. Aquamar practices limited

water exchange for extensive and semi-intensive ponds and had "zero exchange" for intensive and super-intensive ponds during the growout period, after which the water was released. Aquamar is currently participating in WWF's incipient certification program.

Aquamar is located southwest of the mouth of Placencia Lagoon and is connected to coastal waters by several creeks and lagoons. Aquamar's major release point (60 ponds) is into Plantation Creek, which runs for 2 km before entering Plantation Lagoon. The creek has a dense riparian mangrove forest with a main channel and several small side lagoons. Plantation Lagoon is 1 km by 1.5 km and is adjacent to an extensive lagoon system to the south going towards Monkey River village (12 km) and the sea to the east (300 m). Plantation Creek is more saline and slower flowing than Santa Maria and Hensley's creeks. Bel-Euro, a 93 ha extensive shrimp farm, also releases effluent that reaches the western side of Plantation Lagoon. Other Aquamar release points include an outlet behind Harvest Caye (15 ponds) and two outlets into Big Creek (8 ponds).

Methods

Data for this study were collected between 10 June and 5 August 2008. Methods and site locations were adapted from a 2007 pilot study (S. Ledwin, University of Michigan, May-August 2007, unpublished). Seagrass survey data from this pilot study was also used.

Periphyton samples were taken at 25 sampling sites, with 10 and 15 sites respectively located in the effluent receiving waterways of Aquamar (see Figure 1) and BAL (see Figure 2). A series of 1.2 m long, 2.5 cm diameter PVC poles were staked into the bottom sediments using a randomized spatial pattern at the major effluent release points and at control areas adjacent to BAL and Aquamar. Periphyton was sampled from the poles at intervals of 8 days a total 8 times per site. Removal of periphyton was performed using pressurized distilled water. The material and distilled water were immediately pumped through a 0.7 μm Whatman filter (GF/F, 47 mm) (Waukesha, WI, GE Heathcare). Any visible consumer organisms were removed from the filter with sterile forceps. Enough periphyton was collected to cover the filter with a minimum of 5 mm thickness. For poles without sufficient growth on the top 15 cm of the submerged part of the pole, periphyton was also collected from a larger area lower on the pole. After sampling, the poles were scrubbed, cleaned, and replaced. Samples were wrapped in tinfoil and put in a cooler with ice for transport. Periphyton were then separated from the filter, dried at 70 ºC for 24 hours,

ground to a fine powder, and packed into 8x5 mm tin capsules. Analysis of periphyton %N, %C, C/N, and $\delta^{15}N$ was performed at the University of Michigan School of Natural Resources and Environment using a Finnigan Delta Plus isotope ratio mass spectrometer (IRMS) (Bremen, Germany, Thermofinnigan). The $\delta^{15}N$ values were determined by the ratios of light nitrogen compared to the normative standard of N^2 in air (defined as 0 ‰). The $\delta^{15}N$ values were calculated with the equation $[(R_{sample} - R_{standard})/R_{standard}] \times 10^3$ where "R" is the ratio of $^{15}N/^{14}N$ (Peterson and Fry, 1987). Shrimp feed (n=6) from BAL and Aquamar and flocculent from BAL (n=6) were also collected and analyzed for $\delta^{15}N$.

Grayscale analysis was used to estimate maximum periphyton growth on PVC poles. Digital images of each pole were taken every 8 days before periphyton sampling. These TIFF files were then imported into the ImageJ image processing program (Bethesda, MD, National Institute of Health). A 1 cm^2 square patch with the highest percent coverage was chosen on each image for analysis. This area was then scanned into ImageJ creating a histogram scaled from 0 (black) to 255 (white). Analysis was performed at the Eastman Kodak Company in Rochester, NY.

For the purposes of statistical analysis, the 25 periphyton sampling sites were divided into four groups based on their spatial relation to effluent discharge points. At BAL, sites were divided based on their location above (upcurrent or above BAL; sites A-F) or below (downcurrent or below BAL; sites G-O) of Santa Maria Creek. This distinction was based on the fact that Santa Maria Creek is BAL's major effluent outlet and the prevailing water movement is southward. Sites M, N, and O are likely influenced to a greater degree by effluent from Hensley's Creek, which has its mouth at site M (Figure 1). At Aquamar, sites were divided based on their proximity to the effluent release point at site Z (Figure 2). Near (Q – T) and far (U – Z) sites were classified as being respectively within or beyond 2500 m from the effluent release point. This distance was measured along the shortest possible path along the water's surface, reflecting the shortest possible path along which effluent might disperse. At each farm, the two groups were compared for %N, $\delta^{15}N$, C/N, and maximum periphyton growth both overall and by sampling time using a repeated measures mixed-model ANOVA. Additional comparisons between individual sample locations and sampling dates were made using Tukey-Kramer post hoc tests. All analyses were performed using SAS software version 9.13 SP4 (Cary, North Carolina, SAS Institute Inc).

Increased fractionation lowering $\delta^{15}N$ often occurs in high N environments (see Wada and Hottori, 1978; Cifuentes et al., 1989; Yonemaya et al., 1991; Fogel and Cifuentes, 1993; Pennock et al., 1996; Waser et al., 1998; Altabet, 2001; Lake et al., 2001) which can confound the ability to accurately assess uptaken source N (Wada and Hottori, 1978; Cifuentes et al., 1989; Yonemaya et al., 1991; Fogel and Cifuentes, 1993; Pennock et al., 1996; Waser et al., 1998; Altabet, 2001; Lake et al., 2001). Given that previous sampling in 2007 demonstrated this occurrence at sites directly adjacent to the effluent sources (sites G, H, I at BAL and sites Z and Y at Aquamar), group analysis was conducted both with and without these sites for $\delta^{15}N$. All means for %N, $\delta^{15}N$, C/N, and maximum periphyton growth are reported in text with ± 1 standard error (SE).

Water samples were taken every 8 days at 11 of the periphyton sampling sites for a total of 8 samples per site. Whole water samples were collected at the surface, placed in a cooler with ice for transport, and frozen within three hours. Samples were brought to Aquamar for tests of nitrate nitrogen, nitrite nitrogen, phosphate, total suspended solids (TSS), pH, alkalinity, and salinity.

Water samples were analyzed using Hach methods (Hach, 2005) unless otherwise specified. The spectrophotometer used for relevant methods was the Hach Odyssey DR2500 model (Loveland, CO). Nitrate was determined using a cadmium reduction method (8171). Nitrite was determined using a diazotization method (8507). Phosphate was determined using the PhosVer® 3 method (8048). A pH Testr Z (Vernon Hills, IL, Oakton Instruments) was used to determine pH. Alkalinity was determined using a phenolphthalein and total alkalinity method (8203). Total suspended solids (TSS) were quantified using a photometric method (8006). A YSI 6600 V2 Sonde (Yellow Springs, Ohio, YSI Inc) was used to measure dissolved oxygen (DO).

Seagrass was sampled at 80 sites by anchor drops (Danforth anchor 14-3/4”and 10-1/2”fluke length) during the 2007 pilot study. The anchor was dropped at a random location within 15 m of the site at least 3 times per site on a transect line to assess presence or absence of seagrass. Data from these samples, along with data found in Garcia (2003), were entered in Arc GIS 9.2 (Redlands, CA, ESRI). A map of seagrass coverage in the sampled regions was created using Theissen polygons representing each sample site. A Z-test for proportions (Zar, 1999) was used to compare 2003 and 2007 data, with the null hypothesis that the two frequencies were

equivalent ($\alpha = 0.05$). Only presence or absence data were used to ensure methodological consistency with Garcia (2003).

Figure 1. A map of Belize Aquaculture Ltd ponds and sample sites for this study. See Table 9 for UTM coordinates of each site.

Map from Google Earth. Imagery date: 28 November, 2004 (Mountainview, California, Google Corporation).

Figure 2. A map of Aquamar ponds and sample sites for this study. See Table 10 for UTM coordinates of each site.

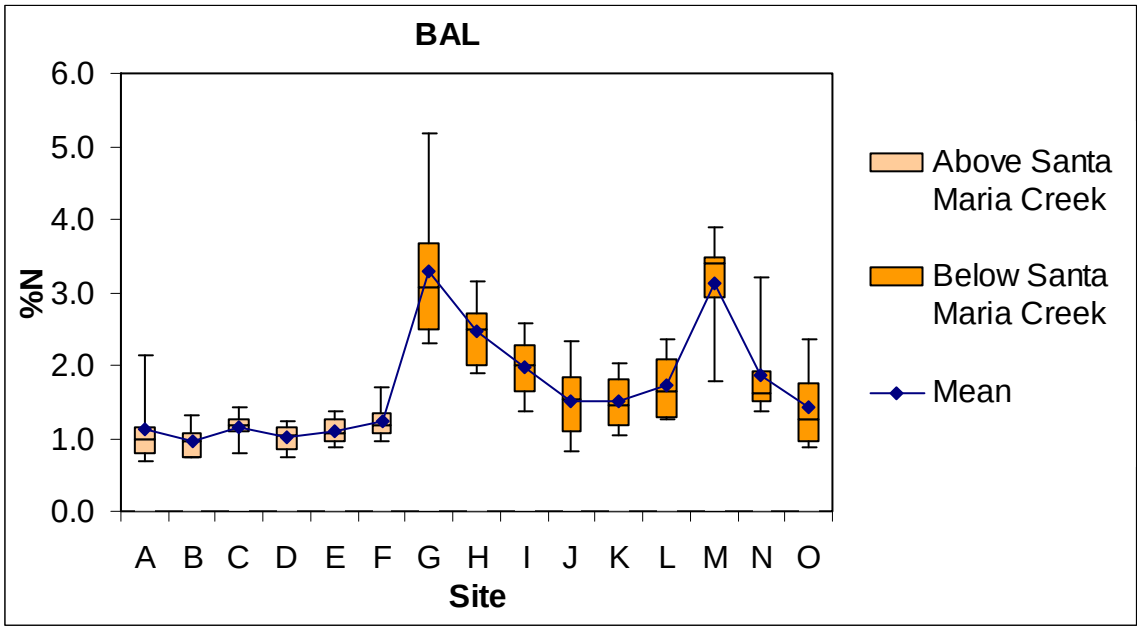
Map from Google Earth. Imagery date: 28 November, 2004 (Mountainview, California, Google Corporation).

Results

Belize Aquaculture Limited

There was a significantly higher level of periphyton %N at sites below Santa Maria Creek compared to sites above, indicating the likelihood of increased nitrogen at these sites. Mean %N values were 1.10 ± 0.04 % above (sites A-F) and 2.02 ± 0.13 % below (sites G-O) Santa Maria Creek (Figure 3). Mean values at sites M (the mouth of Hensley's Creek) and G were significantly higher than all other sites except H (Table 1). These results suggest that there is more biologically available nitrogen below Santa Maria and Hensley's creeks, the sites of BAL's effluent discharge.

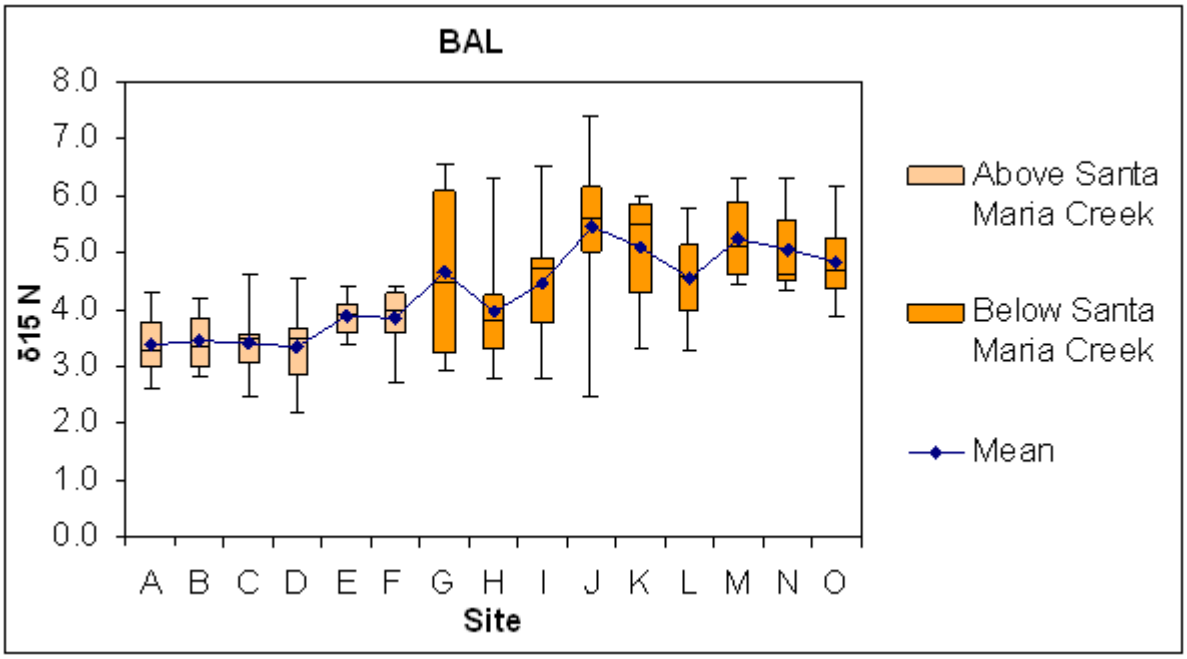
Figure 3. Box and whisker plot of periphyton %N at sample sites above and below BAL effluent creeks. Plots shows mean, median, 25th and 75th quartiles, and range.



There was a significantly higher level of periphyton $\delta^{15}N$ at sites below Santa Maria Creek compared to sites above, indicating that shrimp farm derived nitrogen is likely reaching the downstream sites. Mean $\delta^{15}N$ values were 3.54 ± 0.09 ‰ for sites above and 4.73 ± 0.14 ‰ for sites below Santa Maria Creek (Figure 4). Preliminary data collection in 2007 showed unexpectedly low periphyton $\delta^{15}N$ at sites closest to the effluent source. Increased fractionation (lowering $\delta^{15}N$) can occur in conditions of surplus N (Wada and Hottori, 1978; Cifuentes et al., 1989; Yonemaya et al., 1991; Fogel and Cifuentes, 1993; Pennock et al., 1996; Waser et al.,

1998; Altabet, 2001; Lake et al., 2001) and likely influenced the results at sites closest to the effluent sources. Given this issue, analysis was also conducted omitting samples G, H, and I, which were within the area previously shown to have unexpectedly low $\delta^{15}N$. This modified group had a mean $\delta^{15}N$ value of 5.01 ± 0.15 ‰, which was also significantly elevated compared to the above Santa Maria Creek group. Shrimp feed and pond flocculent at BAL had $\delta^{15}N$ signals of 5.27 ± 0.12 ‰ and 7.37± 0.33 ‰ respectively. Mean $\delta^{15}N$ values at sites J and M were higher than for all other sites (Table 1). These results indicate that shrimp farm effluent is the likely source of most of the extraneous nitrogen below Santa Maria and Hensley's creeks.

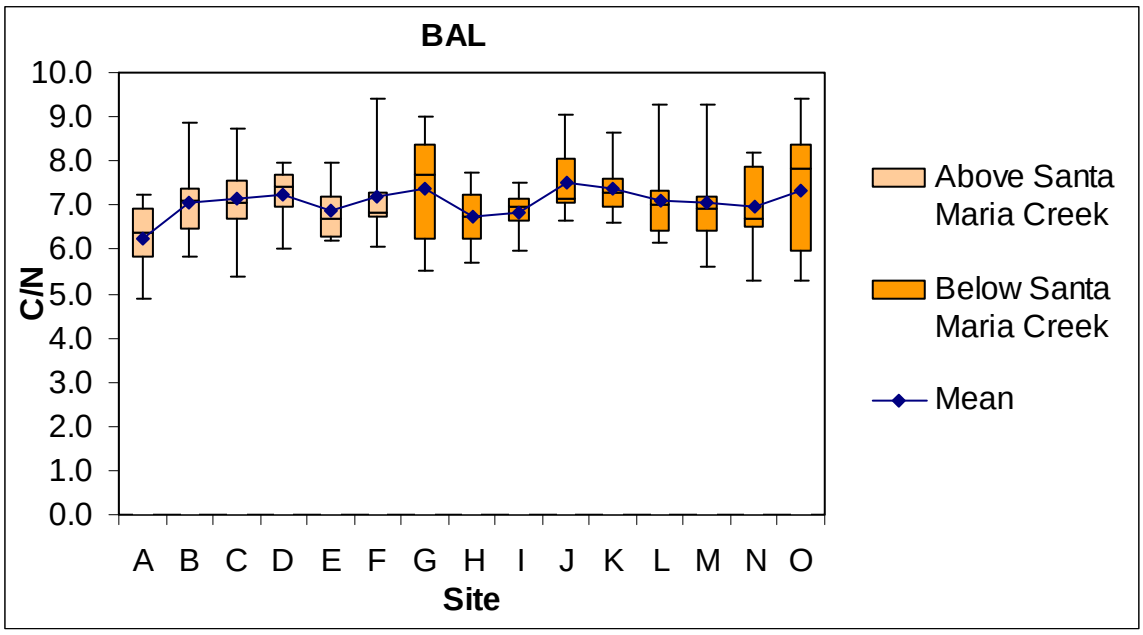
Figure 4. Box and whisker plot of periphyton $\delta^{15}N$ at sample sites above and below BAL effluent creeks.



There was not a significant overall difference between the above and below Santa Maria Creek groups for periphyton C/N ratios. This unexpected outcome may have resulted from increased carbon availability for the heterotrophic component of sampled periphyton given molasses additions on the farm (increasing C/N in samples below BAL) or less nitrogen limitation above the farm (lowering C/N in samples above BAL). Mean C/N ratios were 6.98 ± 0.13 for the above Santa Maria Creek group and 7.15 ± 0.12 for the below Santa Maria Creek group (Figure 5). There were significant temporal differences between sampling dates (see

Table 2) but no strong relationships between dates and likely pond releases.

Figure 5. Box and Whisker plot of periphyton C/N at sample sites above and below BAL effluent creeks.



There was significantly higher maximum periphyton coverage at sites below Santa Maria Creek compared to those above, indicating that there are likely more nutrients available for growth at the former set of sites. Mean grayscale values were 140.10 ± 7.50 for the above and 78.46 ± 4.65 for the below Santa Maria Creek group (Figure 6). Mean values at sites A and B above BAL at the northern end of Placencia Lagoon had significantly less maximum coverage than all sites below Santa Maria Creek (Table 1). These results suggest that there is more rapid growth of periphyton below Santa Maria Creek given the increased nutrients coming from BAL.

Figure 6. Box and whisker plot of maximum periphyton coverage grayscale analysis at sample sites above and below BAL effluent creeks. Scale: 0 (black) to 255 (white).

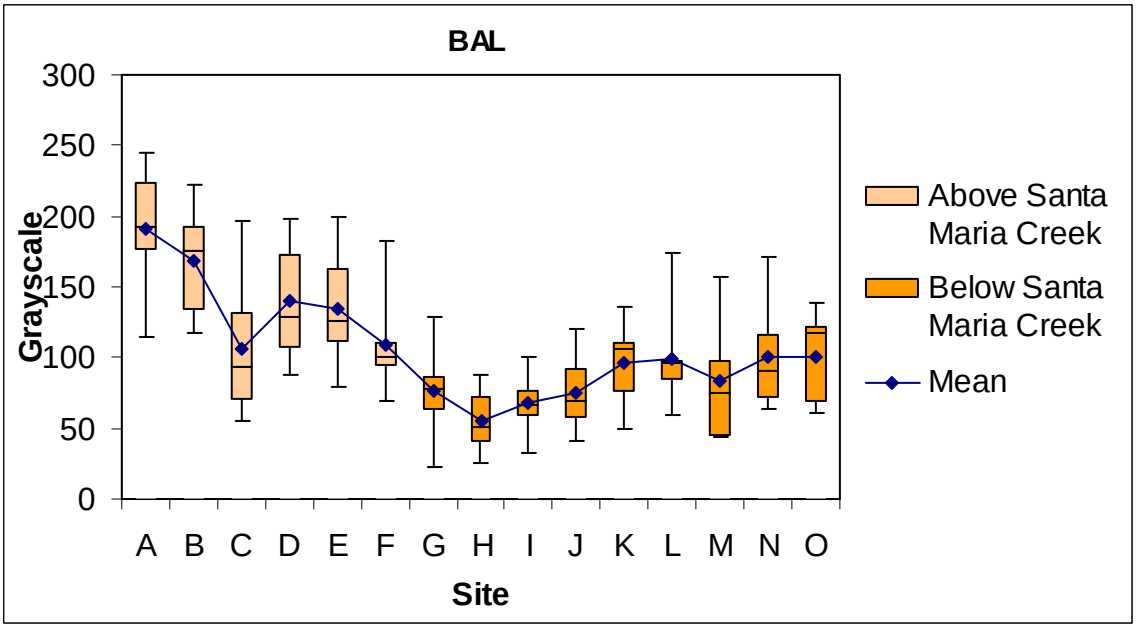


Table 1. Mean values at each site for percent nitrogen, $\delta^{15}N$, carbon/nitrogen ratios, and maximum periphyton grayscale analysis.
Belize Aquaculture Ltd

Site	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
%N	1.1^{a}	1.0^{a}	1.2^{a}	1.0^{a}	1.1^{a}	1.2^{a}	3.3^{d}	2.5^{cd}	2.0^{bc}	1.5^{ab}	1.5^{ab}	1.7^{b}	3.1^{d}	1.9^{bc}	1.4^{ab}
$\delta^{15}N$	3.4^{a}	3.4^{a}	3.4^{a}	3.3^{a}	3.9^{ab}	3.8^{ab}	4.6^{ab}	4.0^{ab}	4.4^{ab}	5.4^{b}	5.1^{b}	4.5^{ab}	5.2^{b}	5.0^{b}	4.8^{ab}
C/N	6.3	7.1	7.1	7.2	6.9	7.2	7.4	6.7	6.8	7.5	7.4	7.1	7.0	7.0	7.3
Grayscale	191.5^{a}	168.1^{a}	106.2^{abc}	139.5^{ab}	135.1^{ab}	109.2^{abc}	75.8^{b}	55.7^{c}	67.5^{c}	75.4^{bc}	96.1^{bc}	99.3^{bc}	84^{bc}	100.2^{bc}	100.0^{bc}

Means with different superscripts were significantly different at $p < 0.05$.

Table 2. Mean values on each sample date for percent nitrogen, $\delta^{15}N$, carbon/nitrogen ratios, and maximum periphyton grayscale analysis.
Belize Aquaculture Ltd

Sampling Date	10-Jun	18-Jun	26-Jun	4-Jul	12-Jul	20-Jul	28-Jul	5-Aug
%N	1.7	1.2	1.7	1.5	1.8	1.3	1.8	1.6
δ15N without sites G, H, and I	4.5	4.2	4.3	4.2	4.0	4.0	4.4	4.5
δ15N	4.1	4.1	4.1	4.1	3.9	3.8	4.4	4.4
C/N	6.5^{c}	7.3^{ab}	6.8^{bc}	7.7^{ab}	6.7^{bc}	7.7^{a}	7.1^{ab}	6.7^{ab}
Grayscale	153.8^{a}	111.1^{ab}	88.0^{b}	80.3^{b}	125.2^{a}	115.2^{ab}	95.9^{b}	127.4^{a}

Means with different superscripts were significantly different at $p<0.05$.

Most water quality parameters showed impacts at the sites directly below BAL in Santa Maria Creek. Mean nitrate, phosphate, and TSS were higher and DO lower at site G (the site closest to BAL in Santa Maria Creek) than at all other sites, although only DO was significantly different (Table 3) for those parameters. At site G, values ranged widely from 0.0-9.7 for nitrate, 0.0-5.45 for phosphate, and 11-320 for TSS. Only alkalinity was significantly different comparing all sites by sample date (Table 4).

Table 3. Mean surface water physical/chemical parameters at each site. Unless specified, units are mg/L.

	Above BAL	Below BAL			
Site	A	G	H	M	O
Salinity (ppt)	17.13^{b}	3.00^{a}	6.00^{a}	6.13^{a}	14.13^{b}
pH	7.86^{b}	7.89^{b}	7.49^{ab}	7.30^{a}	7.64^{ab}
Alkalinity	56.14	24.80	39.17	31.43	46.43
Nitrite	0.00	0.02	0.04	0.00	0.00
Nitrate	0.46	2.17	0.66	0.03	0.03
Phosphate	0.01	1.20	0.49	0.04	0.00
TSS	17.75	56.86	14.13	12.25	17.88
DO	7.02^{c}	1.38^{a}	2.08^{a}	3.73^{b}	7.21^{c}

Means with different superscripts were significantly different at $p < 0.05$.

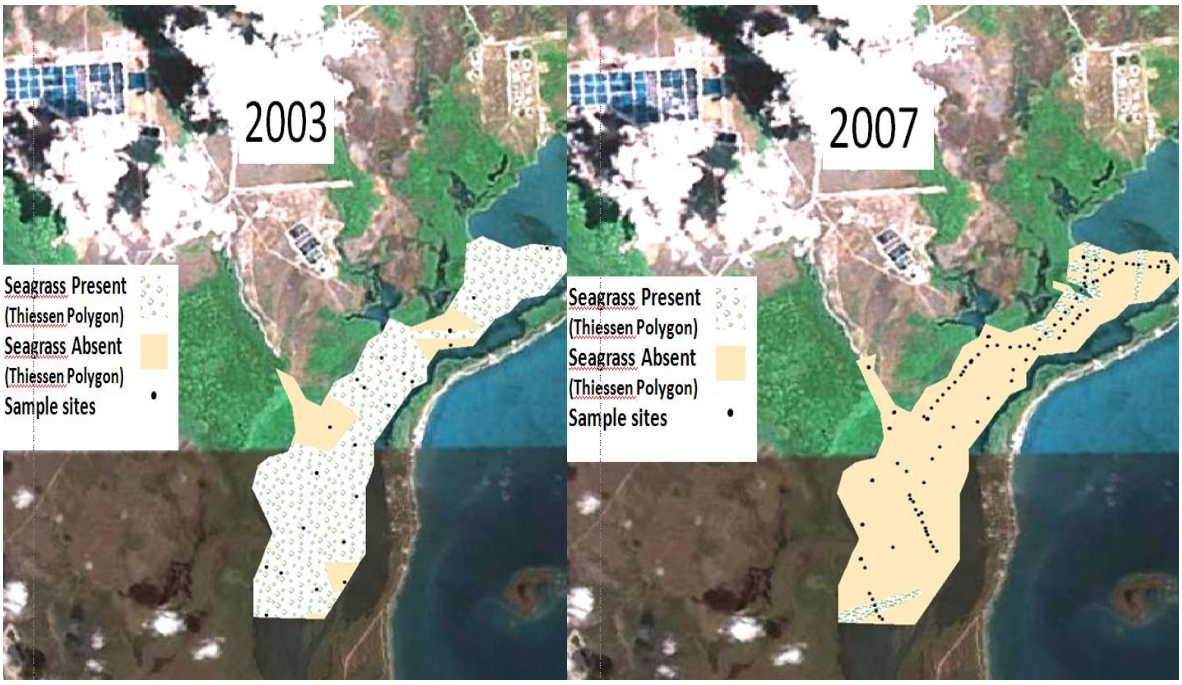
Table 4. Mean surface water physical/chemical parameters at all sampled sites for each sampling date. Unless specified, units are mg/L.

Date	10-Jun	18-Jun	26-Jun	4-Jul	12-Jul	20-Jul	28-Jul	5-Aug
Salinity (ppt)	11.60	12.60	12.80	5.60	9.00	6.75	9.00	7.60
pH	7.30	7.78	7.50	7.80	7.50	7.83	7.48	8.00
Alkalinity	52.60^{ab}	71.67^{b}	n.a.	34.20^{ab}	38.20^{ab}	49.25^{ab}	25.80^{a}	26.20^{a}
Nitrite	0.02	0.08	0.00	0.00	0.00	0.00	0.00	0.00
Nitrate	1.12	3.25	0.90	0.10	0.00	0.00	0.20	0.00
Phosphate	0.39	0.60	1.29	0.00	0.13	0.02	0.04	0.06
TSS	15.00	16.20	32.40	14.40	14.00	13.25	14.00	12.20
DO	4.52	5.39	3.56	4.73	n.a.	3.72	5.12	4.35

Means with different superscripts were significantly different at $p < 0.05$.

Between 2003 and 2007, seagrass distribution shrank significantly in the middle section of Placencia Lagoon (Figure 7). Seagrass coverage within the sampled area was approximately 83% in 2003 and 7% in 2007. In 2007, Seagrass was only present at the northern and southern edges of the sampled area. Production of shrimp at BAL and the development of the surrounding coastal area both accelerated between 2003 and 2007, with BAL operations releasing minimal or no effluent before 2003 (Boyd and Clay, 2002; Smith and Mackie, 2005).

Figure 7. Seagrass sample sites in 2003 (Garcia) and 2007. The map represents results of the Theissen polygon nearest neighbor analysis using presence or absence data.

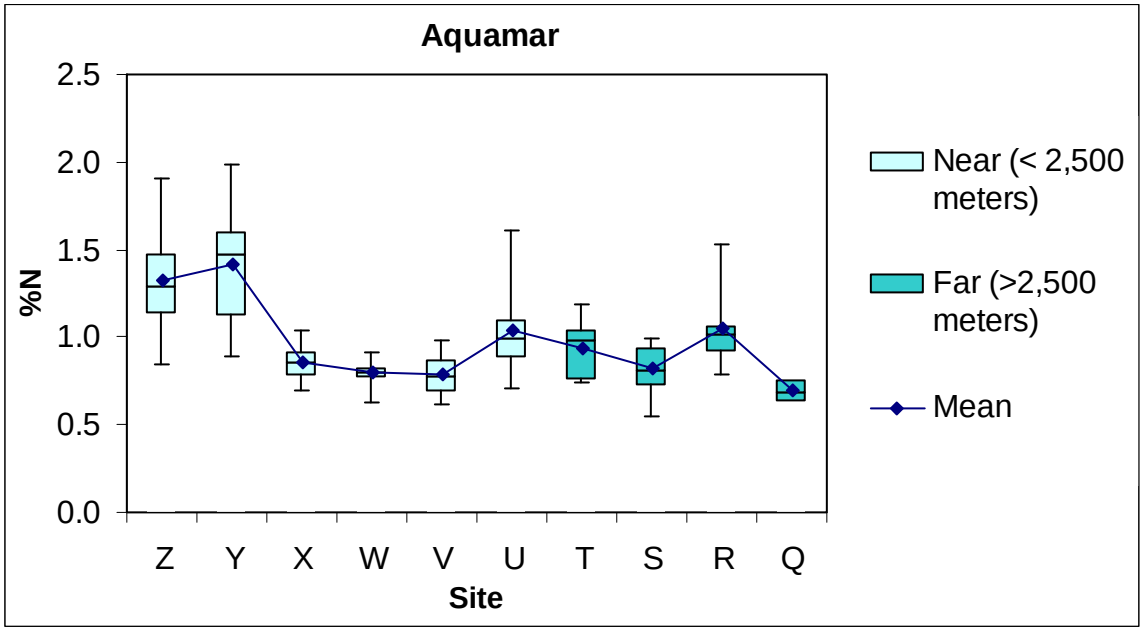


Map from Google Earth. Imagery Date 28 November, 2004 (Mountainview, California, Google Corporation).

Aquamar

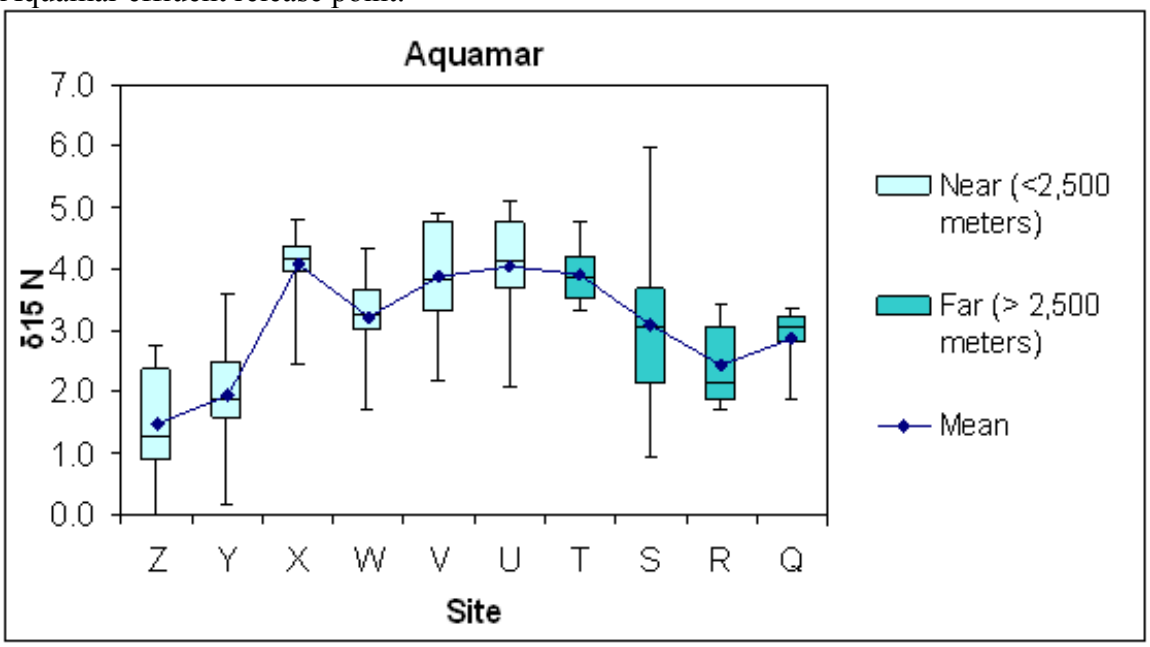
Sites Z and Y, which were within Aquamar's effluent creek, had the highest %N values. There was, however, no significant elevation of %N within the group closest (<2500 m) to the effluent release point compared with the group farther away. Mean %N values were 1.03 ± 0.05 % for the near group and 0.88 ± 0.06 % for the far group (Figure 8). This may indicate a low magnitude of influence on sites outside of the effluent creek.

Figure 8. Box and whisker plot of periphyton %N at sample sites near and far from the largest Aquamar effluent release point.



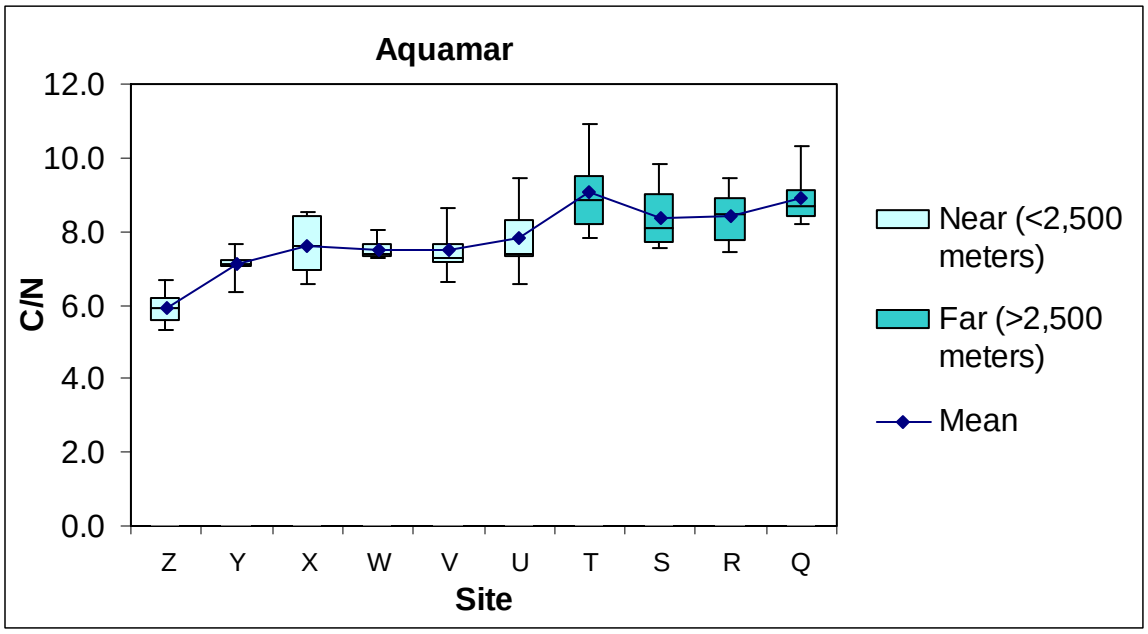
Analysis of $\delta^{15}N$ was performed both with and without sites Z and Y, in order to account for the possibility of perturbed $\delta^{15}N$ signals in high N environments (see Wada and Hottori, 1978; Cifuentes et al., 1989; Yonemaya et al., 1991; Fogel and Cifuentes, 1993; Pennock et al., 1996; Waser et al., 1998; Altabet, 2001; Lake et al., 2001). Similar issues were found at BAL and are explained above. Without Z and Y, there was a significantly higher level of periphyton $\delta^{15}N$ at near sites compared to far sites, indicating that shrimp farm derived nitrogen is likely reaching the former set of sites. When all sites were included, the mean $\delta^{15}N$ values were 3.11 ± 0.24 ‰ for the near group and 3.13 ± 0.18 ‰ for the far group (Figure 8). However, when sites Z and Y were removed, the near group had a mean of 3.8 ± 0.15 ‰. Shrimp feed at Aquamar had $\delta^{15}N$ signals of 3.01 ± 0.12 ‰ for the Areca 25% protein feed and 4.10 ± 0.15 ‰ for the Areca 35% protein feed. The mean $\delta^{15}N$ value at site X (the site directly outside of Aquamar's effluent creek) was significantly higher than values at sites nearest to (Z, Y) and furthest from (Q, R) the effluent discharge point (Table 5). There was also a significant temporal difference between sampling dates (see Table 6), with the lowest mean $\delta^{15}N$ occurring on August 5th after no discharge occurred during the previous eight days (Linda Thornton, Aquamar, August 2008, pers. comm.).

Figure 9. Box and whisker plot of periphyton $\delta^{15}N$ at sample sites near and far from the largest Aquamar effluent release point.



There was a significantly lower C/N ratio for near sites compared to far sites, indicating that nitrogen may be less limited closer to the farm effluent. Mean C/N ratios were 7.24 ±0.13 for the near group and 8.68 ±0.17 for the far group (Figure 10). The mean C/N ratio at site Z was significantly lower than at all other sites (Table 5). The results suggest that N emanating from Aquamar is likely influencing the immediate area around the shrimp farm.

Figure 10: Box and whisker plot of periphyton carbon/nitrogen at sample sites near and far from the largest Aquamar effluent release point.



Near sites had higher mean maximum periphyton coverage than far sites, but the difference was not significant. Mean grayscale values were 129.70 ±6.98 for the near group and 154.68 ± 10.63 for the far group (Figure 11). This result suggests that nutrient additions from Aquamar may not have spurred much additional growth in periphyton. This result may also be due to confounding factors such as the poor adhesiveness of periphyton in the effluent creek. There were significant differences between sites (Table 5), but it is unclear why site V had the highest mean maximum growth.

Figure 11. Box and whisker plot of maximum periphyton coverage grayscale analysis at sample sites near and far from the largest Aquamar effluent release point.

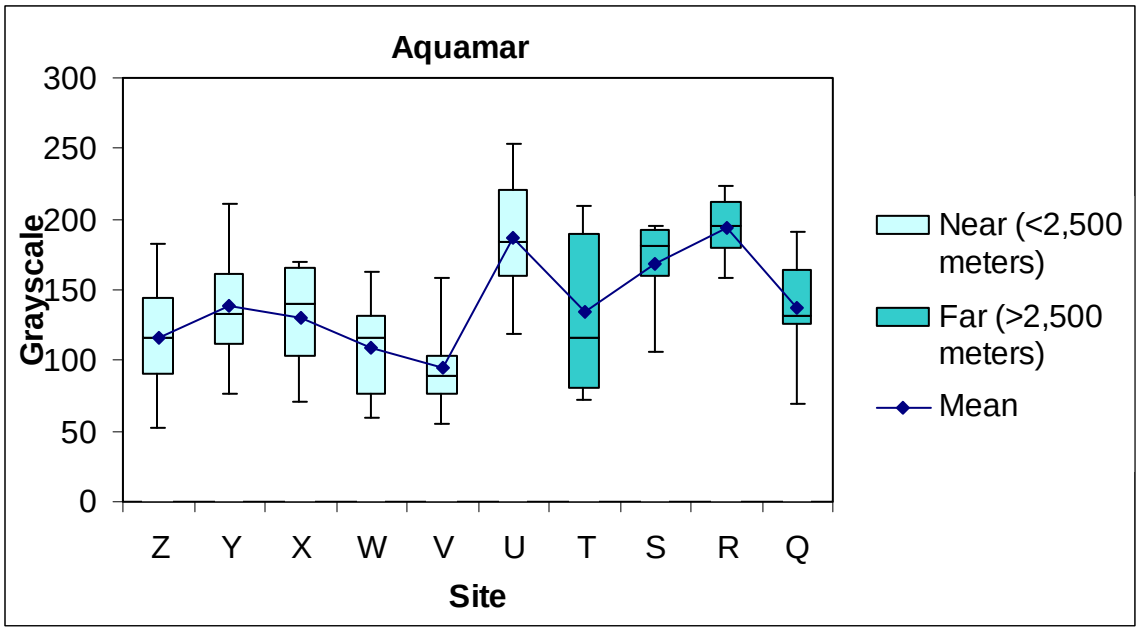


Table 5. Mean values at each site for percent nitrogen, $\delta^{15}N$, carbon/nitrogen ratios, and maximum periphyton grayscale analysis.
Aquamar

Site	Z	Y	X	W	V	U	T	S	R	Q
%N	1.3^{c}	1.4^{c}	0.9^{ab}	0.8^{ab}	0.8^{ab}	1.0^{bc}	0.9^{ab}	0.8^{ab}	1.1^{bc}	0.7^{a}
$\delta^{15}N$	1.5^{a}	1.9^{a}	4.1^{d}	3.2^{bcd}	3.9^{cd}	4.0^{cd}	3.9^{cd}	3.0^{bcd}	2.4^{ab}	2.9^{abc}
C/N	5.9^{d}	7.1^{c}	7.6^{abc}	7.5^{bc}	7.5^{bc}	7.8^{abc}	9.0^{a}	8.4^{ab}	8.4^{ab}	8.9^{a}
Grayscale	116.6^{bc}	138.0^{bc}	130.7^{bc}	109.5^{bc}	94.2^{c}	186.9^{ab}	133.8^{abc}	169.0^{ab}	194.1^{a}	137.0^{bc}

Means with different letters are significantly different at $p < 0.05$.

Table 6. Mean values on each sample date for percent nitrogen, $\delta^{15}N$, carbon/nitrogen ratios, and maximum periphyton grayscale analysis.
Aquamar

Sampling Date	10-Jun	18-Jun	26-Jun	4-Jul	12-Jul	20-Jul	28-Jul	5-Aug
%N	0.9	1.0	0.9	1.0	0.9	1.0	1.0	1.0
δ15N without sites Z and Y	3.5^{ab}	3.3^{ab}	3.4^{ab}	3.6^{ab}	3.7^{b}	3.6^{ab}	4.2^{b}	3.0^{a}
δ15N	2.8	2.8	3.2	3.2	3.5	3.4	3.8	2.2
C/N	7.8	7.7	8.4	8.2	8.0	8.1	7.8	7.5
Grayscale	136.2	115.9	145.2	159.4	138.5	145.2	117.0	179.6

Means with different superscripts were significantly different at $p < 0.05$.

Most water quality parameters showed impacts at the sites directly below Aquamar. Mean nitrate and TSS were much higher at site Z than at all other sites, although not significantly so (Table 7). Site Z had values that ranged widely from 0.0-7.0 for nitrate and 7-85 for TSS. DO was significantly lower throughout Plantation Creek from site Z to Y. Only pH was significantly different comparing all sites by sample date (Table 8).

Table 7. Mean surface water physical/chemical parameters at each site. Values are in mg/L unless otherwise specified.

Site	Z	Y	V	U	T	R
Salinity (ppt)	11.88[a]	17.29[ab]	19.00[ab]	20.50[b]	22.75[b]	23.86[b]
pH	6.94[a]	6.96[a]	7.32[b]	7.63[b]	7.73[b]	7.68[b]
Alkalinity	36.50[a]	47.00[b]	64.00[bc]	66.57[bc]	82.86[c]	85.14[c]
Nitrite	0.03	0.01	0.00	0.00	0.00	0.00
Nitrate	1.11	0.43	0.03	0.29	0.19	0.31
Phosphate	0.01	0.04	0.01	0.04	0.00	0.01
TSS	26.13[a]	6.86[b]	12.33[ab]	8.75[b]	17.25[ab]	12.40[b]
DO	3.53[a]	3.24[a]	6.33[b]	6.70[b]	n.a.	7.26[b]

Means with different superscripts were significantly different ($p < 0.05$).

Table 8. Mean surface water physical/chemical parameters at all sampled sites for each sampling date. Values are in mg/L unless otherwise specified.

Date	10-Jun	18-Jun	26-Jun	4-Jul	12-Jul	20-Jul	28-Jul	5-Aug
Salinity (ppt)	20.80	21.80	22.33	22.00	16.50	19.17	19.00	12.00
pH	7.40[ab]	7.54[ab]	7.45[ab]	7.67[b]	6.83[a]	7.27[ab]	7.47[ab]	7.50[ab]
Alkalinity	74.20	84.67	n.a.	66.83	63.00	69.33	60.67	46.40
Nitrite	0.01	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Nitrate	0.56	1.78	0.43	0.25	0.83	0.20	0.83	0.80
Phosphate	0.00	0.04	0.05	0.00	0.00	0.03	0.00	0.02
TSS	11.20	6.60	11.50	22.00	15.17	9.00	11.17	11.20
DO	5.01	n.a.	5.29	n.a.	n.a.	5.33	4.49	6.49

Means with different superscripts were significantly different ($p < 0.05$).

Discussion

Shrimp farm waste from both BAL and Aquamar had an influence on surrounding waterways. Water quality and periphyton bioindicators revealed spatial and temporal impacts of effluent on these receiving waterways. At BAL, periphyton sampling showed significant elevation of %N, $\delta^{15}N$, and maximum coverage at sites below effluent creeks. Nitrate, phosphate, and TSS were highest and DO lowest at sample sites in Santa Maria Creek, the major effluent release point at BAL. Seagrass distribution has been reduced dramatically in the middle portion of Placencia Lagoon between 2003 and 2007 above and below Santa Maria Creek, coinciding with increased nutrient loading from BAL and adjacent development in the area. At Aquamar, sampling showed significantly higher $\delta^{15}N$ (when sites Z and Y were removed) and significantly lower C/N ratios in the group closest to the effluent source (<2500 m). Nitrate and TSS were highest and DO lowest in Plantation Creek, the major effluent release point for Aquamar. The suite of parameters used in this study helped elucidate the magnitude and extent of the influence of shrimp farm effluent on the two receiving waterways. The results of this study also reveal the strengths and weaknesses of these parameters as monitoring tools.

The %N in periphyton (Stelzer and Lamberti, 2001, Hillbrand and Kahlert, 2001; Lepoint et al., 2007) and plant (Duarte, 1990; Horrocks et al., 1995; Invers et al., 2004) tissue is a potential indicator of biologically available nutrient concentrations. Results addressing %N confirmed expectations that this parameter would be highest at sites closest to effluent sources with rapid attenuation to background levels at increasingly distant sites due to dilution effects, assimilation by organisms, and denitrification. At both farms considered in this study, sites closest to shrimp farm effluent release points had the highest %N. This result was similar to the findings of Lin and Fong (2008) for deployed macroalgae, where increased %N occurred near a shrimp farm effluent release point, but not at intermediate distances away from the farm. BAL had more than twice the mean periphtyon %N as Aquamar at sites closest to the effluent release points and significant elevation of %N at sites outside of the effluent creeks, indicating more intense effects and greater spatial influence.

The $\delta^{15}N$ composition of primary producers often reflects external N sources (Heaton, 1986; Fry, 2006) and can be used to assess the influence of shrimp farm effluent on receiving waterways (Jones et al., 2001; Costanzo et. al., 2004; Lin and Fong, 2008; Piñón-Gimate, 2009). At both farms, feed inputs had higher $\delta^{15}N$ than background waters and periphyton $\delta^{15}N$ was

significantly elevated in the groups closest to effluent release points, but not at the specific sites closest to these points. At Aquamar, both sites in the effluent creek actually had the lowest $\delta^{15}N$ signals. This counterintuitive result may be explained by increased fractionation during the uptake and internal processing of DIN by periphyton under conditions of surplus DIN. Several studies have demonstrated such fractionation occurring in plants, bacteria, and algae under surplus nitrogen conditions (Wada and Hottori, 1978; Cifuentes et al., 1989; Yonemaya et al., 1991; Fogel and Cifuentes, 1993; Pennock et. al., 1996; Waser et al., 1998; Altabet, 2001; Lake et al., 2001). Given high nitrate levels and high self reported levels of TAN from discharge ponds (BAL Annual Supplier Report, 2007), surplus nitrogen levels were the likely cause of depleted $\delta^{15}N$ in periphyton at these sites.

The $\delta^{15}N$ composition at other sites in the below Santa Maria Creek group at BAL was generally in the range of feed, flocculent, and literature values of 4.2-7 ‰ (Jones et al., 2001, Costanzo et al., 2004; Lin and Fong 2008, Piñón-Gimate, 2009). Lower $\delta^{15}N$ values at Aquamar compared to BAL were likely the result of lower initial feed $\delta^{15}N$ as well as less concentrated shrimp waste. These results suggest that periphyton at the sites nearest to the effluent release points derived more of their nitrogen from shrimp effluent than from inorganic fertilizer runoff from the watershed (usually around 0 ‰) (Heaton, 1986). There are no human settlements on Santa Maria or Plantation Creeks so there should be little or no sewage effluent (usually around 10 ‰: Costanzo et al., 2001) While $\delta^{15}N$ generally did not show significant temporal differences during sampling in 2008, the sampling date with the lowest periphtyon $\delta^{15}N$ value coincided with the only sampling period that had no discharge at Aquamar (Linda Thornton, Aquamar, August 2008, pers. comm.). This indicates that $\delta^{15}N$ uptake in periphyton may be a sensitive indicator of nitrogen dispersal from shrimp farms.

The ratios of C/N for periphyton and plant tissue have been found to demonstrate relative nutrient loading in receiving waterways (Atkinson and Smith, 1983; Nelson et al., 2001; Fry et al., 2003; Chessman et al., 2009). Significant group and site differences at Aquamar met expectations given the attenuation of N from the effluent source; results at BAL did not. This is likely due to the character of the farms' respective waterways and the nature of the effluent emanating from each farm. Sampling sites outside of Plantation Lagoon (2500 m away from the Aquamar release point) in Plantation Creek were located in some of the area's most pristine waters and were probably not exposed to significant anthropogenic nitrogen from non-shrimp

farm sources. Placencia Lagoon is more likely less nitrogen limited given adjacent development and agriculture. Fry et al. (2003) had a similar result using green macroalgae tissue where only the least impacted estuary showed an inverse relationship between C/N and DIN values.

The growth of aquatic producers are often limited by nitrogen and/or phosphorus and can be used as indicators of anthropogenic nutrient additions to the environment (Nichols et al., 1986; Fong et al., 1994; Oviatt et al., 1995; Bricker et al, 2003; Cosgrove et al., 2004; Lin and Fong, 2008). The maximum growth of periphyton in effluent exposed areas differed for the two farms. BAL sampling sites down-current of effluent creeks had significantly higher maximum periphtyon growth than upstream sites, while no discernable pattern emerged at the Aquamar sampling sites. While growth responses were most likely linked to nutrient concentrations (higher levels of both N and P inputs and larger spatial extent at BAL), other factors (e.g. differential grazing pressure, water movement, etc.) may have influenced the results.

Traditional physical and chemical water quality parameters indicated that the impacts of shrimp farm effluent on both farms' effluent creeks included increased nitrate, TSS, and significantly lower DO. Santa Maria Creek at BAL had higher maximum nutrient and TSS levels and lower DO than Plantation Creek at Aquamar, including much higher concentrations of phosphate. Higher phosphate levels found in Santa Maria Creek are consistent with in-pond measurements by Burford and effluent measurements at the delta of Santa Maria Creek by BAL (mean soluble phosphorus 9.5 mg/L) (Burford et al., 2003b; BAL Annual Supplier Report, 2007). The effluent in Santa Maria Creek was characterized by a high density of flocculated particles, resembling that of the BAL ponds near harvest. Hensley's Creek at BAL did not have high nutrient levels or TSS, but did experience low DO. Given that elevated %N, $\delta^{15}N$, and maximum periphyton growth occurred at the mouth of the creek, it is likely that traditional sampling missed nutrient pulses. Except for the steady, low DO found in this study in all effluent creeks, the physical and chemical water parameters showed a restricted pattern of spatial and temporal impacts with short term fluctuations similar to other studies (Samocha and Lawrence; 1997; Trott and Alongi, 2000; Wolanski et al., 2000; Costanzo et al., 2004).

Increased epiphyte production, increased turbidity, and anoxic conditions caused by shrimp farms all have the potential to cause seagrass decline (McGlathery, 1995; Burkholder et al., 2007). Nutrient additions generally increase epiphyte production (Harlin and Thorne-Miller, 1981; Wear et al., 1999; Cancemi et al., 2003) limiting the amount of light available to seagrass

for photosynthesis (Sand-Jensen, 1997; Hemminga, 1998), increasing competition for CO^2 (Sand-Jensen et al., 1985), and possibly escalating deleterious herbivory (Karez et al., 2000). Increased turbidity can also limit light available for seagrass to perform photosynthesis. Anoxic conditions can impact seagrass through disruption of ion absorption in their roots (Hemminga, 1998) and by promoting the production of sulfide compounds that are toxic to the plants (Carlson et al., 1994). While findings of low dissolved oxygen and high TSS were limited to the shrimp effluent creeks in this study, the significantly higher periphyton growth in the areas surrounding the effluent creeks is likely a good proxy for increased growth of epiphytes on seagrass blades. BAL released little to no effluent before Garcia completed her seagrass survey of the area in 2003 (Boyd and Clay, 2002; Garcia, 2003; Smith and Mackie; 2005). While Smith and Mackie (2005) documented elevated N levels near the hatchery creek north of BAL and at the mouth of Hensley's Creek, this was relatively local. Given the expansion of the farm since 2004 and the cessation of closed system recirculation, it can be assumed that increased nutrient loading commenced between 2004 and 2007. This increased effluent loading corresponded with seagrass declines but does not infer causation. Short et al. (2006) noted that areas in Placencia Lagoon where mangroves were removed for development showed a reduced abundance of the seagrass *H. baillonii* and that areas adjacent to recent developments had more flowering plants, a possible sign of stress. While limited information makes it impossible to partition contributions to seagrass decline in middle Placencia Lagoon, it is very likely that both increased shrimp effluent from BAL and commercial and residential development were major contributing factors.

Biases and Limitations

There were limitations and inherent biases in this study. Without the appropriate equipment and chemicals, important water quality analyses like TN or TAN were not run, precluding comparison of DIN with periphyton variables. Physical and chemical parameters were only measured at the surface, so despite mixing from wave and wind action, mid-column or benthic impacts may have been underestimated. Lacking accurate farm release data, it was impossible to correlate the temporal aspects of discharge and loading rates with metrics describing the study area during the course of this study. Without knowing the $\delta^{15}N$ of DIN at the study sites, it was difficult to categorically distinguish whether depleted $\delta^{15}N$ values found nearest to the farm effluent points were from the source DIN (e.g. possible contributions from

inorganic fertilizer, N fixation, atmospheric deposition, nitrification) or whether the source N was fractionated during uptake or catabolism (Kendall, 1998; Fry, 2006). The issue of increased fractionation at high DIN concentrations is particularly problematic as $\delta^{15}N$ values misrepresent the N source $\delta^{15}N$ values (Fry et al., 2003). Without explicitly identifying periphyton species or community composition, possible community structure differences at sites could impact measured variables (e.g. more heterotrophic organisms in heavily impacted sites). The aforementioned uncertainties are similar to other studies and these results generally met expectations given farm effluent quality and location, the character of the receiving waterways, and prevailing currents. While individual parameters had site specific limitations, the full suite of parameters showed a robust picture of the spatial extent and magnitude of effluent impacts.

Conclusions

Results of this study indicate that effluent discharge from shrimp ponds at both BAL and Aquamar influenced the farms'respective receiving waterways. Periphyton bioindicators coupled with physical/chemical metrics helped elucidate the extent of such impacts. The extent and intensity of shrimp farm influence varied but was generally strong in effluent creeks closest to the farms and much weaker further into the adjacent lagoons.

The character of the release areas and intensity of the operations at BAL and Aquamar differed, influencing the measured variables in this study. Aquamar has less intensive operations than BAL, drains fewer ponds into the study area due to multiple and dispersed release points, and is located in a more remote location. Plantation Creek at Aquamar probably provided more nutrient buffering capacity than other release points given higher densities of mangroves and a greater increased in-stream residence time. Furthermore, a section of Santa Maria Creek is channelized, so nutrient processing in the creek was reduced. While anecdotal, the presence of seagrass adjacent to Plantation Creek and absence of seagrass adjacent to Santa Maria Creek may be related to different loading intensities and buffering capacity.

If current effluent loading levels continue or increase, BAL may have a significant and lasting impact on its receiving waterways. While commenting on BAL's proposed expansion in 2002, and praising BAL for the farm's many "environmentally friendly" practices, Boyd and Clay (2002) advised that BAL's effluent system stay closed and that any releases should be directed into the sea, rather than Placencia Lagoon. This advice was not heeded; probable

related consequences include increased eutrophication and reduced seagrass abundance in Placencia Lagoon. As the only shrimp farm certified under the Wegmans Food Inc./Environmental Defense Fund purchaser standard (Bruce Hammond, Environmental Defense Fund, August 2007, pers. comm.) the effluent problem should be corrected so that purchaser and consumer confidence in the product can persist.

This study demonstrated that periphtyon bioindicators are a useful tool for monitoring shrimp farm waste, despite certain limitations. Detecting bioavailable N is extremely important when assessing potential anthropogenic impacts of shrimp farms. While the sensitivity of individual parameters (e.g. %N, $\delta^{15}N$) was sometimes site dependent, the suite of parameters gave a fairly robust account of expected impacts. This periphyton method can be performed with readily available materials and without on-site laboratory equipment (such as spectrophotometers), potentially expanding the range of areas that can be sampled. Biological indicators provide many advantages over traditional physical and chemical water sampling (Jones et al., 2001; Constanzo et al., 2004; Lin and Fong; 2008) and should be incorporated into shrimp farm monitoring protocols and ecocertification program standards. This periphyton method provides a novel approach for assessing impacts of shrimp farming, providing researchers and managers with another method in the monitoring toolkit.

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Appendix

Table 9. UTM coordinates of sampling points for BAL.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
East/West	356597	356734	355365	355127	354845	354624	354031	354206	354175	354190	354135	353954	353838	353865	353958
UTM	1840546	1839792	1839331	1838944	1838498	1838186	1838655	1838289	1838060	1837822	1837624	1837388	1836733	1836187	1835682

Table 10. UTM coordinates of sampling points for Aquamar.

	Z	Y	X	W	V	U	T	S	R	Q
East/West	346866	347419	347635	347410	347342	347999	348134	347125	347891	349082
UTM	1822787	1821707	1821453	1821125	1820834	1820902	1820399	1819408	1819151	1822786