An Assessment of the Ecological Impacts from Low Intensity Shrimp Farm Wastewater in the Bang Pakong River Region of Thailand Using Stable Isotope Analysis

Sarah Kempke

in partial fulfillment of the requirements

for the degree of

Master of Science

in the School of Natural Resources and Environment

of the University of Michigan

December 2011

Thesis Committee:

Dr. James S. Diana, Chair

Dr. Michael Wiley

Table of Contents

Acknowledgments

List of Tables

List of Figures

List of Appendices

Abstract

Eutrophication of receiving water is often cited as a significant environmental impact of shrimp aquaculture. However, it is difficult to trace sporadic nutrient inputs from harvests and to separate effects of aquaculture effluent from other nutrient sources. This study attempted to trace impacts of shrimp wastewater in Thailand, where shrimp farming dominates much of the coastal landscape and economy. Stable isotopes of N and C, C:N ratios, and water quality analysis were used to evaluate the impact of shrimp farm effluent from small scale farms using semi intensive management with minimal treatment of discharged water. Farms discharge into the Chucachur Canal, a tributary to the Bang Pakong River in the Northern Gulf of Thailand. There were exceptionally high levels of nutrients in the canal, stemming both from shrimp aquaculture and from other agricultural sources. TP exceeded Thai standards for all sampling points and days; NH_3 exceeded Thai standards near a pig farm source on all days, as well as in a plume of shrimp wastewater on a day when a farm was harvested. Multiple linear regression on stable isotope data indicated that insolation was the most influential variable determining $\delta^{15}N$ and $\delta^{13}C$ in plants in the canal. This analysis also indicated that levels of NO_2 and NH_3 contributed to the variation in isotope ratios measured in periphyton and hyacinth, respectively. Overall, $\delta^{15}N$ isotope ratios indicated that although nitrogen inputs did explain some isotopic concentrations in plants, isotope ratios were driven more by physical processes like insolation and available oxygen than by inputs of particular $\delta^{15}N$ signatures from shrimp farms. My results indicate that although it is likely that shrimp farming on the canal does impact the environment, other factors significantly influence it as well, with other anthropogenic inputs such as pig farming having a more significant and continual impact than temporal pulses of nutrients from pond harvests.

Introduction

As humans continue to exploit Earth's finite resources faster than they are replenished, it is clear that to be sustainable the human race must find a way to feed itself without sacrificing the environment that it depends on to provide its food. One of the areas of food production that has been overly taxed is seafood production. Although wild fish catches have plateaued, demand for seafood continues to rise with human population, and aquaculture continues to grow to fill the gap between supply and demand. Currently, aquaculture is the fastest growing food production system in the world and is expected to continue to expand at a rate of nearly 10% per year (Diana 2009). This increase, coupled with other definitive characteristics of aquaculture, including feeding practices and water treatment methods, implies that aquaculture may have a potentially large and increasing impact on loading anthropogenic nitrogen to sensitive coastal ecosystems. Shrimp aquaculture is one such example of a nutrient intensive production system. The average industry producer uses high protein feed to culture organisms in intensive systems, and a large percentage of nitrogen inputs (35-57%) are discharged with wastewater (Briggs and Funge-Smith 1994; Jackson et al. 2003a). Often this wastewater is discharged directly to receiving creeks, rivers, or estuaries without treatment, contributing significantly to eutrophication (Naylor et al. 1998; Preston et al. 2000). It has been asserted that these nutrient inputs have a significant and increasing detrimental impact on coastal marine nutrient dynamics (Ryther and Dunstan 1971; Howarth and Marino 2006; Smith et al. 2006).

Although nutrient dynamics and the influence of anthropogenic sources of these nutrients in coastal ecosystems are not completely understood, it is known that loading nutrients into a system can have adverse impacts, including increasing prevalence of coastal dead zones due to decomposition of plankton blooms, and the loss of ecologically important sea grass beds (Udy and Dennison 1997; Vitousek et al. 1997; Lapointe et al. 2004). Many governments and aquaculture organizations in developed and developing countries such as Australia, New Zealand, Honduras and Thailand have established water quality parameters that are measured to monitor the impact of the industry on the environment (NEB 1994; Boyd and Green 2002). For instance, goal oriented monitoring programs in Honduras, run as a collaboration between the Honduran government and the Honduran National Association of Aquaculturists, aim for no greater than

3mg/L ammonia (NH_3) and 10mg/L total nitrogen (TN) in discharged water, and coastal waters are monitored to watch for levels of total nitrogen over 0.75 mg/L TN (Boyd and Green 2002). Unfortunately, these methods to monitor water quality can be time consuming and expensive. It is estimated that a startup project would cost an upfront $300,000, with an annual cost of $159,000 thereafter (Boyd and Green 2002). Additionally, discrete sampling events may miss temporal and spatial variability in nutrient concentrations, since farm derived nutrient pulses can vary temporally, with large pulses occurring at harvest or during water exchange. Due to the complicated nature of nitrogen cycling in receiving waters, it can be difficult to estimate the ecosystem impact of nutrient inputs by a simple evaluation of chemical concentrations (Voss and Struck 1997; Wolanski et al. 2000).

Conventional water quality analysis does not differentiate between various sources of nutrients, or their ultimate fate. If a shrimp farm is located downstream from a sewage treatment outfall it is difficult to determine which source may generate the high organic nitrogen concentrations in local waters. Aquaculture could be blamed for eutrophication when there may be more important sources of reactive nitrogen, like agricultural fertilizers or domestic and municipal human sewage (Jones et al. 2001; Burford et al. 2003). Additionally, ecological impacts in the form of nutrients incorporated into growing tissue may also be observed at greater distances from nutrient sources than the zone of elevated nutrient concentrations in the water (Jones et al. 2001; Lin and Fong 2008). This implies that conventional water quality testing may not be a sufficient indicator of ecosystem impact by anthropogenic nitrogen. In response to this concern about the effectiveness and cost of conventional water quality monitoring, stable isotope analysis has been suggested as an alternative indicator to evaluate impacts of anthropogenic nutrients (Tucker et al. 1999; Costanzo et al. 2001; Jones et al. 2001) and to trace sewage and agriculture nutrient effects in coastal areas (Tucker et al. 1999; Daskin et al. 2008).

Stable isotope analysis traces varying isotope ratio signatures found in different organisms to determine nutrient transfer within a system of interest. For instance, isotopic ratios of nitrogen within organisms change because biochemical reactions process heavy isotopes of nitrogen (^{15}N) at slower rates relative to lighter isotopes (^{14}N), essentially concentrating the heavy ^{15}N in tissues during metabolism. This process is called fractionation, and results in characteristic ratios of

^{15}N:^{14}N in different organisms. Since fractionation (and thus altered concentrations of ^{15}N) occurs at each trophic level, organisms at higher trophic levels have higher ratios of ^{15}N:^{14}N. These ratios are compared to a standard ratio of atmospheric nitrogen to give a delta N number (δ^{15}N) which is presented in a per mil difference from the standard ratio and indicates how much fractionation has occurred. On average there is a 3-5 per mil increase from one trophic level to another (Fry 2006). Additionally, other human and biological processes can also significantly influence the δ^{15}N signature of different organisms. For instance, nitrification and denitrification can both have a substantial effect on δ^{15}N values, with increasing fractionation from 10 to 40 per mil (Fry 2006). Furthermore, because all nitrogen fixed in the Haber Bosch process is taken from N_2 in the atmosphere without any fractionation, synthetic fertilizers and plants deriving their nitrogen from this process have very little differentiation from the standard (Fry 2006).

Ratios of carbon isotopes (^{12}C and heavy ^{13}C) also provide information about biochemical processes characteristic of various organisms, and shed light on the dynamics of an ecosystem. Because heavy ^{13}C proceeds through photosynthesis at a slower rate (fractionating the carbon isotopes in the resulting tissue), autotrophic organisms have ratios of ^{12}C to ^{13}C that are different from the ratio available in the atmosphere. This fractionation is generally determined by the biochemical and physical traits of the plant, especially on land, where CO_2 diffuses quickly through the air, and lighter CO_2 with ^{12}C atoms can be preferentially taken up. δ^{13}C values vary significantly with type of photosynthesis, with C_3 photosynthesis, used by a majority of plants, fractionating to -28 per mil on average, and C_4 photosynthesis, used by corn, sugar cane and many tropical grasses, fractionating to an average of -13 per mil. However, a study by Finlay et al. (1999) demonstrated that water velocity and rate of diffusion of CO_2 past the boundary layer of photosynthesizing algae had a significant impact on δ^{13}C values in varying California stream habitats. In productive streams, algae from pools (where water velocity was slow) showed much less fractionation of carbon isotopes, with average values ranging from -17.9 to -21.7 per mil δ^{13}C. However, in fast moving riffles of the productive streams δ^{13}C was more fractionated (from -25.6 to -27.5).

Another indicator of nutrient dynamics in a system are the ratio of carbon to nitrogen (C:N) found in the tissues of organisms. C:N is the total ratio of carbon to nitrogen atoms found in a tissue, and

changes with the biology and chemistry of an organism. Higher carbon in relation to nitrogen is found in plant material, which is made of cellulose and other molecules that contain large amounts of carbon. Lower values of carbon in relation to nitrogen are found in heterotrophs and other protein rich organisms, which contain more nitrogen. C:N ratios have been used with stable isotopes to trace sources of organic matter in aquatic and marine environments (Andrews et al. 1998; Usui et al. 2006). However, difficulties in tracing sources and differences in decomposition rates can make assessment difficult (Meksumpun et al. 2005). Additionally, an experiment by Dorenbosch and Bakker (2011) demonstrated that the C:N ratio of aquatic macrophytes decreased when plants were grown in high nutrient environments, in this case with 2.2 mg KH_2PO_4 L^{-1} and 10.4 mg NH_4NO_3 L^{-1}.

These characteristic differences in stable isotope ratios can be useful in determining nutrient sources for organisms. For instance, nitrogen isotope ratios have been used to trace anthropogenic nitrogen sources through the ecosystem (Kao and Liu 2000; Cole et al. 2004; Meksumpun et al. 2005; Fry 2006; Daskin et al. 2008; Kon et al. 2009; Pinon-Gimate et al. 2009). Human sewage inputs generally produce considerably higher concentrations of N^{15} than shrimp farm effluent, with human sewage averaging 7 to 10 per mil $\delta^{15}N$, and shrimp aquaculture averaging 5 to 6 per mil $\delta^{15}N$. Both are also significantly higher than agricultural runoff, which averages -3 to 3 per mil $\delta^{15}N$ (Voss and Struck 1997; Tucker et al. 1999; Costanzo et al. 2001; Jones et al. 2001; Pinon-Gimate et al. 2009). Additionally, it is challenging to determine the fate of nutrients from shrimp ponds because volatilization, nitrification and denitrification in the ponds change the normal effluent isotope ratios produced by feeds used to grow shrimp (Handley and Raven 1992; Alongi et al. 2000). Costanzo et al. (2004) evaluated effluents from an isolated flow-through shrimp farm on the Northern coast of Australia, finding a signal of about 6 per mil $\delta^{15}N$ in the receiving ecosystem. The study utilized anchored growth chambers containing a fast growing macroalgae, which would quickly assimilate isotopes and worked well to establish a spatial gradient of dilution as the nutrients entered the bay. A later study of isotopes in anchored macroalgae samples in a bay in French Polynesia indicated that stable isotopes were the most sensitive indicator of impacts from a shrimp farm there, which also had an isotopic signature between 5 and 6 per mil (Lin and Fong 2008).

Stable isotopes have been successfully used to trace a signature from shrimp farms in the receiving environment in the past, but the procedure was complicated. Instead, it has been suggested that simply providing a surface for periphyton to adhere to may be an analogous alternative to collect plant material from receiving waters to analyze for stable isotope composition (Ledwin 2010). This technique was implemented on shrimp farms in Belize and detected elevated $\delta^{15}N$ levels near the outfalls of shrimp farms, indicating a perceivable zone of influence for the shrimp farms. However, some unexpectedly low N^{15} results indicated that nitrogen fixing blue green algae may become established in the periphyton and influence the $\delta^{15}N$ ratio results by fixing atmospheric nitrogen (Ledwin 2010). While there was success with tracing elevated levels of ^{15}N from these shrimp farms, the lack of other human impacts in these study areas meant that there were few other inputs of ^{15}N isotopes. Most areas where shrimp farming occurs also host many other industries and users that discharge nitrogen into the receiving water (e.g. livestock culture, agriculture, human and industrial waste) which would potentially complicate the evaluation of shrimp farm effluent on isotope values in periphyton. However, the unique fractionation of different organisms in theory supports the evaluation of nitrogen inputs from different sources by tracing their unique signatures in the receiving environment (Fry 2006).

The purpose of this study was to evaluate the impacts of shrimp farming on water quality and aquatic ecosystem processes in the Bang Pakong River Region of Thailand. Specific objectives were to: 1) determine if there was a common isotope and water quality signature from shrimp farms in the area; 2) try to trace this signature in canal water and isotopic signatures of plants grown in that water; and 3) determine what water quality and physical parameters influenced the isotope signature.

I expected that there would be a common $\delta^{15}N$ isotope signature from shrimp farms, which should be 2 to 5 per mil higher than the feed signatures. I also expected that this signature would be different than other sources of nitrogen, so it would be perceivable in plants and animals growing in the receiving environment and would be more diluted with distance from the source of the nutrients. Additionally, higher levels of $\delta^{15}N$ should be correlated with levels of organic nitrogen parameters in the water, but I expected the isotope signature from shrimp farms to be perceivable at a greater distance than elevated nutrient levels.

Study Site

The study site was chosen to represent an area with shrimp farms using wastewater treatment practices common to the area. Expert opinion and data from a region-wide farmer survey conducted separately indicated that shrimp farming in the Bang Pakong River region was dominated by small-scale, privately owned and operated farms which were semi-intensively managed. There are approximately 10,000 of these small-scale farms in the region. *Litopenaeus vannamei* (Pacific white shrimp) were commonly stocked at medium to low densities (20-100 post larvae/m^2), fed formulated shrimp feed daily, and grown for 3-4 months before harvest. Minimal preconditioning of water was practiced, little water exchange took place, and water was directly discharged without treatment during harvest. There were many other agricultural and manufacturing industries in the region, as well as large population centers, which also contributed nutrients to the system.

The Bang Pakong Basin is characterized by a diurnal tide, which greatly influences the flow of the river twice daily. The low topography of the region leads to tidal influence extending nearly 100km upriver. The region is also highly altered hydraulically to provide water for inland farms. Numerous irrigation canals are maintained by the government and were originally constructed to provide water for rice farming. These canals have subsequently been used to supply water to shrimp farms. It is against Thai federal regulations to discharge harvest water directly to the canals without treatment (NEB 1994). A 3 km irrigation canal connected to the Bang Pakong River, the Chucachur Canal, was selected as the representative site to evaluate nutrient inputs from shrimp farms. It is located about 45 km upstream from the mouth of the Bang Pakong River. The main canal was always inundated with water, but depth and the direction of flow changed twice daily under influence of the tide. Numerous side channels branch off from the main canal, connecting additional agriculture and aquaculture farms, but most did not have significant flow. Farms along the canal all used similar management practices, including pond size, stocking and feed application. Closer to the mouth of the canal more shrimp farms were connected by small connecting canals, and thus the density of farms increased further downstream.

Methods

PVC poles were used to passively collect periphyton, and subsequently used to measure ambient isotope ratios available in the environment at each sample site. To do this, poles were driven into the substrate of culture ponds and in the canal at regular intervals. Feed was also collected to determine isotope ratios of inputs to the shrimp farms. Because periphyton was initially difficult to grow on Chucachur Canal, water hyacinth (*Eichhornia crassipes,* an introduced aquatic plant which grows vigorously throughout the Bang Pakong River) was used there as an additional isotope-collecting organism. Benthic mollusks (Order: *Basommatophora*, Family: *Physidae* and Order: *Unionoida* Family: *Unionidae*) were also collected from the canal for stable isotope analysis.

All samples for stable isotope analysis were rinsed, dried for 48 hrs at 60 degrees C, ground, and packed into 5mm tin capsules. Samples were processed for heavy ^{13}C and ^{15}N isotope values at the UC Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). δ^{15}N and δ^{13}C were reported in per mil difference from experimental standards. Total mass of C and N in the sample were reported, and C:N ratio was determined by dividing total C by total N.

Sampling points were selected at regular intervals along the canal. The points proximal to the river (C4-C14) were accessed by small boat, and the points distal to the river (C1-C3) were accessed by road and foot. On 26 June 2009, a 1.5-meter hollow PVC pole was driven into the channel bottom at each point to passively collect periphyton. However, with the vast tidal variation in depth, water was not consistently in contact with any part of the pole throughout the tidal cycle and periphyton did not grow. After seven days without periphyton growth, floating cages were designed and constructed which surrounded the pole and floated up and down with water level. Water hyacinth was collected from canals on the Asian Institute of Technology campus, 80 km from the study site. After two days in fresh water, the newest leaf on each plant was collected and dried for isotope analysis as the pre-placement value. On 10 July 2009 a basket containing a hyacinth plant was placed at each sampling point along the Chucachur Canal. While

deployed, the flotation devices maintained constant contact with the water as depth changed with the tide. On 22 July 2009, periphyton was observed growing on the flotation devices and was then included in stable isotope analysis. Insolation at each sampling point was estimated one time from approximate cover of the 50 meters above and below each point (100 meters total).

Hyacinth and periphyton samples were collected on 22 July, 13 August, and 29 August 2009, at as close to regular intervals as logistics would allow. These days coincided with 2 weeks (July 22), 2 days (13 August) and 0 days (August 29) after known harvests from at least one shrimp farm along the canal. Hyacinth and periphyton samples were collected at each point whenever possible, however, some samples were lost during high water events, or due to problems with access during sampling.

Snail and mussel tissue were collected from accessible points on 22 July 2009 by rinsing sediment collected by hand from the canal bottom through a mesh sieve. Two species were sampled, one known as a chedi snail (6 collected, probably *Melanoides tuberculatus)* and one small unknown bivalve species (5 collected). *M. tuberculatus* feeds on algae, detritus and particulate matter. It is tolerant of a wide range of conditions and is a very common snail in Thai waters.

Additionally, seven farms directly adjacent to the Chucachur Canal were monitored using periphyton isotopes, but not hyacinth because it did not survive in the saline environment. Samples of feed were also taken from these farms to evaluate the stable isotope ratios input by shrimp farms. Unfortunately, most shrimp farms were harvested without prior notice and no samples of shrimp tissue were collected from the farms.

Water quality was also measured to evaluate the dynamics of wastewater and stable isotopes in the system. On each sampling day, water temperature, pH, secchi depth and dissolved oxygen were recorded at five evenly spaced sampling points in the canal (C1, C3, C6, C10 and C14). Water column samples were also collected at these points using an integrated water sampler. Due to logistical constraints water quality on the farms was limited to collections from F2, F7 and F8 on 13 August 2009.

Once collected, water samples were immediately placed in a lightproof insulated box containing melting ice and transported to a laboratory at the Asian Institute of Technology (AIT) for analysis of TSS, TAN, NO_2, NO_3, TKN, TP, and Chlorophyll A. Total suspended solid (TSS) was measured gravimetrically by filtering a well-stirred water sample through pre-washed, oven dried at 105 °C and pre-weighed GFC filter (Whatman, 1.2 μm pore size, 4.7 cm in diameter) under suction. The residue retained on the filter paper was dried to constant weight at 105 °C for 24 hrs and reweighed after cooling in a desiccator (APHA 1998).Total ammonia-nitrogen (TAN) was analyzed according to the modified Phenate method described in Parsons et al. (1984). The analytical procedure for nitrite-nitrogen (NO_2) used the method described in Parsons et al. (1984). Nitrate nitrogen (NO_3) was determined using the cadmium reduction method (APHA 1998). Total Kjeldahl Nitrogen (TKN) was determined using the standard method (APHA 1998). Total phosphorus (TP) content was measured by autoclaving sample water at 15 to 20 psi for 30 minutes. The water sample was then treated with potassium persulphate in an acidic medium and followed by the ascorbic acid method according to APHA (1998). Chlorophyll A biomass in water samples were measured spectrophotometrically (CECIL – CE 1021) by filtering a 100 ml sample through a GFC filter. Chlorophyll A was then extracted from the filter with 90% acetone according to APHA (1998).

To facilitate spatial and temporal comparison between different water quality measures, each parameter was ranked with respect to its impact on the environment. Thailand has water quality standards for both surface water and inland aquaculture effluent, but they use a limited number of water quality parameters (NEB 1994; MNRE 2007). Therefore, rankings were determined as a measure of the ecological functioning of the ecosystem, also taking Thai water quality measures into account. Each parameter was ranked on a 5-point scale with 1 being least impaired and 5 being the most impaired. Detailed descriptions of the rankings are found in the first column of Table 1. Individual water quality parameters were categorized as described below (see also Table 1).

Thai inland aquaculture effluent standard for NH_3 calls for less than 1.1 mg/L in effluents for Standard B (moderate regulation) (MNRE 2007) and Thai surface water quality standards call for less than 0.5 mg/L NH_3 in Class 3 water bodies (which include the Bang Pakong River and the

Chucachur Canal) (NEB 1994). Boyd (2000) stated that concentrations of NH_3 less than 0.25 mg/L indicate unpolluted waters, above 1 mg/L indicate polluted waters, and 5 to 10 mg/L are not uncommon in highly polluted waters. Thus, NH_3 was categorized as 1: 0-0.249 mg/L, 2: 0.25-0.49 mg/L, 3: 0.5–0.749 mg/L, 4: 0.75–1.49 mg/L, 5: $\geq$ 1.5 mg/L.

Thai inland aquaculture effluent standards do not regulate NO_3, but Thai surface water quality standards call for less than 5.0 mg/L NO_3 in Class 3 water bodies (NEB 1994). Chapman (1996) stated that natural concentrations seldom exceed 0.1 mg/L in un-impacted waters, and that human influence can raise these levels up to 5 mg/L, but often less than 1 mg/L. Thus NO_3 was categorized as 1: 0-0.249 mg/L, 2: 0.25-0.49 mg/L, 3: 0.5–0.749 mg/L, 4: 0.75–1.49 mg/L, 5: $\geq$ 1.5 mg/L.

Thai aquaculture effluent and surface water quality standards do not provide guidelines for NO_2. However, Chapman (1996) indicates that NO_2 concentrations are usually low and rarely exceed 0.05 mg/L, but may reach higher concentrations in polluted and anoxic water. Thus NO_2 was ranked with 1: 0.0–0.0249 mg/L, 2: 0.025-0.049, 3: 0.05–0.09 mg/L, 4: 0.10–0.149 mg/L, 5: $\geq$ 0.15.

The Thai inland aquaculture effluent standard calls for less than 1.1 mg/L TP in effluents for Standard B (MNRE 2007) but Thai surface water quality standards do not address target TP concentrations. Chapman (1996) and Boyd (2000) indicate that TP in natural surface water generally ranges from 0.005 to 0.020 mg/L, and seldom exceeds 5 mg/L except in hyper-eutrophic waters or wastewater. Thus, TP was ranked 1: 0-0.029 mg/L, 2: 0.03-0.049 mg/L, 3: 0.05–0.09 mg/L, 4: 0.1–0.39 mg/L, 5: $\geq$ 0.40.

Thai water quality standards do not address Chlorophyll A concentrations, but Chlorophyll A is considered a good indicator of eutrophication, with 5-140 mg/m^3 indicating eutrophic conditions, and levels in excess of 300 also occurring (Chapman 1996; Boyd and Green 2002). Thus, Chlorophyll A ranking was established at 1: 0-9.9 mg/m^3, 2: 10–29.9 mg/m^3, 3: 30-59.9 mg/m^3, 4: 60-89.9 mg/m^3, and 5: $\geq$ 90 mg/m^3. However, these values may be confounded by high productivity and hyacinth growing in the environment.

Dissolved Oxygen (DO): Thai surface water quality standards call for more than 4.0 mg/L DO in Class 3 water bodies (NEB 1994). Thus DO was ranked as 1: $\geq$ 9 mg/L, 2: 7-8.9 mg/L, 3: 5-6.9 mg/L, 4: 3-4.9 mg/L, 5: 0-2.9 mg/L.

Table 1: Values used for ranking of water quality parameters by their degree of impairment on the receiving water.

	TKN (ppm)	NH$_3$ (ppm)	NO$_3$ (ppm)	NO$_2$ (ppm)	TP (ppm)	Chlorophyll A (mg/m^3)	DO (mg/L)
1. Impairment unlikely	0-0.09	0-0.249	0-0.249	0-0.0249	0-0.029	0-9.9	$\geq$ 9
2. Impairment possible	0.1-0.49	0.25-0.49	0.25-0.49	0.025-0.049	0.03-0.049	10-29.9	7-8.9
3. Impairment likely	0.5-0.99	0.5-0.749	0.5-0.749	0.05-0.09	0.05-0.09	30-59.9	5-6.9
4. Impairment expected	1-1.99	0.75-1.49	0.75-1.49	0.10-0.149	0.1-0.39	60-89.9	3 - 4.9
5. Well beyond impairment levels	$\geq$ 2	$\geq$ 1.5	$\geq$ 1.5	$\geq$ 0.15	$\geq$ 0.40	$\geq$ 90	0 - 2.9

In order to determine the influence of shrimp farms on the canal environment, the following steps were followed: 1) shrimp feed isotopes (δ^{13}C and δ^{15}N, and C:N) were analyzed to determine expected baseline values of shrimp farm derived isotopes; 2) Stable isotopes from periphyton grown on shrimp ponds were analyzed to determine the levels of fractionation occurring due to processing by shrimp, bacteria and chemical processes in the ponds, and what would be expected as an isotopic signature from ponds in the canal; 3) Water quality parameters were evaluated to determine if they showed significant variation temporally or spatially; 4) Stable isotopes from hyacinth grown in the canal were analyzed and compared to pre-placement isotope values to determine if there was a discernable change in isotopic signature in the canal; 5) Hyacinth and periphyton isotopes from the canal were evaluated and compared to determine if there was similar fractionation of isotopes there; 6) Mollusk tissue was also compared to periphyton and hyacinth isotopes to further evaluate whether shrimp farms influenced another trophic level in the canal; 7)

Periphyton and hyacinth isotope data were analyzed to determine if they varied spatially; 8) Canal periphyton was compared with on farm periphyton to see if canal isotopes were influenced by farms; and 9) Multiple linear regression was used to evaluate what physical and chemical parameters influenced the isotope ratios of periphyton and hyacinth.

Data was assessed for normality and adjusted with a log 10 transformation as needed to meet assumptions of statistical tests. All statistical tests were run using the statistical program PASW Statistics 18, Release Version 18.0.0. (PASW 2009).

Because many hyacinth samples were lost, few points had samples collected on all days, and isotopes of hyacinth before and after placement in the canal (after 12, 31 and 50 days) were compared with the Paired-Sample T-Test. Similar issues also lead to small number of replicates in comparing isotopes from canal hyacinth and periphyton, so again the Paired-Sample T-Test was used. Additionally a Pearson Correlation was used to evaluate the similarity between hyacinth and periphyton isotope values. Mollusk isotope content was compared to canal periphyton and hyacinth with a One-way Between Groups ANOVA.

Since the assumption of equal variance was violated in some data sets, non-parametric tests were also used. The Wilcoxon Rank Test was used to compare feed and farm periphyton. Due to small sample size a p value of 0.1 was considered significant for this comparison. When the canal and farms were compared, the Mann-Whitney Test was used to evaluate significant differences. However, when three or more different groups were compared, for example each independent point on the canal and farms, the Kruskal-Wallis test was used to evaluate differences between the groups. The Tukey HSD Test was used to evaluate significant variation in isotopes from point to point along the canal.

Differences in water quality were also evaluated with non-parametric tests due to non-normal distributions in the data. The Friedman Test was used to evaluate differences in water quality on the canal between days. The Mann-Whitney U Test was used to compare farm water quality and canal water quality on the days when there were and were not harvests. The Kruskal-Wallis Test was used to evaluate differences in water quality parameters from point to point along the canal.

Multiple linear regressions were performed on transformed data to evaluate the influence of water quality and physical parameters like insolation and proximity to shrimp ponds on isotopic signatures in the canal to determine if shrimp pond waste had a significant impact on nutrient processing in the canal. An alpha value of 0.05 was considered significant, except in the case where replication was extremely low and thus the significant alpha was relaxed to 0.1. Finally, the relationship between isotopes in each species was evaluated using a Pearson Product-moment Correlation Coefficient.

Results

Shrimp feed from farms on the canal showed a characteristic isotope signature. Isotope values for feed were an average of -24.96 per mil for δ^{13}C, an average of 4.59 per mil for δ^{15}N, and an average of 7.71 for C:N.

Nitrogen isotope values and C:N ratios were considerably different between periphyton grown in ponds and shrimp feed, indicating fractionation by attached algae. There was a consistent difference in isotope values between feed and periphyton growing on farms (Figure 1). Although there was not a significant difference between δ^{15}N isotope values of periphyton from different shrimp farms, δ^{15}N values from periphyton did show significant increases from feed values, indicating that significant fractionation occurred in the ponds (Wilcoxon Rank Test: $z = -1.753$, $p = 0.080$). C:N also showed significant decreases from feed to periphyton values (Figure 2, Wilcoxon Rank Test: $z = -2.023$, $p = 0.043$). δ^{13}C isotopes from periphyton grown on shrimp farms showed no significant differences between farms or from feed. δ^{13}C values from all farm periphyton and feed had a mean of -27.04 with a standard deviation of 2.62.
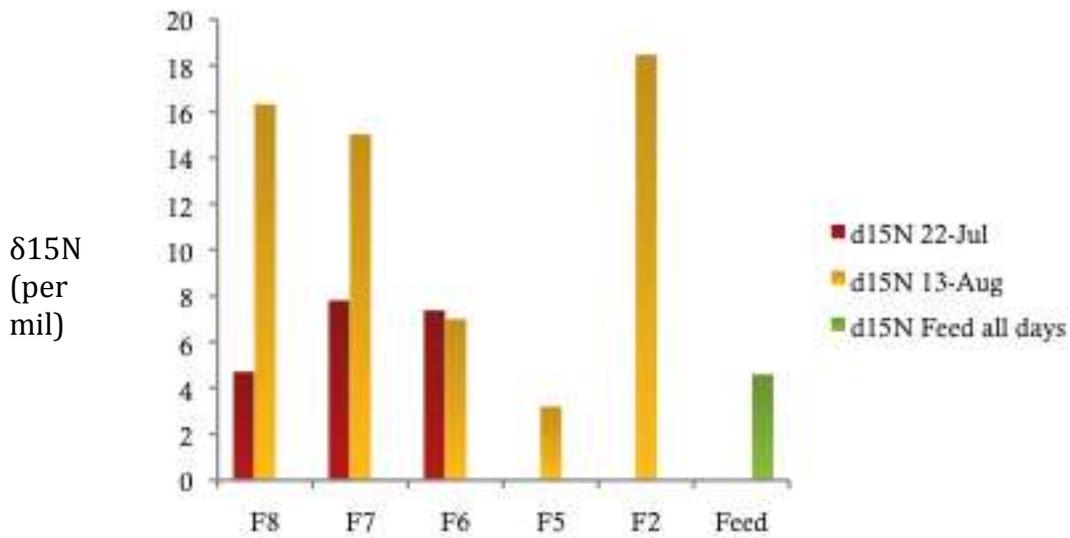
Figure 1. $\delta^{15}N$ of periphyton grown in shrimp ponds and feed used for five farms on two sampling days
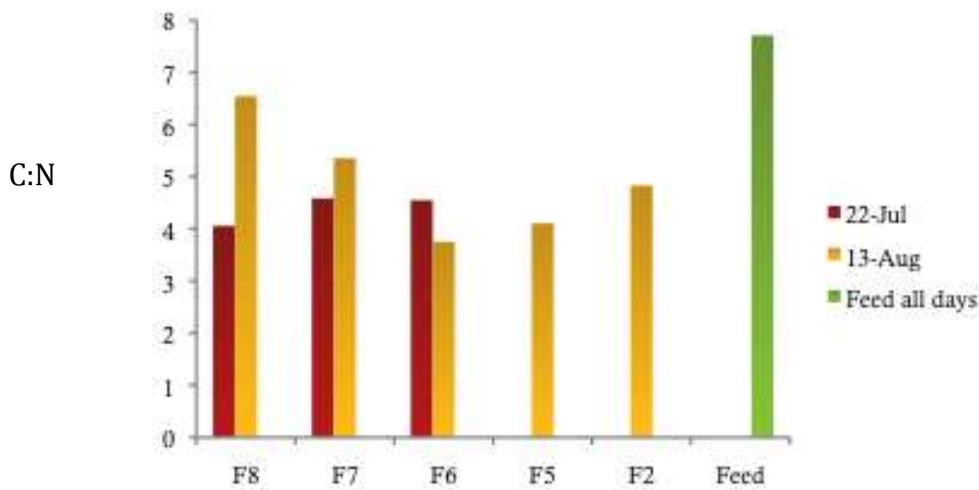
Figure 2. C:N of periphyton grown in shrimp ponds and feed used for five farms on two sampling days.

I found differences between farm and canal periphyton isotope values, indicating that although farms could be the source of canal isotopes, there were differences in nutrient dynamics between the two systems. There was no significant difference in $\delta^{15}N$ between farm periphyton values

(Median = 9.99) and ALL canal periphyton values (Median =11.86) (Mann-Whitney U = 104.00, p = 0.357), indicating that farm nitrogen was likely a major source of the canal nitrogen. However, there was a significantly lower C:N for periphyton from farms (Median = 4.72) than from all canal points (Median = 6.36, Mann-Whitney U = 29.00, p = 0.001). Additionally, $\delta^{13}C$ of the canal periphyton was significantly higher than $\delta^{13}C$ of farm periphyton for all days (Canal $\delta^{13}C$ Median = -22.60, Farm $\delta^{13}C$ Median = -27.57, Mann-Whitney U = 41.00, p = 0.002). Furthermore, although there was no significant difference in average $\delta^{15}N$ between periphyton from the farm and canal, variance in canal $\delta^{15}N$ was quite large (Figure 3). There was a significant difference in periphyton $\delta^{15}N$ at each location along the canal with highest values at the sampling locations most proximal and distal to the river (C1, C13 and C14), and lower values in the middle of the canal (C4, C7 and C8).
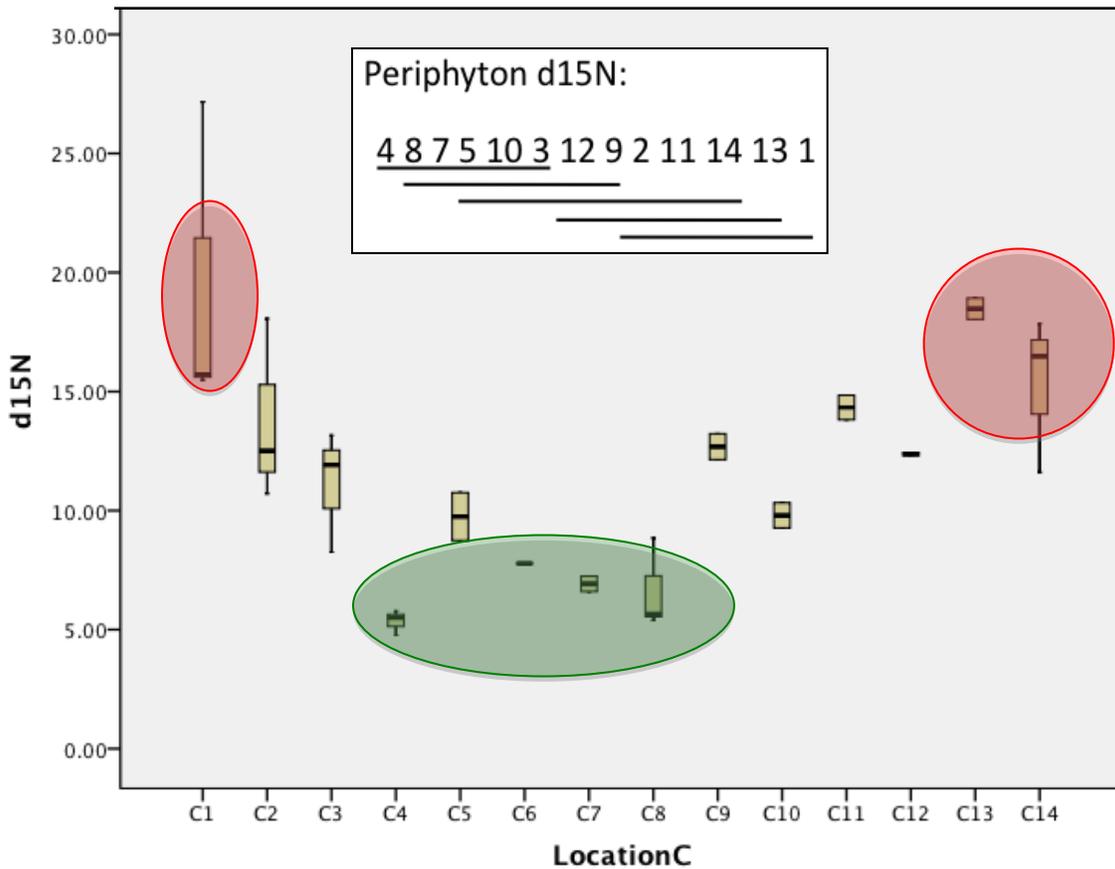
Figure 3. Box plots for $\delta^{15}N$ values of periphyton grown at each location on the canal. Red shaded points were significantly high values, green shaded values significantly low values. Sample points underlined at the same level in the inset figure at top were not significantly different (p>0.05, Tukey HSD Test).

Comparing $\delta^{15}N$ of farm periphyton with periphyton from each point on the canal indicated that a few points on the canal had less fractionation than farms, while most had more fractionation. Evaluating $\delta^{15}N$ for periphyton from each individual point, only three points on the canal (C4, C7 and C8) had $\delta^{15}N$ levels lower than farm values (Kruskal Wallis Test, p = 0.028), while the other 11 points on the canal had higher $\delta^{15}N$ levels in periphyton tissue. Thus, although overall there were not significant differences in $\delta^{15}N$ of periphyton between farms and the canal as a whole, there was great variation in values, and large differences in mean values of each location which made it difficult to trace the isotopic signature.

Hyacinth isotope values showed a change in nitrogen isotope ratios and increased eutrophication in the canal. Hyacinth tissue grown in the canal had significantly different $\delta^{15}N$ values compared to pre-sample tissue. Pairwise comparisons showed an average increase of 9.05 per mil $\delta^{15}N$, which is on the same order of magnitude as both the change in $\delta^{15}N$ between feed and periphyton on farms, and the change in fractionation between high and low periphyton in the canal. Pairwise comparisons also showed C:N for hyacinth grown in situ significantly decreased from pre-sample values, with an average of -8.21 per mil.

Hyacinth grown in the canal showed a significant difference in $\delta^{15}N$ spatially with lowest values in the middle of the canal (C4, C7 and C8), and highest values at the end of the canal proximal to the river (C13 and C14, Figure 4). C1, C3, C6, and C10 were removed from analysis because there was only one sample at these points. The increase in $\delta^{15}N$ was expected as more farm effluent entered the canal as it progressed downstream, while the high levels at C1 were not expected.
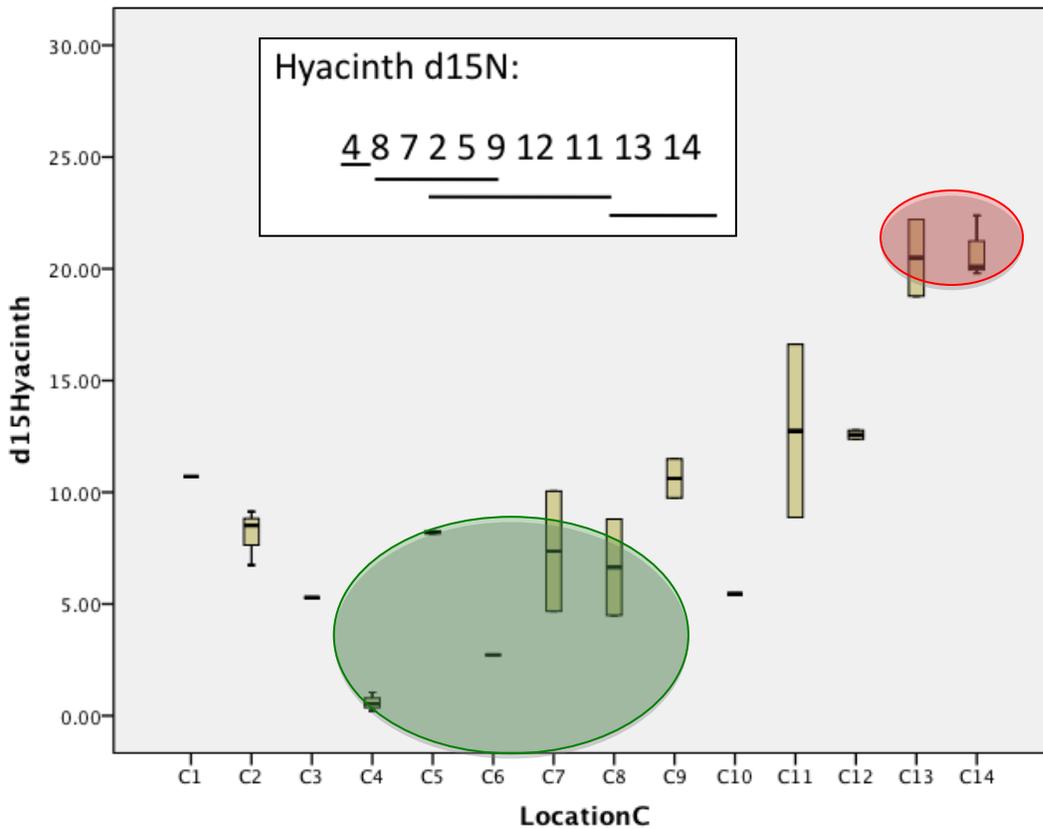
Figure 4. Boxplots of δ^{15}N values for hyacinth grown at each location on the canal. Notations as in Figure 3.

δ^{15}N values from hyacinth and periphyton collected at the same points on the canal were different in magnitude, but were highly correlated (Pearson Correlation = 0.660, p <0.0005), indicating a similar response to the ambient environment nitrogen. Periphyton δ^{15}N was consistently higher than hyacinth δ^{15}N, and a Paired-Sample T-Test showed a significant difference in mean of +2.039 per mil δ^{15}N for periphyton compared to hyacinth.

In contrast to δ^{15}N trends, δ^{13}C was significantly different but uncorrelated between periphyton and hyacinth values. A paired sample t-test showed a statistically significant difference (p <0.0005) between periphyton and hyacinth (+9.293 per mil), but an insignificant Pearson

18

Correlation. $\delta^{13}C$ also had a significantly different mean between farm and canal periphyton of +4.97 (Mann-Whitney Test, p = 0.002). A correlation matrix of all isotope types between themselves and with water quality parameters is found in Appendix 1A and 1B.

Mollusks collected from the canal were not significantly different from periphyton and hyacinth in $\delta^{15}N$ and $\delta^{13}C$ (Table 2), but showed less variation (with the exception of $\delta^{13}C$ in hyacinth). They did have lower C:N values than plants, which is expected in as they are consumers rather than producers.

Table 2. Means, standard deviations (SD) and coefficients of variance (CV) for $\delta^{13}C$, $\delta^{15}N$, and C:N of mollusks, periphyton and hyacinth collected in the canal. Samples for snails and mussels were collected on 22 July, samples for hyacinth and periphyton include all sampling dates combined.

	$\delta^{15}N$		$\delta^{13}C$		C:N	
	Mean (SD)	CV	Mean (SD)	CV	Mean (SD)	CV
Snail	8.908 (0.959)	0.103	-23.853 (0.892)	0.033	3.912 (0.219)	0.012
Mussel	11.873 (2.496)	0.525	-27.790 (2.563)	0.236	4.482 (0.539)	0.065
Periphyton	11.859 (4.937)	2.055	-22.596 (4.442)	0.873	6.362 (0.972)	0.148
Hyacinth	9.999 (6.445)	4.155	-30.961 (1.258)	0.051	9.295 (1.123)	0.136

Water Quality

Water in ponds of all three farms sampled met NH_3 standards for Thai inland aquaculture effluent (less than 1.1 mg/L, rank 2 or less). However, TP far exceeded the Thai inland aquaculture effluent standard of 0.05 mg/L for all farms. TKN, NH_3, NO_2, TSS and Chlorophyll A varied considerably from farm to farm with some farms achieving the Thai standard and others exceeding it (see Figure 5d).

The canal water quality was degraded and pollutant concentrations often exceeded Thai surface water quality standards as well as standards for inland shrimp farm effluent (Figure 5). Pearson-moment correlations for water quality parameters compared with isotope values and other water

quality parameters are found in Appendix 1B and 1C. NH_3 exceeded the surface water quality standard at C1 and C3 two days after a harvest (13 August), and at all canal locations on a harvest day (29 August). NH_3 also exceeded the limit for inland aquaculture effluent at C1 and C3 on 13 August and at C1, C3, and C6 on 29 August. Values for TP also exceed the Thai inland aquaculture effluent limits at all points on every sampling day. Although DO was below the Thai surface water quality standard of 4 mg/L at point C1 on 29 August, these samples were taken during daylight hours when photosynthesis would cause an increase in DO levels compared to a predawn minimum DO.

The level of NH_3 in canal water was significantly different between sampling days, with highest levels on the day of a harvest, and lower values in samples taken two days and two weeks after a harvest (chi squared = 8.40, p = 0.015). On the day of a harvest (29 August) the median NH_3 was 1.85 ppm, two days after a harvest (13 August) it was 0.231 ppm, and 14 days after a harvest (22 July) it was 0.111 ppm.

DO, TSS and Chlorophyll A were significantly different between the farms and canal two days after a harvest (13 August). Specifically, DO values were significantly higher (p<0.05) on the farm (Median = 9.3, n = 3) than in the canal (Median = 4.78, n = 5), undoubtedly because farms were oxygenated with aerators. Also, TSS values varied and were significantly higher on the farms (Median = 10.30, n = 3) than in the canal (Median = 1.70, n = 5). Additionally, Chlorophyll A values were also significantly higher on the farms (Median = 219.0, n = 3) than the canal (Median = 24.06, n = 5).

Interestingly, comparing water quality between farms and the canal on the day of a harvest (29 August), DO, TSS, and Chlorophyll A were not significantly different, indicating farm effluent may be transforming the water quality of the canal to shrimp farm levels. However, NH_3 was significantly higher in the canal on a harvest day compared to farm values overall. Either the farm harvested on 29 August had significantly elevated levels of NH_3, or there was another source of NH_3 in the canal. However, water quality was not evaluated on the harvested farm. Although NH_3 was elevated at C1 and C3 in the canal on days when no farms were harvested and on 29 August

(the day of harvest), NH_3 was also elevated at C6 on 29 August, where there was a visible plume of shrimp farm wastewater.

Spatially, NO_2 was statistically different from point to point (Kruskal-Wallis Test, Chi squared = 10.711, p = 0.030) with the highest mean ranking found at the upstream end of the canal (distal to the river, C1), and decreasing to the lowest mean ranking at the last sampling point in the river (C14). Additionally, the highest concentration ranking of TP was also found at the upstream end of the canal, and decreased to the lowest mean ranking in the river. On-farm water quality was also significantly higher than most points along the canal for both NO_2 (Chi squared = 12.797, p = 0.025) and TP (Chi squared = 13.023, p = 0.029).
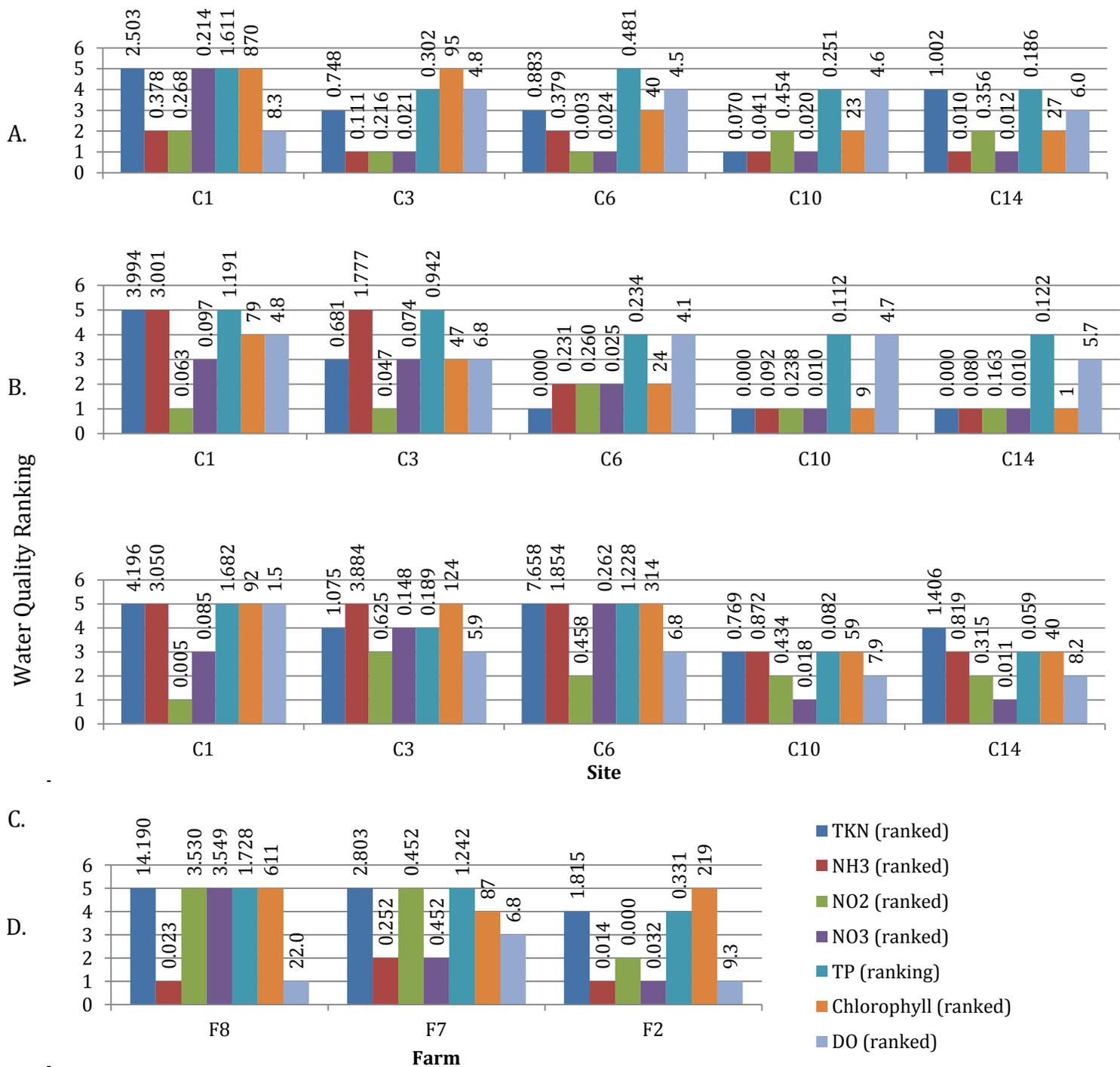
Figure 5. Ranked water quality measures for each sampling site on Chucachur Canal 22 July (A), 13 August (B) and 29 August (C) and on adjacent farms on 13 August (D) by sampling site and farm. Actual values for each parameter are listed above each bar (in ppm). Values of 2 or 1 indicate compliance with Thai water quality standards.

Multiple linear regressions of periphyton $\delta^{15}N$ indicated that $\delta^{15}N$ decreased (showed less fractionation) with increasing shade on the canal ($R^2 = 0.442$, $p < 0.0005$, see Table 3 and Appendix 2A for full statistical results). Additionally, % shade and NO_2 explained the periphyton $\delta^{15}N$ data better than % shade alone, indicating that less shade and more NO_2 increased periphyton $\delta^{15}N$ ($R^2 = 0.589$, $p = 0.007$, see Table 3 and Appendix 2B for full statistical results). Periphyton $\delta^{13}C$ was also significantly correlated with % shade, with increasing shade related to lower $\delta^{13}C$ values ($R^2 = 0.196$, $p = 0.006$, See Table 3 and Appendix 2C for full statistical results).

Hyacinth $\delta^{15}N$ showed the most interesting results from multiple linear regressions. The best model indicated that $\delta^{15}N$ increased with decreasing shade, increasing number of shrimp farms and increasing NH_3. This multiple linear regression of hyacinth $\delta^{15}N$ with % shade, number of shrimp farms and NH_3 had an adjusted R^2 value of 0.998, with a highly significant ANOVA ($p < 0.0005$, see Table 3 and Appendix 2D for full statistical results).

Table 3. Results of significant linear regressions of periphyton or hyacinth isotopes with physical and chemical characteristics of each canal site.

Variable	Standardized Coefficient (β)	P
Periphyton $\delta^{15}N$: $R^2 = 0.442$, $P < 0.0005$		
% shade	-0.678	<0.0005
Periphyton $\delta^{15}N$: $R^2 = 0.589$, $P = 0.007$		
% shade	-0.731	0.005
NO_2	0.455	0.044
Periphyton $\delta^{13}C$: $R^2 = 0.196$, $P = 0.006$		
% shade	0.470	0.006
Hyacinth $\delta^{15}N$: $R^2 = 0.998$, $P < 0.0005$		
% shade	-0.520	<0.0005
# shrimp farms	0.940	<0.0005
NH_3	0.541	<0.0005

Discussion

Evidence from shrimp farms along the Chucachur Canal indicated that wastewater was elevated in nitrogen and TP, which could have a significant impact on the receiving ecosystem. Although there was a significant change in isotopes from feed to periphyton growing at the shrimp farms, there was a similar common signature between all farms monitored. However, isotope values on farms were significantly different from canal isotope values, indicating that additional factors including other inputs, biological processing of excessive nutrients, and physical characteristics of the environment all had impacts that made it impossible to trace the farm isotope signature in the canal in isolation.

Significant increases in $\delta^{15}N$ between feed inputs and periphyton growing in the pond indicated significant fractionation of $\delta^{15}N$ in the ponds as expected. The average 2 per mil change (from 4.59 to 7.60) on the study farms was similar to a 2 per mil change (from 5.27 to 7.37 per mil) in $\delta^{15}N$ found in pond flocculent on shrimp farms in Belize (Ledwin 2010). It is likely that these changes stem from the cumulative effects of nitrification, denitrification and volatilization of organic nitrogen wastes in the ponds (Handley and Raven 1992). Since there was no water exchange on the study farms, and the highest values of $\delta^{15}N$ were recorded closest to the end of the culture cycle, fractionation (and thus values of $\delta^{15}N$) appeared to increase as processing of nitrogen continued over the shrimp grow out. $\delta^{15}N$ values were not significantly different from farm to farm, indicating that processes on farms were comparable and that comparable signatures should be emanating from farms as expected, and could be compared to canal isotope values to determine the extent of impact of farm nutrients on the receiving ecosystem.

Additionally, although water quality at the farms could not be measured over time and varied somewhat between farms, TP values and organic nitrogen were high, as expected. Water quality on these farms was comparable to water quality on similar studies of semi-intensive shrimp farming (Islam et al. 2004). Differences in water quality between farms sampled are likely to reflect different stocking time and management practices between farms.

Canal water quality was similar to average farm water quality on the day a farm was harvested. This was expected and similar to results of other studies where shrimp farm effluent was found to be a source of eutrophication in receiving waters (Preston et al. 2000; Wolanski et al. 2000; Jones et al. 2001; Pinon-Gimate et al. 2009; Ledwin 2010). I expected canal water to show the maximum change in stable isotopes and the worst water quality near the downstream end. However, station C1 consistently had values similar to stations C13 and C14, even though it was the most upstream station. A pig farm located at C1 appeared to have a significant impact on water quality in that portion of the canal on all days, which added confusion to these results. On days when no farm was harvested, high values of organic nitrogen at C1 decreased with distance, and were relatively low by C6. However, on the day of a harvest, although water quality values at C1 and C3 remained high, C6 had the highest levels of TKN, NH_3, NO_2 and TP on the canal. Since these results corresponded to a visible plume of shrimp harvest water at this point it was determined that shrimp effluent did impact canal water quality in the canal with a magnitude that rivaled the input from the pig farm on the day of a harvest. However, since the pig farm had a significant impact on the canal on all days and no impact from shrimp farms was observed on days without a harvest, shrimp farms were clearly not the only contributor to eutrophication measured. This is similar to previous studies which determined that multiple sources of nutrients influenced eutrophication in receiving waters (Pinon-Gimate et al. 2009).

Levels of TP were consistently above inland aquaculture standards for all points, indicating that this was a significant contributor to eutrophication on the canal. Spatially, the decrease in both TP and NO_2 along the canal, and the high values of both nutrients on farms indicated that farms were a source of these nutrients, which may have been processed or diluted as the water moved into the main river. C1 was ranked high in both parameters, and higher than farms for TP, which indicated other inputs (like the pig farm) were also important inputs to the canal.

Stable isotopes of periphyton and hyacinth from the canal give another means to trace shrimp farm effluent in the receiving system. Significant differences in hyacinth $\delta^{15}N$ and C:N before and after placement in the canal, as well as correlation in hyacinth and periphyton $\delta^{15}N$ values on the canal indicate that both were successfully representing the isotope concentration of available nutrients as anticipated. However, isotope results varied substantially from what I expected. $\delta^{15}N$

25

in both hyacinth and periphyton were more spatially variable than expected, with differences for each of about 20 per mil δ^{15}N from the highest to lowest points on the canal. This was similar to some previous studies of δ^{15}N of point-source sewage inputs into coastal regions (Jones et al. 2001) and wide gradients of eutrophication in Swedish boreal streams (Bergfur et al. 2009) , but also had greater variation than studies with smaller effluent inputs (Ledwin 2010) or more diffuse inputs (Pinon-Gimate et al. 2009). Additionally, δ^{15}N values did not show evidence of dilution as expected with increasing distance from the point of input as seen in other studies (Jones et al. 2001; Ledwin 2010). Instead, fractionation first decreased, reached a low in the middle of the study area, and then increased again for the rest of the study area. Some of the highest δ^{15}N values were found at C1 (near the pig farm) and at C13 and C14 (in the main river) and lowest values were found near C4 (in the center of the canal). Although farm δ^{15}N values were contained within the range of canal values, δ^{15}N values were both higher and lower on the canal than those from farms. High δ^{15}N values at the end of the canal distal to the river (C1) correspond to high levels of organic nitrogen from the pig farm there, indicating that processing of these nutrients drove fractionation of nitrogen. However, high values of δ^{15}N at the end of the canal proximal to the river (C13 and C14) did not correspond to high levels of nutrients. Instead it is likely that increasing re-processing of chemical forms of nitrogen drove these fractionated values since nitrogen fractionates further in each phase of microbial decomposition (Handley and Raven 1992). However, since there were also more farms toward the end of the canal and there may have been a more frequent influence from shrimp farm harvests there, the increase may also be a cumulative signature of many shrimp farms being harvested.

The extremely low value of δ^{15}N in the canal at C4 for both hyacinth and periphyton further complicated nature of evaluating nutrient and isotope dynamics in the system. However, it is likely that the low δ^{15}N values reflect an input of synthetic fertilizer near this point, possibly from fertilized agriculture. This is supported by the values of δ^{15}N near zero in hyacinth at this point (Fry 2006). The values of periphyton were about 5 per mil, but were still the lowest value of δ^{15}N in the canal periphyton, which indicates that bacteria in the periphyton was potentially fractionating the nitrogen as it incorporated it into tissue.

The similarity of mollusk $\delta^{15}N$ values to the canal plant values and the decrease in variance in mollusk tissue isotopes indicate that mollusks consumed an average diet of plant material in the canal, and were not influenced by the significant variation found in plant $\delta^{15}N$, indicating that differences in mechanisms within the plants influence their $\delta^{15}N$ values. This was similar to invertebrate response to isotope values in basal resources of boreal streams over a gradient of eutrophication, which found that invertebrate $\delta^{15}N$ to be positively correlated with basal resources and nutrient enrichment (Bergfur et al. 2009). This supports the concept that $\delta^{15}N$ is a robust indicator of nutrient enrichment over various trophic levels in a system.

Multiple linear regressions indicated that insolation was important in explaining $\delta^{15}N$, and that less shade (or more sunlight) led to more fractionation of nitrogen in both hyacinth and periphyton. This was not expected, as sunlight should not directly influence microbial processing of nitrogen. However, sunlight should increase photosynthesis in the eutrophied environment, and increasing average dissolved oxygen at sunny points may allow more aerobic nitrification to occur. Additionally, as expected, nitrogen was also influential in plant $\delta^{15}N$ values, with increases in NH_3 explaining some of the increases in hyacinth $\delta^{15}N$ and increases in NO_2 explaining some increases in periphyton $\delta^{15}N$. This indicates that nitrogen concentrations did influence fractionation in the canal, and since NO_2 is normally a short-lived intermediary of nitrification in aerobic systems, areas with high periphyton $\delta^{15}N$ may either be anaerobic or areas where nitrification was occurring quickly. These increases in $\delta^{15}N$ with increases in nitrogen are similar to other studies which found that $\delta^{15}N$ was elevated in areas with NH_3 and NO_2/NO_3 were elevated due to inputs from sewage and shrimp farm effluents (Jones et al. 2001) and over a gradient of nutrient concentrations in various boreal streams, where $\delta^{15}N$ was positively correlated with TN (Bergfur et al. 2009).

Increased fractionation of $\delta^{13}C$ in periphyton and its positive relationship with insolation indicates that abundant nutrients and high rates of primary productivity as well as different colonizing species may influence $\delta^{13}C$ trends in periphyton. Different characteristics of the canal may influence periphyton $\delta^{13}C$, including a difference in colonizing species, a difference in nutrient dynamics, or a difference in physical parameters like insolation, water flow or DO that leads to this change. Since hyacinth and farm periphyton showed very little variation in $\delta^{13}C$, and canal

periphyton had a great deal of variation, limitation of CO_2 supply to the canal periphyton (due either to oxygen limitation of microbial respiration or to limited diffusion of CO_2 at the boundary layer of the periphyton) may be responsible for the variation in $\delta^{13}C$. This result, with less fractionation when CO_2 taken in during photosynthesis, is similar to results in productive California streams, where areas of low water velocity (and thus low diffusion of CO_2 past the boundary layer of photosynthesizing algae) showed much less fractionation of carbon isotopes (Finlay et al. 1999). Furthermore, experimental shading of seagrass plots in Tampa Bay, Florida also found lower levels of $\delta^{13}C$ in shaded plots of grass, indicating a reduction in carbon demand decreased the influence of a diffusion dependent carbon availability on the enzymatic discrimination against $\delta^{13}C$ (Durako and Hall 1992). This should be somewhat different than straightforward limitation of CO_2 in the system, since it is the rate of diffusion of CO_2 that slows photosynthesis enough to allow more ^{13}C to be utilized. However, alkalinity parameters were unfortunately not taken in this study, and could have helped to determine whether CO_2 could be limiting photosynthesis. Given the large carbon inputs from shrimp farms and other human activities, it is unlikely that CO_2 alone limited primary production in the canal.

Results of multiple linear regressions of physical and chemical canal parameters and isotope values indicate that sunlight contributed to both $\delta^{15}N$ and $\delta^{13}C$. Thus it is likely that photosynthesis rates: 1) caused $\delta^{13}C$ fractionation, driving fractionation down (making $\delta^{13}C$ values less negative) when photosynthesis was high, similar to results of shading in seagrass (Durako and Hall 1992) and 2) kept oxygen concentrations high enough to allow aerobic nitrification and thus increase fractionation of $\delta^{15}N$ in these areas, similar to experimental results indicating increasing nitrification with increasing oxygen in the water column (Handley and Raven 1992; Rysgaard et al. 1994) .

This study highlights significant problems with using naturally growing periphyton to trace stable isotopes, because nitrifying or denitrifying bacteria probably colonize the periphyton depending on environmental conditions, thus influencing $\delta^{15}N$ in the collected tissue. This was also an issue in the study in Belize, where unexpectedly low $\delta^{15}N$ near an effluent release point were attributed to different processes occurring in the presence of excess nitrogen (Ledwin 2010).

However, this study does provide information about the spatial magnitude of impacts from various sources. For instance, since highly fractionated δ^{15}N decreased to a low in the 1225 meters from C1 to C4, the influence of pig farm nutrients did not extend far beyond about a kilometer, or else the signature would show an average between both inputs. Shrimp pond effluent may also have very localized impacts in the canal due to limited flushing of the system, which may also influence the variability of nitrogen isotopes and contribute to reprocessing of isotopes at the end of the canal.

This emphasizes the fact that changes in stable isotope ratios in organisms do not necessarily indicate deleterious impacts on the system, but simply an assimilation of available nutrients. In fact, the nutrient inputs may be within the assimilative capacity of the system, and thus avoid adverse environmental impacts. Although the lack of benthic organism diversity sampled indicated that the system is somewhat degraded, to determine an adverse impact of eutrophication the canal would need to be compared to a similar, un-impacted control system or a pre-development reference point.

Future studies of the impact of shrimp farm effluent on a receiving water should choose simpler systems with fewer inputs to make tracing the impacts from shrimp farms more feasible. However, in any system where there are multiple inputs of waste, water quality and isotopic ratio of other nutrient sources could be more carefully enumerated and compared with shrimp farm signatures using mixing models and models of nutrient processing. Alkalinity should also be evaluated to aid in the evaluation of potential CO_2 limitation. Additionally, the species of algae and bacteria colonizing periphyton should be identified, possibly with DNA analysis, to determine if periphyton species composition impacts periphyton isotopes. Laboratory studies should also be conducted to evaluate the changes in isotopic ratios of periphyton and hyacinth under differing conditions including ranges of nutrient pollutants, insolation and water velocity to provide controlled estimations of the processes proceeding in the canal. Additionally, more frequent sampling over multiple seasons of harvest would give a better picture of the patterns occurring in such a system.

It is likely that shrimp ponds along the Chucachur Canal strongly influence the receiving environment, and that efforts to treat wastewater before it is discharged would reduce the amount of NH_3 entering the system. For instance, a study in Australia found that two days in a settling pond decreased TP and TN by 35% and 23%, respectively (Jackson et al. 2003b). A study in fish ponds in Portugal indicated that settling ponds in semi intensive systems improved benthic species diversity in the receiving water (Carvalho et al. 2009). However, it is also clear that other industries have significant impacts on the environment and the burden of reducing impacts should not be placed on shrimp aquaculture alone.

Literature Cited

Alongi, D.M., Johnston, D.J., Xuan, T.T., 2000. Carbon and nitrogen budgets in shrimp ponds of extensive mixed shrimp–mangrove forestry farms in the Mekong delta, Vietnam. Aquaculture Research 31, 387-389.

Andrews, J.E., Greenaway, A.M., Dennis, P.F., 1998. Combined carbon isotope and C/N ratios as indicators of source and fate of organic matter in a poorly flushed, tropical estuary: Hunts Bay, Kingston Harbour, Jamaica. Estuarine, Coastal and Shelf Science 46, 743-756.

APHA (American Public Health Association), 1998. Standard methods for the examination of water and wastewater. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, D.C.

Bergfur, J., Johnson, R., Sandin, L., Goedkoop, W., 2009. Effects of nutrient enrichment on C and N stable isotope ratios of invertebrates, fish and their food resources in boreal streams. Hydrobiologia 628, 67-79.

Boyd, C.E., 2000. Water quality: An introduction. Kluwer Academic Publishers, Boston, USA, 330 pp.

Boyd, C.E., Green, B.W., 2002. Water quality monitoring in shrimp farming areas: an example from Honduras, Shrimp Farming and the Environment. Report prepared under the World Bank, NACA, WWF and FAO Consortium Program on Shrimp Farming and the Environment, Auburn, USA, pp. 29.

Briggs, M.R.P., Funge-Smith, S.J., 1994. A nutrient budget of some intensive marine shrimp ponds in Thailand. Aquaculture and Fisheries Management 25, 789-811.

Burford, M.A., Costanzo, S.D., Dennison, W.C., Jackson, C.J., Jones, A.B., McKinnon, A.D., Preston, N.P., Trott, L.A., 2003. A synthesis of dominant ecological processes in intensive shrimp ponds and adjacent coastal environments in NE Australia. Marine Pollution Bulletin 46, 1456-1469.

Carvalho, S., Falcao, M., Curdia, J., Moura, A., Serpa, D., Gaspar, M.B., Dinis, M.T., Pousao-Ferreira, P., Cancela Da Fonseca, L., 2009. Benthic dynamics within a land-based semi-

intensive aquaculture fish farm: The importance of settlement ponds. Aquaculture International 17, 571-587.

Chapman, D., 1996. Water quality assessments: A guide to the use of biota, sediments and water in environmental monitoring. UNESCO/WHO/UNEP, London, UK.

Cole, M.L., Valiela, I., Kroeger, K.D., Tomasky, G.L., Cebrian, J., Wigand, C., McKinney, R.A., Grady, S.P., Carvalho da Silva, M.H., 2004. Assessment of a delta15N isotopic method to indicate anthropogenic eutrophication in aquatic ecosystems. Journal of Environmental Quality 33, 124-132.

Costanzo, S.D., O'Donohue, M.J., Dennison, W.C., 2004. Assessing the influence and distribution of shrimp pond effluent in a tidal mangrove creek in north-east Australia. Marine Pollution Bulletin 48, 514–525.

Costanzo, S.D., O'Donohue, M.J., Dennison, W.C., Loneragan, N.R., Thomas, M., 2001. A new approach for detecting and mapping sewage impacts. Marine Pollution Bulletin 42, 149-156.

Daskin, J.H., Calci, K.R., Burkhardt III, W., Carmichael, R.H., 2008. Use of N stable isotope and microbial analyses to define wastewater influence in Mobile Bay, AL. Marine Pollution Bulletin 56, 860-868.

Diana, J.S., 2009. Aquaculture production and biodiversity conservation. BioScience 59, 27-38.

Dorenbosch, M., Bakker, E.S., 2011. Herbivory in omnivorous fishes: effect of plant secondary metabolites and prey stoichiometry. Freshwater Biology 56, 1783-1797.

Durako, M.J., Hall, M.O., 1992. Effects of light on the stable carbon isotope composition of the seagrass *Thalassia testudinum*. Marine Ecology Progress Series 86, 99-101.

Finlay, J.C., Power, M.E., Cabana, G., 1999. Effects of water velocity on algal carbon isotope ratios: implications for river food web studies. Limnology and Oceanography 44, 1198-1203.

Fry, B., 2006. Stable isotope ecology. Springer US, New York, USA.

Handley, L.L., Raven, J.A., 1992. The use of natural abundance of nitrogen isotopes in plant physiology and ecology. Plant, Cell & Environment 15, 965-985.

Howarth, R.W., Marino, R., 2006. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: evolving views over three decades. Limnology and Oceanography 51, 364-376.

Islam, M., Jahangir Sarker, M., Yamamoto, T., Abdul Wahab, M., Tanaka, M., 2004. Water and sediment quality, partial mass budget and effluent N loading in coastal brackishwater shrimp farms in Bangladesh. Marine Pollution Bulletin 48, 471-485.

Jackson, C., Preston, N., Thompson, P.J., Burford, M., 2003a. Nitrogen budget and effluent nitrogen components at an intensive shrimp farm. Aquaculture 218, 397-411.

Jackson, C.J., Preston, N., Burford, M.A., Thompson, P.J., 2003b. Managing the development of sustainable shrimp farming in Australia: the role of sedimentation ponds in treatment of farm discharge water. Aquaculture 226, 23-34.

Jones, A.B., O'Donohue, M.J., Udy, J., Dennison, W.C., 2001. Assessing ecological impacts of shrimp and sewage effluent: biological indicators with standard water quality analyses. Estuarine, Coastal and Shelf Science 52, 91-109

Kao, S.J., Liu, K.K., 2000. Stable carbon and nitrogen isotope systematics in a human-disturbed watershed (Lanyang-Hsi) in Taiwan and the estimation of biogenic particulate organic carbon and nitrogen fluxes. Global Biogeochemical Cycles 14, 189-198.

Kon, K., Kawakubo, N., Aoki, J.-I., Tongnunui, P., Hayashizaki, K.-I., Kurokura, H., 2009. Effect of shrimp farming organic waste on food availability for deposit feeder crabs in a mangrove estuary, based on stable isotope analysis. Fisheries Science 75, 715-722.

Lapointe, B.E., Barile, P.J., Matzie, W.R., 2004. Anthropogenic nutrient enrichment of seagrass and coral reef communities in the Lower Florida Keys: discrimination of local versus regional nitrogen sources. Journal of Experimental Marine Biology and Ecology 308, 23-58.

Ledwin, S., 2010. Assessment of the ecological impacts of two shrimp farms in Southern Belize, Master's Thesis, School of Natural Resources and Environment. University of Michigan, Ann Arbor.

Lin, D.T., Fong, P., 2008. Macroalgal bioindicators (growth, tissue N, [delta]15N) detect nutrient enrichment from shrimp farm effluent entering Opunohu Bay, Moorea, French Polynesia. Marine Pollution Bulletin 56, 245-249.

Meksumpun, S., Meksumpun, C., Hoshika, A., Mishima, Y., Tanimoto, T., 2005. Stable carbon and nitrogen isotope ratios of sediment in the Gulf of Thailand: Evidence for understanding of marine environment. Continental Shelf Research 25, 1905-1915.

MNRE (Minestry of Natural Resources and the Environment), 2007. Notification of the Ministry of Natural Resources and Environment, Effluent Standard for Inland Aquaculture Royal Government Gazette. Ministry of Natural Resources and Environment, Bangkok, Thailand.

Naylor, R.L., Goldburg, R.J., Mooney, H., Beveridge, M., Clay, J., Folke, C., Kautsky, N., Lubchenco, J., Primavera, J., Williams, M., 1998. Nature's subsidies to shrimp and salmon farming. Science 282, 883-884

NEB (National Environmental Board), 1994. Notification of the National Environmental Board, No. 8, B.E. 2537 National Environmental Board, Bangkok, Thailand.

Parsons, T.R., Maita, Y., Lalli, C.M., 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford, UK.

PASW (Predictive Analytics SoftWare), 2009. PASW Statistics 18. SPSS, Inc., Chicago.

Pinon-Gimate, A., Soto-Jimenez, M.F., Ochoa-Izaguirre, M.J., GarcÌa-Pages, E., Paez-Osuna, F., 2009. Macroalgae blooms and δ15N in subtropical coastal lagoons from the Southeastern Gulf of California: Discrimination among agricultural, shrimp farm and sewage effluents. Marine Pollution Bulletin 58, 1144-1151.

Preston, N.P., Jackson, C., Thompson, P.A., Austin, M., Burford, M., 2000. Prawn farm effluent: composition, origin and treatment. Fishing Research and Development Corporation, Canberra, Australia.

Rysgaard, S., Risgaard-Petersen, N., Sloth, N., Jensen, K., Nielsen, L., 1994. Oxygen regulation of nitrification and denitrification in sediments. Limnology and Oceanography 39, 1643-1652.

Ryther, J.H., Dunstan, W.M., 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. Science 171, 1008-1013.

Smith, V.H., Joye, S.B., Howarth, R.W., 2006. Eutrophication of freshwater and marine ecosystems. Limnology and Oceanography 51, 351-355.

Tucker, J., Sheats, N., Giblin, A.E., Hopkinson, C.S., Montoya, J.P., 1999. Using stable isotopes to trace sewage-derived material through Boston Harbor and Massachusetts Bay. Marine Environmental Research 48, 353-375.

Udy, J.W., Dennison, W.C., 1997. Physiological responses of seagrasses used to identify anthropogenic nutrient inputs. Marine and Freshwater Research 48, 605-614.

Usui, T., Nagao, S., Yamamoto, M., Suzuki, K., Kudo, I., Montani, S., Noda, A., Minagawa, M., 2006. Distribution and sources of organic matter in surficial sediments on the shelf and slope off Tokachi, western North Pacific, inferred from C and N stable isotopes and C/N ratios. Marine Chemistry 98, 241-259.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications 7, 737-750.

Voss, M., Struck, U., 1997. Stable nitrogen and carbon isotopes as indicator of eutrophication of the Oder River (Baltic sea). Marine Chemistry 59, 35-49.

Wolanski, E., Spagnol, S., Thomas, S., Moore, K., Alongi, D.M., Trott, L., Davidson, A., 2000. Modelling and visualizing the fate of shrimp pond effluent in a mangrove-fringed tidal creek. Estuarine Coastal and Shelf Science 50, 85-97.

Appendix 1A. Pearson product-moment correlations between Chucachur Canal isotope, physical and chemical parameters for Periphyton d13C, d15N, and C:N; as well as Hyacinth d13C and d15N.

		d13C Periph	d15N Periph	C:N Periph	d13 Hya-cinth	d15 Hya-cinth	CN Hya-cinth	% cover	# shrimp farms	DO	TSS	ChlA	TKN	NO3	NO2	NH3	TP
d13C Periph	Pearson Correlation	1	.372*	.342	.293	.105	-.094	-.470**	.045	.350	-.092	.686*	.101	-.009	.362	-.400	.291
	Sig. (2-tailed)		.033	.052	.138	.603	.640	.006	.805	.265	.777	.014	.754	.977	.248	.198	.359
	N	33	33	33	27	27	27	33	33	12	12	12	12	12	12	12	12
d15N Periph	Pearson Correlation	.372*	1	.143	.471*	.660**	.110	-.678**	.133	.282	.101	.789**	.477	-.111	.591*	-.033	.528
	Sig. (2-tailed)	.033		.427	.013	.000	.585	.000	.460	.374	.755	.002	.117	.732	.043	.919	.078
	N	33	33	33	27	27	27	33	33	12	12	12	12	12	12	12	12
C:N Periph	Pearson Correlation	.342	.143	1	.255	.643**	.086	-.433*	.747**	.471	-.440	-.237	-.361	.096	-.499	-.492	-.515
	Sig. (2-tailed)	.052	.427		.200	.000	.671	.012	.000	.122	.152	.459	.249	.768	.098	.104	.087
	N	33	33	33	27	27	27	33	33	12	12	12	12	12	12	12	12
d13 Hya-cinth	Pearson Correlation	.293	.471*	.255	1	.528**	-.444*	-.482*	.136	.838*	-.040	.292	.577	.220	.255	.440	.234
	Sig. (2-tailed)	.138	.013	.200		.005	.020	.011	.499	.019	.932	.525	.175	.635	.581	.324	.613
	N	27	27	27	27	27	27	27	27	7	7	7	7	7	7	7	7
d15 Hya-cinth	Pearson Correlation	.105	.660**	.643**	.528**	1	-.066	-.697**	.745**	.625	-.204	-.113	.237	.307	-.142	.335	-.171
	Sig. (2-tailed)	.603	.000	.000	.005		.744	.000	.000	.133	.662	.809	.609	.503	.762	.463	.714
	N	27	27	27	27	27	27	27	27	7	7	7	7	7	7	7	7

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 1B. Pearson product-moment correlations between Chucachur Canal isotope, physical and chemical parameters for Hyacinth C:N, % cover, # shrimp farms, DO and TSS.

		d13C Periph	d15N Periph	C:N Periph	d13 Hyacinth	d15 Hyacinth	CN Hya-cinth	% cover	# shrimp farms	DO	TSS	ChlA	TKN	NO3	NO2	NH3	TP
CN Hyacinth	Pearson Correlation	-.094	.110	.086	-.444*	-.066	1	-.217	.048	-.825*	-.237	-.214	-.595	-.330	-.151	-.551	-.142
	Sig. (2-tailed)	.640	.585	.671	.020	.744		.277	.814	.022	.610	.644	.159	.469	.746	.200	.761
	N	27	27	27	27	27	27	27	27	7	7	7	7	7	7	7	7
% cover	Pearson Correlation	-.470**	-.678**	-.433*	-.482*	-.697**	-.217	1	-.270	-.046	.003	-.183	-.221	.268	.017	.081	-.252
	Sig. (2-tailed)	.006	.000	.012	.011	.000	.277		.111	.870	.991	.514	.429	.334	.953	.773	.365
	N	33	33	33	27	27	27	36	36	15	15	15	15	15	15	15	15
# shrimp farms	Pearson Correlation	.045	.133	.747**	.136	.745**	.048	-.270	1	.279	-.360	-.403	-.396	.272	-.564*	-.564*	-.678**
	Sig. (2-tailed)	.805	.460	.000	.499	.000	.814	.111		.314	.187	.137	.144	.326	.028	.028	.005
	N	33	33	33	27	27	27	36	36	15	15	15	15	15	15	15	15
DO	Pearson Correlation	.350	.282	.471	.838*	.625	-.825*	-.046	.279	1	-.430	.424	-.028	.431	.256	-.177	-.148
	Sig. (2-tailed)	.265	.374	.122	.019	.133	.022	.870	.314		.110	.116	.922	.109	.358	.527	.599
	N	12	12	12	7	7	7	15	15	15	15	15	15	15	15	15	15
TSS	Pearson Correlation	-.092	.101	-.440	-.040	-.204	-.237	.003	-.360	-.430	1	.120	.527*	-.061	.287	.263	.436
	Sig. (2-tailed)	.777	.755	.152	.932	.662	.610	.991	.187	.110		.671	.044	.829	.299	.343	.104
	N	12	12	12	7	7	7	15	15	15	15	15	15	15	15	15	15

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 1C. Pearson Product-moment correlations between Chucachur Canal isotope, physical and chemical parameters for Chlorophyll A (ChlA), TKN, NO₃, NO₂, NH₃ and TP

		d13C Periph	d15N Periph	C:N Periph	d13 Hyacinth	d15 Hyacinth	C:N Hyacinth	% cover	# shrimp farms	DO	TSS	ChlA	TKN	NO3	NO2	NH3	TP
ChlA	Pearson Correlation	.686*	.789**	-.237	.292	-.113	-.214	-.183	-.403	.424	.120	1	.381	.122	.747**	.019	.617*
	Sig. (2-tailed)	.014	.002	.459	.525	.809	.644	.514	.137	.116	.671		.162	.665	.001	.948	.014
	N	12	12	12	7	7	7	15	15	15	15	15	15	15	15	15	15
TKN	Pearson Correlation	.101	.477	-.361	.577	.237	-.595	-.221	-.396	-.028	.527*	.381	1	-.029	.770**	.508	.731**
	Sig. (2-tailed)	.754	.117	.249	.175	.609	.159	.429	.144	.922	.044	.162		.919	.001	.053	.002
	N	12	12	12	7	7	7	15	15	15	15	15	15	15	15	15	15
NO₃	Pearson Correlation	-.009	-.111	.096	.220	.307	-.330	.268	.272	.431	-.061	.122	-.029	1	.246	.038	-.402
	Sig. (2-tailed)	.977	.732	.768	.635	.503	.469	.334	.326	.109	.829	.665	.919		.377	.893	.137
	N	12	12	12	7	7	7	15	15	15	15	15	15	15	15	15	15
NO₂	Pearson Correlation	.362	.591*	-.499	.255	-.142	-.151	.017	-.564*	.256	.287	.747**	.770**	.246	1	.499	.704**
	Sig. (2-tailed)	.248	.043	.098	.581	.762	.746	.953	.028	.358	.299	.001	.001	.377		.058	.003
	N	12	12	12	7	7	7	15	15	15	15	15	15	15	15	15	15
NH₃	Pearson Correlation	-.400	-.033	-.492	.440	.335	-.551	.081	-.564*	-.177	.263	.019	.508	.038	.499	1	.458
	Sig. (2-tailed)	.198	.919	.104	.324	.463	.200	.773	.028	.527	.343	.948	.053	.893	.058		.086
	N	12	12	12	7	7	7	15	15	15	15	15	15	15	15	15	15
TP	Pearson Correlation	.291	.528	-.515	.234	-.171	-.142	-.252	-.678**	-.148	.436	.617*	.731**	-.402	.704**	.458	1
	Sig. (2-tailed)	.359	.078	.087	.613	.714	.761	.365	.005	.599	.104	.014	.002	.137	.003	.086	
	N	12	12	12	7	7	7	15	15	15	15	15	15	15	15	15	15

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 2A. Multiple linear regression results for Chucachur Canal isotopes - Periphyton δ¹⁵N with % shade

Correlations – Periphyton d15N with % shade

		d15N	% cover
Pearson Correlation	d15N	1.000	-.678
	% cover	-.678	1.000
Sig. (1-tailed)	d15N	.	.000
	% cover	.000	.
N	d15N	33	33
	% cover	33	36

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.678[a]	.459	.442	3.68871

a. Predictors: (Constant), % cover
b. Dependent Variable: d15N

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	358.119	1	358.119	26.319	.000[a]
	Residual	421.805	31	13.607		
	Total	779.924	32			

a. Predictors: (Constant), % cover
b. Dependent Variable: d15N

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.	95.0% Confidence Interval for B Lower Bound	95.0% Confidence Interval for B Upper Bound	Correlations Zero-order	Correlations Partial	Correlations Part	Collinearity Statistics Tolerance	Collinearity Statistics VIF
1	(Constant)	17.317	1.243		13.937	.000	14.782	19.851					
	% cover	-13.185	2.570	-.678	-5.130	.000	-18.427	-7.943	-.678	-.678	-.678	1.000	1.000

a. Dependent Variable: d15N

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	6.1092	17.3166	11.8594	3.34533	36
Std. Predicted Value	-1.719	1.631	.000	1.000	36
Standard Error of Predicted Value	.643	1.292	.881	.227	36
Adjusted Predicted Value	5.9497	18.0470	11.9704	3.52884	33
Residual	-5.95400	11.16192	-.14317	3.63234	33
Std. Residual	-1.614	3.026	-.039	.985	33
Stud. Residual	-1.643	3.152	-.035	1.021	33
Deleted Residual	-6.43696	12.10795	-.11102	3.90619	33
Stud. Deleted Residual	-1.692	3.761	-.018	1.095	33
Mahal. Distance	.003	2.955	.972	1.013	36
Cook's Distance	.000	.421	.038	.083	33
Centered Leverage Value	.000	.092	.030	.032	36

a. Dependent Variable: d15N

Normal P-P Plot of Regression Standardized Residual
Dependent Variable: d15N; Independent Variable: % cover

Scatterplot
Dependent Variable: d15N; Independent Variable: % cover
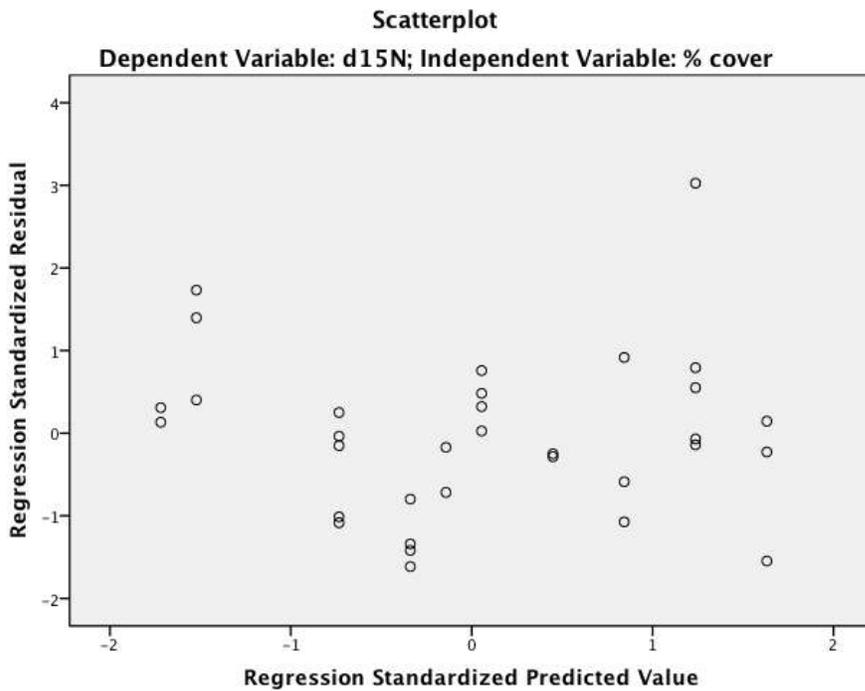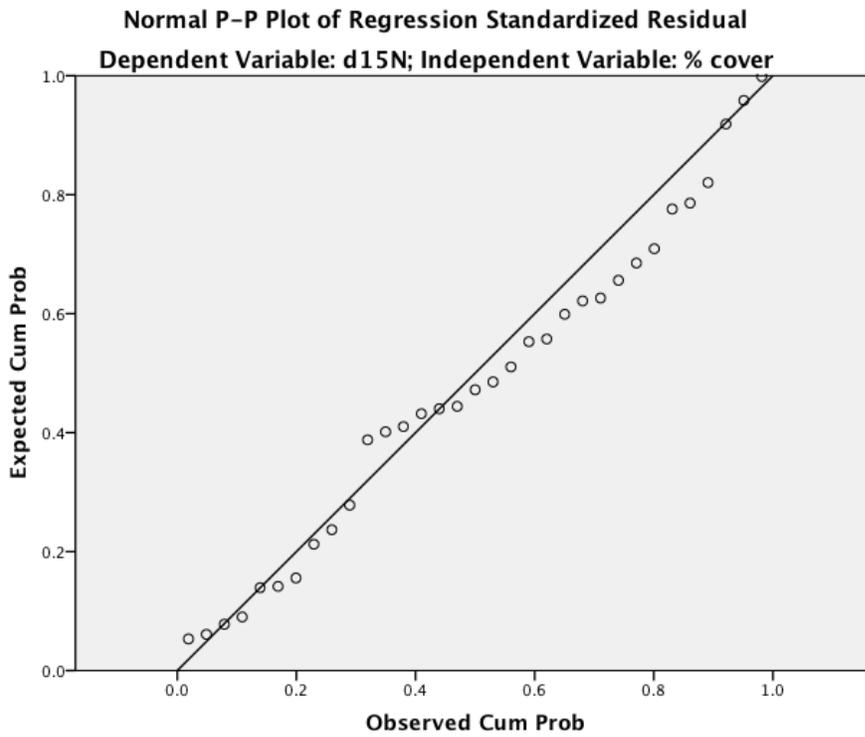
Appendix 2B. Multiple linear regression results for Chucachur Canal isotopes - Periphyton δ¹⁵N with % shade and NO₂

Correlations – Periphyton d15N with % shade and NO2

		d15N	% cover	COMPUTE Log10NO2=LG 10(NO2)
Pearson Correlation	d15N	1.000	-.678	.370
	% cover	-.678	1.000	.116
	COMPUTE Log10NO2=LG10(NO2)	.370	.116	1.000
Sig. (1-tailed)	d15N	.	.000	.118
	% cover	.000	.	.340
	COMPUTE Log10NO2=LG10(NO2)	.118	.340	.
N	d15N	33	33	12
	% cover	33	36	15
	COMPUTE Log10NO2=LG10(NO2)	12	15	15

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.815[a]	.664	.589	3.16571

a. Predictors: (Constant), COMPUTE Log10NO2=LG10(NO2), % cover
b. Dependent Variable: d15N

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	177.903	2	88.952	8.876	.007[a]
	Residual	90.196	9	10.022		
	Total	268.099	11			

a. Predictors: (Constant), COMPUTE Log10NO2=LG10(NO2), % cover
b. Dependent Variable: d15N

Coefficients[a]

Model		Unstandardized Coefficients B	Std. Error	Standardized Coefficients Beta	t	Sig.	95.0% Confidence Interval for B Lower Bound	Upper Bound	Correlations Zero-order	Partial	Part	Collinearity Statistics Tolerance	VIF
1	(Constant)	24.165	3.440		7.024	.000	16.382	31.948					
	% cover	-14.216	3.788	-.731	-3.753	.005	-22.785	-5.648	-.678	-.781	-.726	.986	1.014
	COMPUTE Log10NO2=LG10(NO2)	4.474	1.913	.455	2.338	.044	.146	8.803	.370	.615	.452	.986	1.014

a. Dependent Variable: d15N

Normal P-P Plot of Regression Standardized Residual

Dependent Variable: d15N; Independent Variables: % cover, NO2

Scatterplot

Dependent Variable: d15N; Independent Variables: % cover, NO2
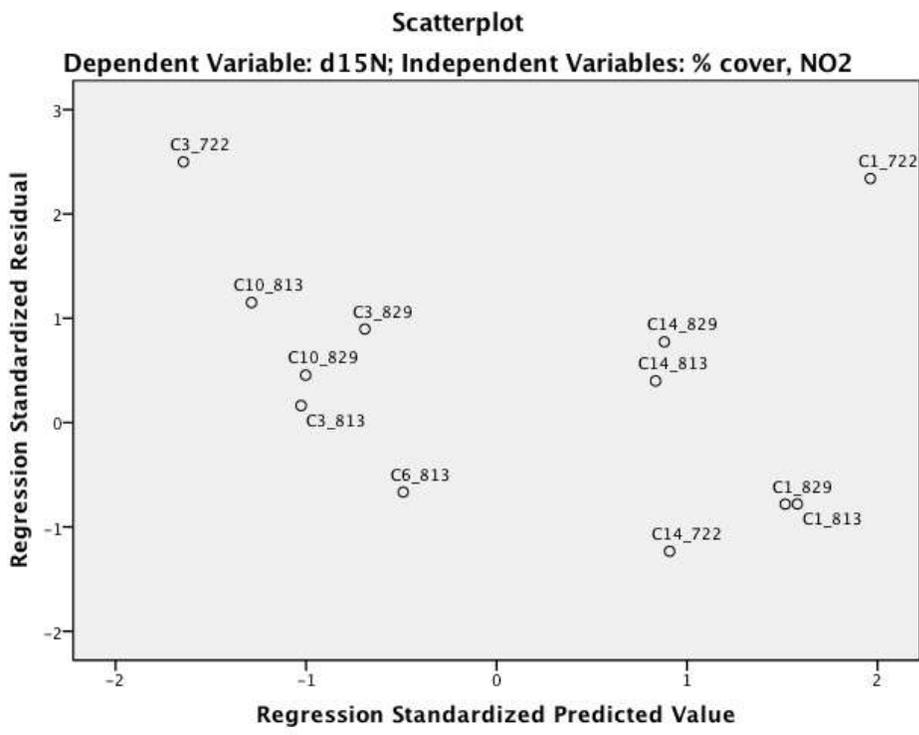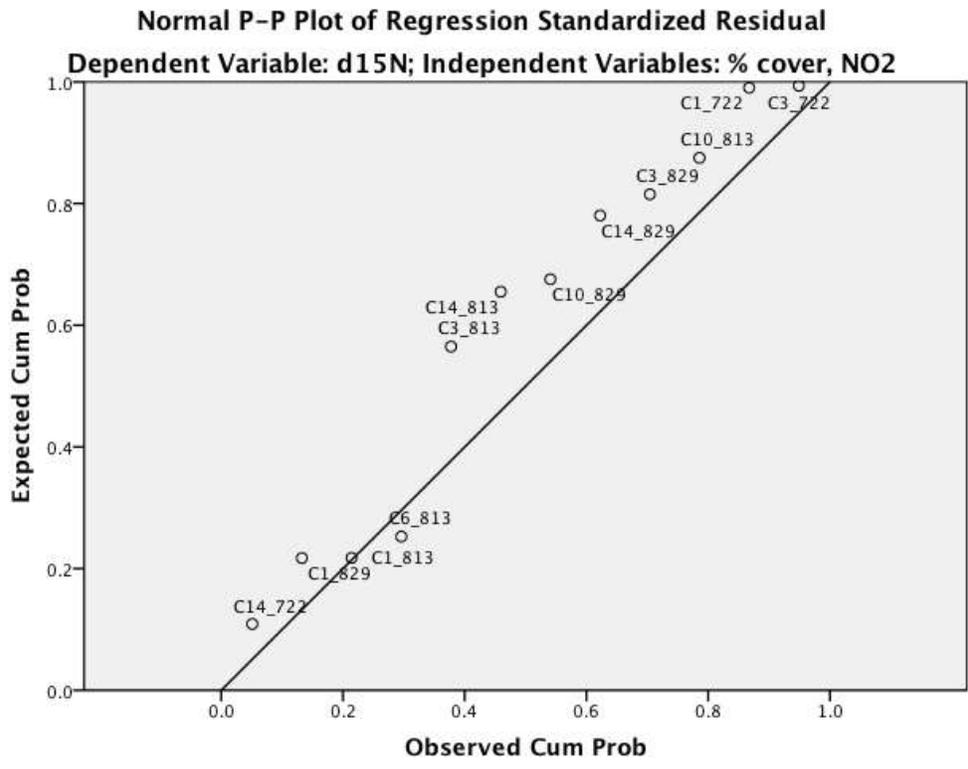
Appendix 2C. Multiple linear regression results for Chucachur Canal isotopes - Periphyton δ¹³C with % shade

Correlations – Periphyton d13C with % shade (cover)

		d13C	% cover
Pearson Correlation	d13C	1.000	-.470
	% cover	-.470	1.000
Sig. (1-tailed)	d13C	.	.003
	% cover	.003	.
N	d13C	33	33
	% cover	33	36

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.470[a]	.221	.196	3.98315

a. Predictors: (Constant), % cover
b. Dependent Variable: d13C

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	139.589	1	139.589	8.798	.006[a]
	Residual	491.830	31	15.865		
	Total	631.419	32			

a. Predictors: (Constant), % cover
b. Dependent Variable: d13C

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B		Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-19.188	1.342		-14.302	.000	-21.925	-16.452					
	% cover	-8.232	2.775	-.470	-2.966	.006	-13.892	-2.572	-.470	-.470	-.470	1.000	1.000

a. Dependent Variable: d13C

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	-26.1855	-19.1884	-22.5955	2.08858	36
Std. Predicted Value	-1.719	1.631	.000	1.000	36
Standard Error of Predicted Value	.694	1.395	.951	.245	36
Adjusted Predicted Value	-25.9402	-18.6238	-22.4469	2.16107	33
Residual	-6.80844	9.02156	-.08939	3.92104	33
Std. Residual	-1.709	2.265	-.022	.984	33
Stud. Residual	-1.780	2.359	-.030	1.015	33
Deleted Residual	-7.38549	9.78618	-.14859	4.17353	33
Stud. Deleted Residual	-1.848	2.562	-.025	1.042	33
Mahal. Distance	.003	2.955	.972	1.013	36
Cook's Distance	.000	.236	.033	.048	33
Centered Leverage Value	.000	.092	.030	.032	36

a. Dependent Variable: d13C

Normal P-P Plot of Regression Standardized Residual

Dependent Variable: d13C; Independent Variable: % cover

Scatterplot

Dependent Variable: d13C; Independent Variable: % cover
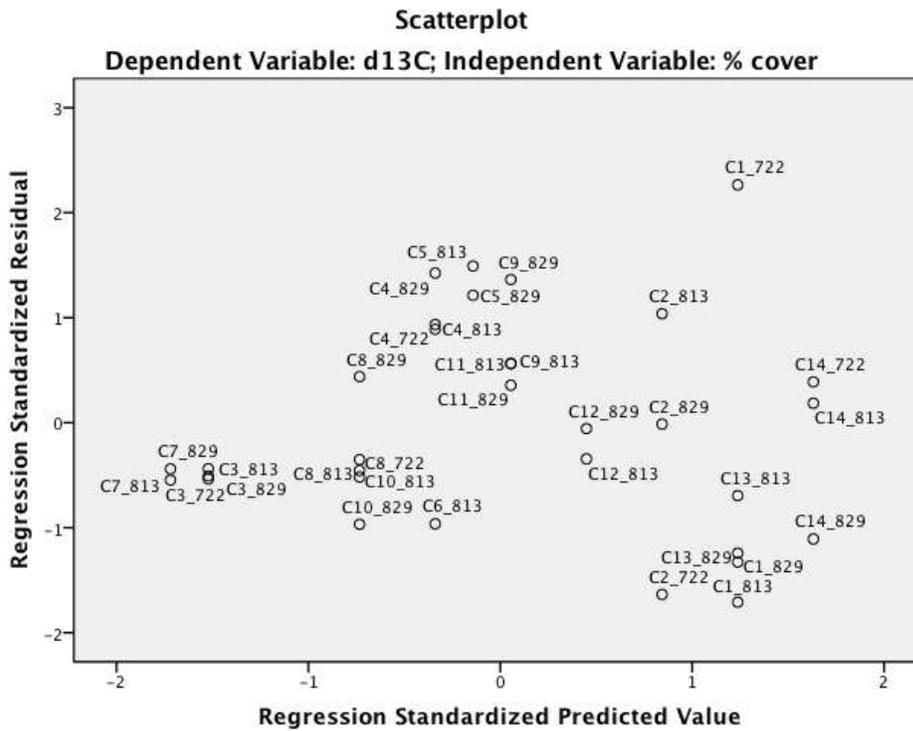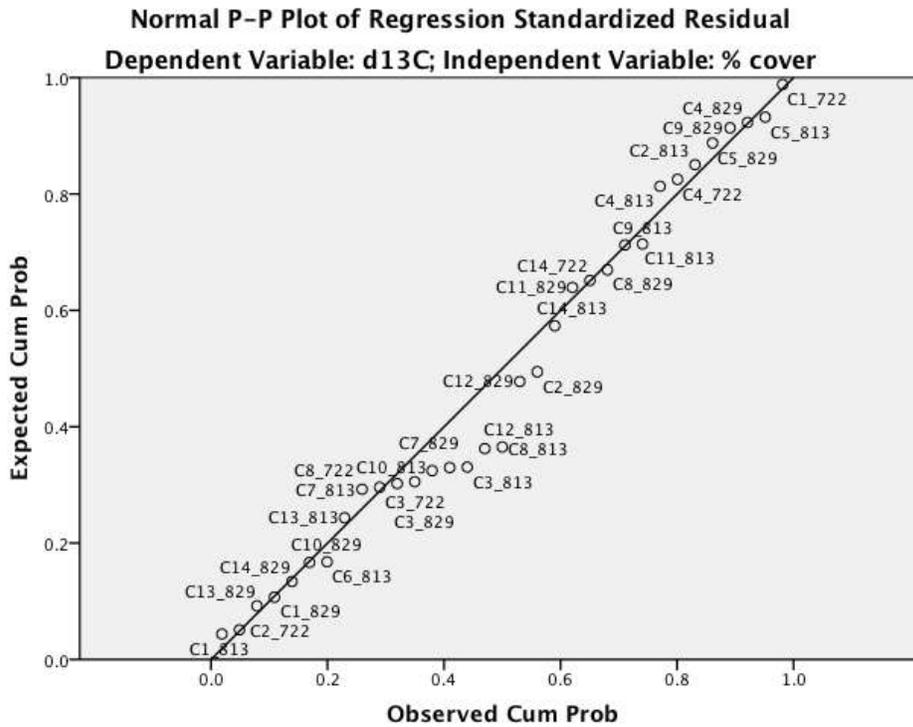
Appendix 2D. <u>Multiple linear regression results for Chucachur Canal isotopes -</u>

<u>Hyacinth δ^{15}N with % shade, number of shrimp farms and NH$_3$</u>

Correlations – For MLR of d15N with # farms, % shade and NH3

		d15Hyacinth	# shrimp farms	% cover	COMPUTE Log10NH3=LG 10(NH3)
Pearson Correlation	d15Hyacinth	1.000	.745	-.697	-.118
	# shrimp farms	.745	1.000	-.270	-.621
	% cover	-.697	-.270	1.000	.143
	COMPUTE Log10NH3=LG10(NH3)	-.118	-.621	.143	1.000
Sig. (1-tailed)	d15Hyacinth	.	.000	.000	.401
	# shrimp farms	.000	.	.056	.007
	% cover	.000	.056	.	.305
	COMPUTE Log10NH3=LG10(NH3)	.401	.007	.305	.
N	d15Hyacinth	27	27	27	7
	# shrimp farms	27	36	36	15
	% cover	27	36	36	15
	COMPUTE Log10NH3=LG10(NH3)	7	15	15	15

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	1.000[a]	.999	.998	.28757

a. Predictors: (Constant), COMPUTE Log10NH3=LG10(NH3), % cover, # shrimp farms

b. Dependent Variable: d15Hyacinth

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	249.005	3	83.002	1003.706	.000[a]
	Residual	.248	3	.083		
	Total	249.253	6			

a. Predictors: (Constant), COMPUTE Log10NH3=LG10(NH3), % cover, # shrimp farms

b. Dependent Variable: d15Hyacinth

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B		Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	9.022	.316		28.525	.000	8.016	10.029					
	# shrimp farms	.319	.008	.940	39.343	.000	.293	.345	.745	.999	.717	.581	1.722
	% cover	-13.218	.481	-.520	-27.492	.000	-14.748	-11.688	-.697	-.998	-.501	.926	1.080
	COMPUTE Log10NH3=LG10(NH3)	4.501	.193	.541	23.271	.000	3.885	5.116	-.118	.997	.424	.613	1.630

a. Dependent Variable: d15Hyacinth

Normal P–P Plot of Regression Standardized Residual

Dependent Variable: d15Hyacinth

Scatterplot

Dependent Variable: d15Hyacinth