Tracing Anthropogenic Nutrient Inputs Using $\delta^{15}N$ Levels in Algae Tissue Koh Tao, Thailand

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Introduction

Coastal development can have a profound impact on nearby marine ecosystems. This is especially true for coral reefs that occupy a narrow niche of environmental conditions, such as temperature, nutrients and light requirements. It has been estimated that up to 25% of the oceans coral reefs are under threat from increased sediments and nutrients that are washed from the terrestrial environment due to the clearance and development of nearby land (Burke, 2011). Jackson et al. (2001) suggested that, in combination with overfishing, nutrient enrichment was the major factor in the decline of coral reefs worldwide.

With the pressure on coral reefs increasing, effective mapping of tropical coastal areas affected by anthropogenic nutrient input is essential in conserving existing reefs and to also restore reefs that may already be degraded (Lin et al., 2007). To map anthropogenic nutrient input as part of a successful management strategy both the level of enrichment and the source of nutrient input is required. It is therefore recommended for water quality assessments that biological assays (bioassays) and chemical data are collected as well as biological and habitat data (US EPA, 2002, from Dailer et al., 2010).

A bioassay involves the use of a biological organism to test for the relative strength of a substance within the natural environment. A well established bioassay technique is the use of naturally stable Nitrogen (N) isotope ratios in algae tissue, which can be used as a proxy for anthropogenic nutrient inputs from human sewage and also help detect the source of these nutrient inputs into the coastal ecosystem. Naturally stable N isotope ratios (^{15}N:^{14}N, expressed as $\delta^{15}N$) are useful in the detection of anthropogenic nutrient input into coastal waters as wastewater derived from sewage is enriched in the heavier isotope, ^{15}N. This enrichment is due to nitrogen transformations that occur before as well as after discharge of these wastes (Risk, et al., 2009), with bacteria preferring the use of the lighter ^{14}N isotope (Heaton, 1986).

It is also becoming evident that macroalgae take up N from the environment with little isotopic fractionation (processes that may affect the relative abundance of isotopes) and without discrimination of the source, i.e. whether anthropogenic or natural (Gartner et al., 2002; Cohen and Fong, 2005). This is especially true in the tropics where natural sources of N are low (Dailer, 2010). This apparent lack of predilection for any particular isotope is why using macroalgae isotope composition is one of the most suitable bio-indicators for assessing the point source of nutrient input on to coral reef ecosystems (Cooper et al. 2009, Risk et al. 2009).

To give an example, Lin et al. (2007) found that algal $\delta^{15}N$ values were a better proxy for detecting anthropogenic N input than both the tissue N content and C:N ratios. The researchers found that the N content and C:N ratios of algae showed little variation between the values of species growing near the sewage-affected transects and the reference transects; whereas values of $\delta^{15}N$ showed a clear spatial pattern of higher to lower values moving away from the sewage-affected area. The same paper also documented little variation in $\delta^{15}N$ values and the type of thallus form sampled, meaning a range of algae forms could be sampled and compared in $\delta^{15}N$ analysis. $\delta^{15}N$ measurements are also fast, cost effective and can be used to detect N sources and sewage stress in a variety of spatial (0-80 km) and temporal (days to weeks) scales (Risk et al., 2009).

This study attempts to quantify the amount, and find the source, of nutrient inputs into the marine environment on a developing tourist island in Thailand. Chemical data in the form of nutrient concentrations are used as an indication of the amount of nutrients present; while $\delta^{15}N$ values in algae tissue are used to indicate the source of nutrients and whether or not they are of anthropogenic origin. A more detailed review of the current literature on tracing anthropogenic nutrient inputs using these methods is given below. Also reviewed is the current knowledge of the effects of nutrients on a coral reef ecosystem along with the various mechanisms involved.

Literature Review

$\delta^{15}N$ values of macroalgae

$\delta^{15}N$ values of macroalgae tissue found in pristine oceanic water will tend to fall in the range of 2-4‰ (Costanzo et al. 2001, Titlyanov et al. 2011). Macroalgae exposed to anthropogenic nutrient input will have values deviating from this range, with the exact value depending on the source of anthropogenic nutrient input. Macroalgae exposed to fertilisers from agricultural practices will tend to have a low range of $\delta^{15}N$ values of -3-2‰ (Owens 1987, Derse 2007); compared to values of between 5‰-38‰ when exposed to nutrients from human sewage (Heaton 1986, Constanzo et al. 2001, Constanzo et al. 2004 Dailer et al. 2010). The greater the degree of sewage treatment will usually correspond to a higher $\delta^{15}N$ value. Wastewater containing raw sewage or primary treated sewage (e.g. from cesspools or septic tanks) would tend to have a lower $\delta^{15}N$ value (4‰-10‰) (McClelland et al. 1997, Dailer et al. 2010) compared to secondary or tertiary treated sewage (>10‰) (Lapointe & Krupa 1995, Gartner et al. 2002, Dailer et al. 2010).

Due to $\delta^{15}N$ values differing in macroalgae depending on the nutrient source, the $\delta^{15}N$ signature of macroalgae is a good proxy for establishing the source of nutrient input (e.g.

whether natural, from fertiliser or from treated/untreated sewage). It should not initially be used as a proxy for the degree of nutrient loading, due to the fact that the size of the $\delta^{15}N$ value does not directly equal the level of nutrient input. However, when the nutrient source and range of $\delta^{15}N$ values are established for a site, $\delta^{15}N$ values of macroalgae have then been used as a proxy for various environmental parameters. For example, Huang et al. (2013) found that $\delta^{15}N$ values of macroalgae were positively correlated with macroalgal cover and negatively correlated with the abundance of live coral. Parsons et al. (2008) found a positive correlation between $\delta^{15}N$ values of macroalgae and the abundance of dead coral. Huang et al (2013) also attempted to correlate $\delta^{15}N$ values with indicators of actual coral condition and found negative correlations between $\delta^{15}N$ values in macroalgae and live coral cover, species richness and juvenile coral density. Although research papers into the direct affects of $\delta^{15}N$ values on the reef environment are few, the link between elevated $\delta^{15}N$ values and anthropogenic nutrient enrichment is well established (Lin et al., 2007, Cooper et al. 2009, Risk et al. 2009) and there is a wide array of literature that discusses the affects of anthropogenic nutrient enrichment on the reef ecosystem.

Nutrient enrichment and macroalgae abundance

There are many research papers that positively correlate nutrient concentrations in the water column with macroalgal abundance (e.g. Hughes 1994, Lapointe 1997, Schaffelke and Klumpp 1998, Jackson et al. 2001). It has been put forward that nutrient enrichment can increase the density of macroalgae on a coral reef through an increase in macroalgae productivity, subsequently leading to a shift from a coral dominated environment to one dominated by algae (Lapointe, 1997; Schaffelke & Klumpp, 1998; Schaffelke, 1999). A study in the Great Barrier Reef used responses of bioindicators to decreasing water quality to demonstrate that with increasing turbidity and nutrients, there was a progressive shift from reef communities that were dominated by phototrophic animals, to those dominated by both phototrophs and macroalgae and then those that were dominated by macroalgae and very few phototrophs (Fabricius et al. 2012). Once established, macroalgae can further encourage coral mortality through processes such as shading, chemical disturbance, a reduction in water exchange (McCook, 2001) and through increased microbial abundance (Alber & Vailela, 1994). These processes can also have negative impacts on fisheries and benthic community composition (Dailer, et al., 2010).

However, it is not an open-and-shut case to say that increased nutrients on to a reef will always have a direct and positive affect on macroalgae abundance and the assumption that all algae on a coral reef is nutrient limited is too simplistic. For example, nutrient input may stimulate the growth of some algae while other species will show no increased growth at all (Stimson and Larned 2001, Smith et al. 2001, Nugues and Bak 2007). Algal species located in an area of anthropogenic nutrient enrichment may also be growth limited from a micronutrient, such as iron, rather than the main macronutrients of N and P. If this limiting micronutrient is not available then the algal species may not be in a position to utilise the increased levels of macronutrients (Suzuki, et al., 1995).

In reality, there is probably no universal 'silver bullet' for a shift to a macroalgae dominated reef and there will always be a number of factors simultaneously at play. Where there is high nutrients from anthropogenic sources in a tropical coastal environment you will usually find a number of other anthropogenic stressors and it is therefore hard to sometimes pinpoint the actual cause for the increase in macroalgae abundance. Furthermore, the replacement of corals by macroalage may actually be a sign of previous coral mortality through other external drivers such as sedimentation and overfishing, or even from global problems, such as heat/light stress and ocean acidification (McCook, 2001). For example, poor water quality in Eilat, Red Sea, caused by sewage discharge and the spillage of apatite (phospate) dust from loading fertiliser onto cargo ships, contributed to a fourfold increase in mortality in the coral species *Stylophora pistillata* and an increase in algal growth. The authors concluded that algae was not a direct cause of the coral mortality but rather colonised the area rapidly after the corals decline. Sediment trapping by the macroalgae prevented any coral recovery and also led to further coral tissue loss (Walker and Ormond, 1982). This example shows that nutrient enrichment can have a direct and negative effect on coral health. These effects along with the various mechanisms that cause them are reviewed below.

Nutrient enrichment and coral health

There is strong evidence to suggest that nutrient enrichment on a coral reef can encourage coral mortality, although there has been much debate in the literature on the mechanisms for this process (Szmant, 2002; Bell et al., 2007). Recently it has been demonstrated that corals that have been exposed to high levels of dissolved inorganic nitrogen (DIN) were more prone to bleaching when exposed to heat and light stress. Somewhat paradoxically, the authors suggested this was in part caused by nutrient starvation of zooxanthellae, which results from accelerated growth brought on by the increased DIN levels (Wiedenmann, et al., 2012). There is also strong evidence to suggest an increase in coral disease with nutrient enrichment (Bruno, et al., 2003). An experiment by Vega Thurber et al. (2013) on the relationship between nutrients and disease found that by increasing levels of nitrogen and phosphorus on a coral reef (but still keeping ambient nutrient concentrations well within the range found on many degraded reefs) increased both the ‘prevalence and severity of disease, compared to control plots’. On a more positive note, the authors found that corals recovered rapidly after the stress was reduced suggesting that simple management techniques to improve water quality would be an effective way to mitigate against future loss of coral cover.

For more indirect effects, it has been shown that increased nutrient input can stimulate phytoplankton that promote filter feeders and increase bio-eroders (Holmes et al., 2000). In the Grand Cayman Islands, untreated fecal sewage led to a sixfold increase in bacterial biomass and a fivefold increase in internal bioerosion by the boring sponge species, *Cliona delitrix.* Increases in both filter feeders and bio-eroders can lead to space competition with corals and can also lead to the structural weakening of the coral reef, increasing erosion. Large phytoplankton blooms can also have other indirect consequences on reef health. In a recent study, an experiment found that nitrate enrichment in the presence of phytoplankton actually led to lower nitrate concentrations in the water and increased coral mortality compared to un-enriched controls (Fabricius et al. 2013).

Increased phytoplankton densities have also been found to have negative effects on coral through other mechanisms such as; the release of mucus and algal toxins, large amounts of decomposing organic material that can lead to oxygen depletion (Guzman et al. 1990) and and an increased supply of food for the larvae of the crown-of-thorns starfish (*Acanthaster planci)* that increases coral mortality through predation by the corallivorous adults (Baird et al. 2013, Fabricius et al. 2010). According to Fabricius (2005) arguably the most significant direct effect of terrestrial runoff to shallow coral reef environments is a reduction in the recruitment success of corals, together with the promotion of macroalgae and *A. planci*.

Not all studies have found a positive correlation between increased nutrient levels and coral mortality. Dunn, et al. (2012) documented that slightly increased nutrient levels may result in increased growth performance of corals. Wiedenmann et al. (2013) also demonstrated that if all essential nutrients are available at sufficient concentrations to ensure chemically balanced growth then increased nutrient levels may not have a negative affect on the performance of zooxanthellae. In healthy coral reefs with abundant herbivores, top down control processes could restrict the negative impacts of elevated nutrient levels (D'Angelo and Wiedenmann 2014). The problem with this idea is that where you find a large amount of anthropogenic nutrient input it is incredibly rare to find a healthy coral reef that is not under other anthropogenic stresses, such as sedimentation and overfishing. These additional anthropogenic stresses would, more often than not, reduce the chances of any top down buffering that a more resilient, healthy reef may have.

Thoughts on Literature Review

It seems from the literature that there has been a continuous and ongoing debate between scientists from different corners on what may be causing the decline of coral reefs worldwide. The problem here may well be trying to find a 'silver bullet' that may not necessarily exist. This search would seem rather dangerous and somewhat fruitless with coral reefs in rapid decline. What is needed, urgently as time is running out, may be less trying to find what the biggest cause of coral decline is and more immediate action in bringing the reefs back to a natural resilient state. On a local level, this means tracing and reducing anthropogenic effects of pollution, sedimentation and overfishing. Reducing these effects locally would be a step in the right direction and would create a more resilient coral reef in a time when global risks, such as bleaching and disease, are on the rise.

Study Location

Koh Tao is an island in the Gulf of Thailand, located in Surat Thani province in southern Thailand (Figure 1). It is a small island with a size of approximately 21 km^2 (Weterings, 2011). Koh Tao's economy is almost entirely reliant on the coral reef that surrounds the island. Out of the 300,000 visitors per year an estimated 90% will use the reef for snorkelling and 60% will use the reef for diving (Scott, 2010). Koh Tao is known as one of the cheapest places for beginners to get their diving certification and so is the place to dive among the travellers to South-East Asia; where it qualifies more open water divers annually than anywhere else in Asia (SATLP, 2009). It is estimated that scuba diving alone generates $62 million annually (Larpnun et al., 2011). It is therefore essential that the coral reefs that surround the island are managed correctly and successfully in order to protect both the ecological and socio-economical services that the reef provides.

The diving 'boom' on Koh Tao has only happened in approximately the last 20 years and, like many other developing tourist destinations, the growth has come at the expense of the natural environment. Like many other tropical tourist destinations, the reefs are under high pressure from recreation, pollution, sedimentation and overfishing (Weterings, 2011; Scott, 2008).

The rapid development on the island has come in response of meeting tourist demand and, for the majority, has been unplanned and spontaneous. This has led to poor wastewater management on the island with lodgings at best using basic cesspool and septic tank systems, which eventually travel to waterways that lead straight out on to the nearshore reef environment. To date, the extent of anthropogenic nutrient input into the marine environment from these wastewater outlets has not been studied.   Tracing the extent and impact of wastewater pollution is urgently needed in the efforts to protect Koh Tao's coral reefs and the important ecological and economic services that they provide.

Figure 1: Map showing location of Koh Tao. Situated within the Gulf of Thailand, South East Asia

Methodology

Site descriptions

Three different sites were chosen on Koh Tao that were thought to be influenced by human activities to varying degrees (Figure 2). Each site was selected as either a high human impact, medium human impact or low human impact area depending on the level of coastal development and whether wastewater outlets were evident at each site. Each site is discussed in more detail below.

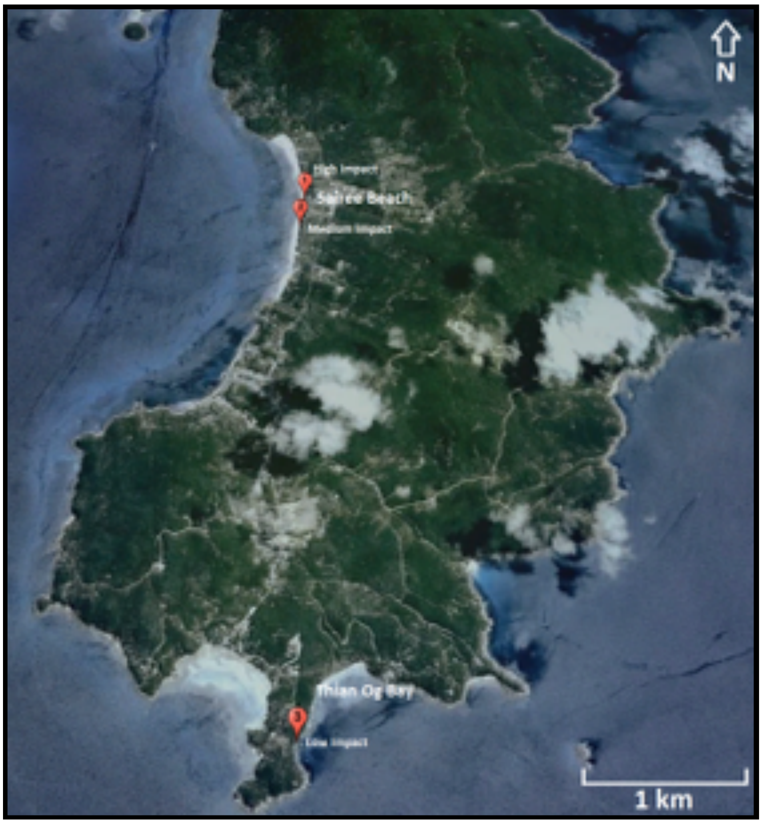

Figure 2: Aerial view of Koh Tao showing the locations of each site location. Site 1 (10°5'55.12"N,99°49'41.85"E) and Site 2 (10°5'46.57"N,99°49'41.38"S) are located on the heavily populated area of Sairee Beach. Site 3 (10°3'42.38"N, 99°49'50.10"S) is situated in the lower human impact area of Thian Og Bay. (Photo courtesy of Google Earth).

Site 1 (High Impact)

Site 1, located on the north of Sairee Beach (co-ordinates: 10°5'55.12"N,99°49'41.85"E), was predicted to be an area of high human impact. Sairee Beach is the most heavily populated area of Koh Tao, where the majority of bars, restaurants and lodgings are situated. Site 1 is located on the north of Sairee Beach by the main wastewater outlet on the island (Figure 3A & 3B). This wastewater outlet flows directly on the beach due to the outlet pipe being broken and in need of repair. Although the study was carried out during the dry season, with no significant rainfall occurring for approximately one month, there was a constant outflow of wastewater from the outlet at Site 1. The majority of this wastewater was predicted to be in the form of grey water (shower water, washing up water, etc.) and it was unclear to what degree raw/primary treated sewage was contained within this wastewater, which could be increasing the nutrient loading on to the nearshore reef.

Figure 3: Photos A & B shows the wastewater outlet at Site 1 (High Impact). Photo C shows beach developments at Site 2 (medium impact). A seasonal wastewater outlet flows just to the right of these developments. Photo D shows the beach bungalows at Site 3 (low impact).

Site 2 (Medium Impact)

Site 2 was situated on the south of Sairee Beach (co-ordinates: 10°5'46.57"N, 99°49'41.38"S) and was located by a smaller wastewater outlet that passed between two beach developments (Figure 2C). There was minimal flow during the time of sampling due to the severe dry period the island was having. A greater flow of wastewater would be expected to flow during the monsoon season or after a heavy downpour. Site 2 was also situated near some of the the main beach bars that had beach parties occurring most nights (Figure 4). Due to the sheer number of people occupying this area at night there was a chance that the underground disposal of waste through cesspools/septic tanks could be over-burdened leading to an overflow of waste to the ocean.

Figure 4: A beach party occurring at a beach bar near to Site 3 (medium impact). Photo courtesy of: Global Travel Mate. Available at: http://www.globaltravelmate.com/asia/thailand/koh-tao/koh-tao-nightlife

Site 3 (low impact)

Site 3 (Figure 3D) was located at Thian Og Bay (co-ordinates 10°3'42.38"N, 99°49'50.10"S) away from the more populated areas on Koh Tao. Although it was selected as a low impact area, there are cheap beach bungalows located directly on the shoreline that empty wastewater from the bungalows directly out to sea (Figure 2D). Any anthropogenic nutrient loading if found would be expected to be lower than the more populated areas of Sairee Beach and would therefore be a good comparison with Site 1 and Site 2.

Data Collection

Chemical Data

Water samples were taken at four different sample points at each site. Sample points were located 25 metres offshore, 100 metres offshore, 100 metres south and 100 metres north of the predicted source of nutrient input. At each sample point two water samples

(50 ml) were taken at 1-2 metres depth, immediately filtered and frozen within one hour of collection. The samples were then analysed for dissolved inorganic nitrogen (DIN) (ammonium, nitrate and nitrite) and phosphorus. The chemical data would be use to ascertain the degree of nutrients within the nearshore waters at each site.

δ^{15}N analysis

At each site location δ^{15}N values of the macroalgae species *Turbinaria ornata* (Figure 5) were obtained along a nearshore transect and an offshore transect at 25 metre intervals. *T. ornata* was selected for this study as it was abundant at each site and has been found to be a sensitive indicator of nutrient pollution (Titlyanov et al., 2011). The nearshore transect consisted of a horizontal transect parallel to the shoreline, stretching 100 metres in either direction away from the source of predicted anthropogenic nutrient input. The offshore transect began at the shoreline and went to 100 metres offshore, perpendicular to the shoreline. Due to a sandy substrate out to 50 metres at Sairee Beach no *T. ornata* individuals were found within 50 metres of the shoreline. This meant that the nearshore sampling for Site 1 and Site 2 could only be carried out at 50 metres from the shoreline and the offshore transect could also only be started at 50 metres from the shoreline.

For the collection of algal samples, three healthy looking individuals were collected at each sample point and transported in zip-lock bags filled with seawater. Samples were then washed of any epiphytes and sediment particles in fresh water. Algal samples were oven-dried at 45°C for 48 hours, then ground to a homogeneous powder using a pestle and mortar and stored in scintillation vials. To measure the N isotopes of *T. ornata*, dried weighed samples(about 2.0–2.5 mg) were analyzed using an isotope ratio mass spectrometer at Scripps Institute of Oceanography, La Jolla.

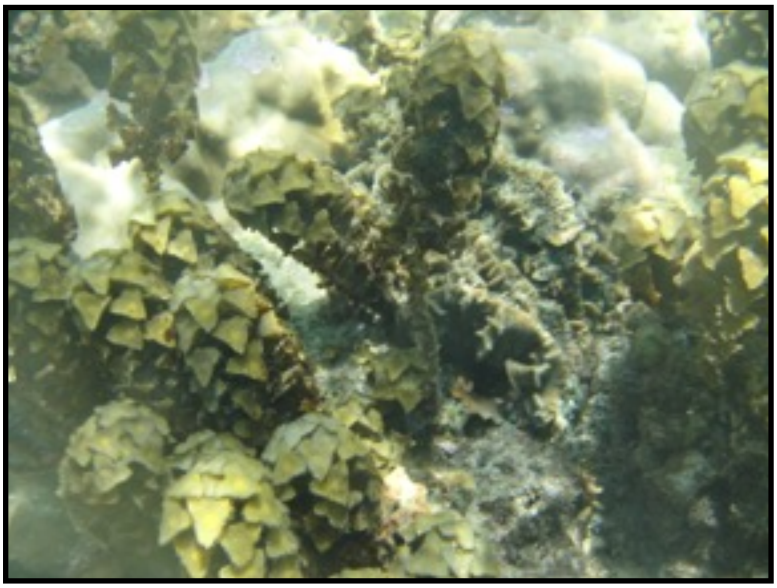

Figure 5: Photo taken on Koh Tao showing the macroalgae species *Turbinaria ornata*

The $\delta^{15}N$ signatures of the algal samples would be used to try to ascertain the point source of any increased nutrients in the nearshore waters that may be found from the chemical analysis. If the $\delta^{15}N$ signatures were found to be higher than background levels at any of the sites, and were also decreasing away from the predicted source, this would be strong evidence to suggest that anthropogenic N input is occurring in the coastal waters surrounding the island.

Results

Table 1 lists the DIN concentrations (ammonium, nitrate and nitrite) and phosphorus for each of the Site locations. For comparison the ASEAN Marine Water Quality Criteria (AMWQC) standard are given as the recommended safe concentration levels (ASEAN Secretariat, 2008).

Parameter	Distance from source	Site 1 (High Impact)	Site 2 (Med. Impact)	Site 3 (Low Impact)	AMWQC
NH_4	50m offshore	31	26	40	70
	100m offshore	36	28	36	
	100m north	22.5	27	35	
	100m south	28	57	41	
	Mean				
NO3	50m offshore	14	14	19	60
	100m offshore	14.5	18	22	
	100m north	12.5	18	9.5	
	100m south	8.5	8.5	10.5	
	Mean				
NO_2	50m offshore	3	2.5	4	55
	100m offshore	2.5	2.5	3	
	100m north	2.5	1.5	3	
	100m south	3	4.5	2.5	
	Mean				
P04	50m offshore	18	17.5	23	15
	100m offshore	13.5	19.5	22	
	100m north	16.5	19.5	24	
	100m south	13.5	19	18.5	
	Mean				

Table 1: DIN concentrations and phosphorus for each of the three Site locations. All concentrations are in $\mu g\ L^{-1}$. ANOVA significance test: * = $P < 0.05$

Table 1 shows no significant difference between DIN concentrations at the three sites and all DIN measurements were lower than the recommended AMWQC standard. Phosphorus was higher than the AMWQC standard at all of the three sites but no significant difference was found between the sites.

Table 2 lists the $\delta^{15}N$ values for the sample points at each study site. The data revealed significant variation in $\delta^{15}N$ values between each of the study sites. With mean $\delta^{15}N$ values decreasing from high to low impact areas (Site 1 > Site 2 > Site 3).

Parameter	Distance from source	Site 1 (High Impact)	Site 2 (Med. Impact)	Site 3 (Low Impact)
$\delta^{15}N$ (‰)	50m offshore	5.6	4.8	2.8
	75m offshore	5.0	3.7	3.0
	100m offshore	4.6	3.2	2.8
	25m north	4.6	4.6	3.5
	50m north	5.3	4.1	3.4
	75m north	5.5^	4.2	3.5
	100m north	5.6	4.2	3.7
	25m south	5.0	4.0	3.3
	50m south	5.0	4.2	2.8
	75m south	4.6	3.9	2.9
	100m south	4.0	3.9	2.6
	Mean	**5.0***	**4.1***	**3.1***

Table 2: Showing $\delta^{15}N$ values for the sample points at each study site. ANOVA significance test: * = $P < 0.05$

In an attempt to confirm the predicted source of anthropogenic nutrient input, $\delta^{15}N$ values were mapped for each site location (Figures 6, 7 & 8). Red points on each Figure symbolise $\delta^{15}N$ values of 4.0‰ and over, while green points symbolise $\delta^{15}N$ values under 4.0‰. The $\delta^{15}N$ value of 4.0‰ was selected as a threshold for standard background levels of seawater not affected by human wastewater. This value was conservatively selected from the the upper range of pristine ocean conditions of 2-4‰ from previous literature (Costanzo et al. 2001, Titlyanov et al. 2011).

Site 1 (High Impact) Mapping of $\delta^{15}N$ values (‰)

Figure 6: Aerial view of Site 1 (High Impact) with $\delta^{15}N$ values (‰) added for each sample point. There is a decrease in $\delta^{15}N$ values both offshore and to the south of the wastewater outlet. $\delta^{15}N$ values increase to the north. Aerial view courtesy of Google Earth.

Site 2 (Medium Impact) Mapping of $\delta^{15}N$ values (‰)

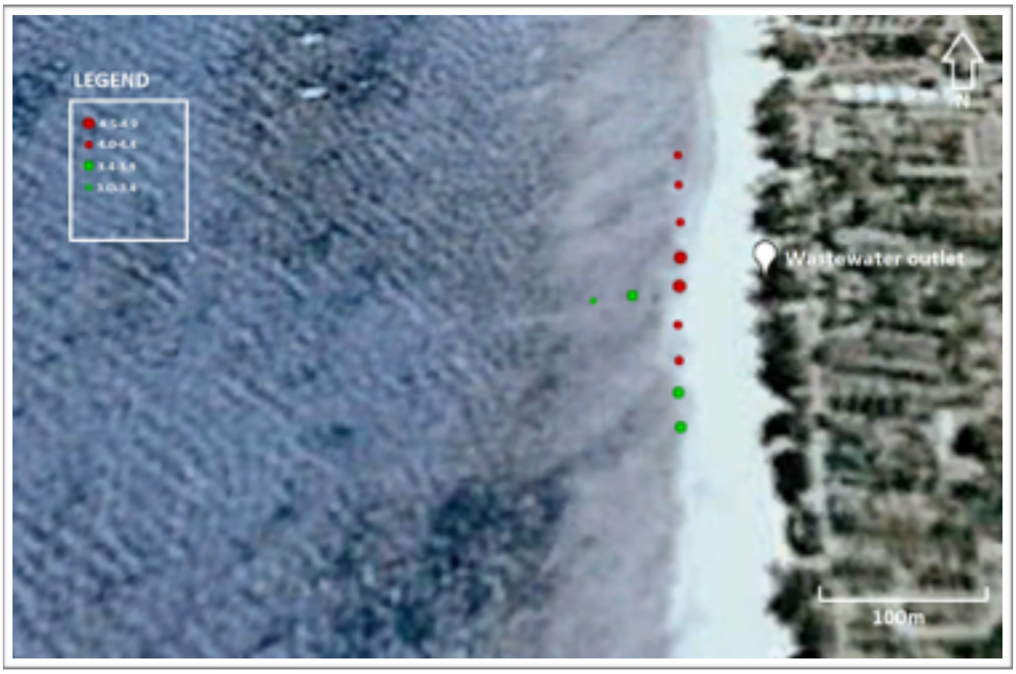

Figure 7: Aerial view of Site 2 (Medium Impact) with $\delta^{15}N$ values (‰) added for each sample point. Aerial view courtesy of Google Earth.

Site 3 (Low Impact) Mapping of $\delta^{15}N$ values (‰)

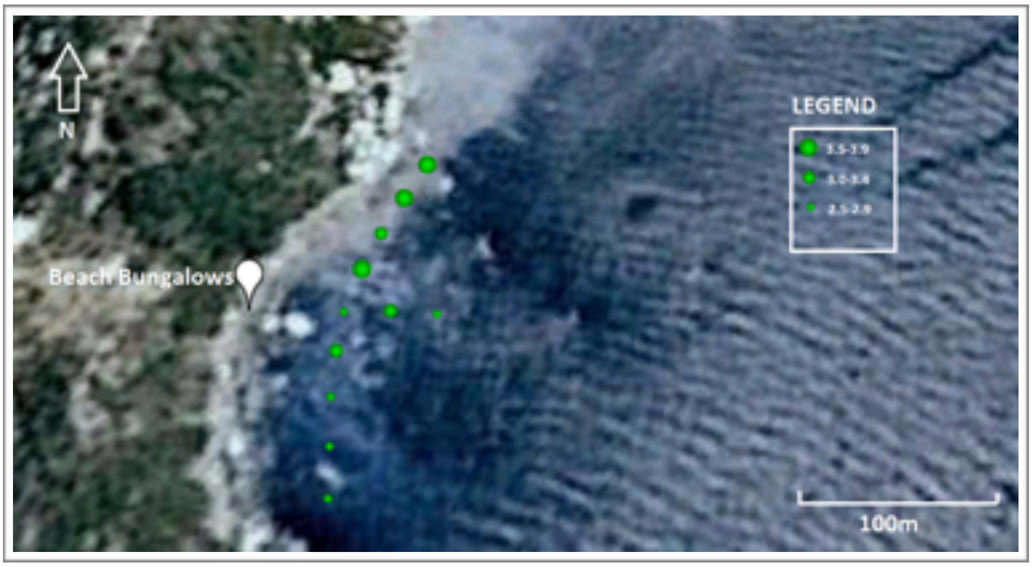

Figure 8: Aerial view of Site 3 (Low Impact) with $\delta^{15}N$ values (‰) added for each sample point. Aerial view courtesy of Google Earth.

At Site 1 and Site 2 $\delta^{15}N$ values decreased with distance from the shoreline (Figure 5 & 6). At Site 1, $\delta^{15}N$ values also decreased away from the wastewater outlet to the South. The same pattern was not found to the north of the outlet, with $\delta^{15}N$ values actually increasing with distance from the outlet. $\delta^{15}N$ values at Site 2 did decrease away from the outlet in both the north and south direction. At Site 1, $\delta^{15}N$ values were above standard levels at all sample points, while at Site 2 $\delta^{15}N$ values were within standard levels by 75 metres offshore and 75 metres to the south. To the north of Site 2, $\delta^{15}N$ values remained over but close to background levels up to the end of the 100 metre transect. At Site 3 there is a slight increase in $\delta^{15}N$ values to the north but all $\delta^{15}N$ values fall within standard levels for seawater not severely affected by anthropogenic nutrient input.

Discussion

δ^{15}N values

The highest values of δ^{15}N were found nearest to the wastewater outlet at Site 1. It was already evident at Site 1 that there was a large amount of wastewater being directly loaded into the coastal environment. It was unclear however whether this wastewater was contributing to any of the anthropogenic nutrient loading found in the area. The pattern of δ^{15}N values at Site 1 and Site 2 (Figure 6 & 7) decreasing away from the outlet both offshore and to the south for Site 1 and in all directions at Site 2, would suggest that the wastewater outlets are a main source of anthropogenic nutrients entering the nearshore reef of Sairee Beach. The increasing δ^{15}N values heading north of the wastewater outlet at Site 1 and Site 2 can be explained by the northerly current that tends to flow along the western coastline of Koh Tao (Scott, 2014, pers. comm.).

The δ^{15}N values nearest to the outlet at Site 1 (5.5 - 6‰) is of greater value than pristine ocean conditions (2-4‰) and close to double the value of the low impact site of Site 3. Although slightly lower (4.5-4.9‰), Site 2 also has δ^{15}N values above background levels in proximity to the smaller wastewater outlet. The δ^{15}N signature found in macroalgae in the proximity of the wastewater outlets at both Site 1 and Site 2 is a similar signature to what would be found from wastewater containing raw sewage or from sewage discharged from septic tanks or cesspools (McClelland et al. 1997, Dailer et al. 2010). Furthermore, the δ^{15}N values at Site 1 are above background concentrations at all sample points, meaning that anthropogenic input from the wastewater outlet stretches over a radius greater than 100m. At Site 2, δ^{15}N values are above background levels close to the outlet but are at, or close to, background levels by 75 metres away from the outlet. The low impact location of Site 3 has $\delta^{15}N$ values within standard ocean conditions and with no pattern of decreasing values away from the shoreline would suggest that this area is not impacted significantly by anthropogenic nitrogen input.

Nutrient inputs

During this study DIN concentrations at the three sites were found to have no significant differences and all DIN measurements were lower than the recommended AMWQC standard. While it is positive news that DIN concentrations do seem to be low around the island, the scope and time restrictions of this project meant that only minimal chemical data collection was carried out and so it cannot be concluded that DIN concentrations will always remain low throughout the year. A major point to consider is that this study was carried out in April, which is during the dry season in Koh Tao. It is unclear whether the wet season would produce a greater amount of nutrient loading through runoff at these sites. It may be the case that there is an increased loading of nutrients at the sites during the wet season, especially at Site 2 due to the seasonality of the wastewater outlet. Reopanichkul et al. (2010) found that pollution was exacerbated during the wet season around Phuket, Thailand. However, Lin, et al., (2007) found that there was no increase in sewage flow in Dakwan Bay, Taiwan, from the wet season to the dry season, which they put down to the increase in tourists to the Bay during the dry season.

It is also often difficult to detect the source and amount of nutrients entering the reef ecosystem due to the rapid uptake of nutrients by marine organisms, especially in the dry season when photosynthetic rates will be significantly increased. Dilution and mixing of nutrients through currents, wave activity and other general mixing events will also tend to dilute any signal of nutrient input from land (Dailer, et al., 2010). Without continuous seasonal monitoring it is hard to predict the temporal changes in nutrient loading, and related seasonal algae blooms, that may be occurring around the island.

Additional stressors (sedimentation)

Along with nutrient inputs, additional anthropogenic stressors are evident on Koh Tao with sedimentation also being documented as a major stressor on the reefs surrounding the island (Surasawadee 2012). This is especially evident on reefs close to heavily developing areas such as Sairee Beach (Figure 9). This is hardly surprising as sediment and nutrient stress generally go hand-in-hand with coastal development (Risk et al. 2009). Once macroalgae is established following a bloom there can be a continuos and positive feedback effect with increased macroalgae leading to greater capture of sedimentation, shading and chemical disturbance. This can further inhibit the settlement and growth of coral in early life stages and severely reduce the chance of the reef ever recovering after such an event. Coastal development is undoubtably the greatest concern for this small island, along with many other islands worldwide. Although no direct measurements for sedimentation were taken during this study it is obvious that coastal development is not only affecting nutrient levels in coastal waters but is also leading to major sedimentation on the reefs of Koh Tao.

Figure 9: Photo of a building development directly on the shoreline of Sairee Beach. High levels of loose sediments are evident.

Recommendations

Continuous seasonal monitoring of anthropogenic nutrient inputs from land during both the dry and wet season is recommended to fully understand the temporal dynamics of nutrient loading on Koh Tao and also how this may affect seasonal algal blooms. Due to the difficulty in measuring nutrient concentrations in coastal water directly, it is recommended that bioassay assessments using alga would be a suitable technique for nutrient and pollution monitoring in the future and therefore should be considered in any future management strategies of Koh Tao. Predicting seasonal nutrient loading patterns and dispersals will help towards an efficient management strategy for the coral reefs of Koh Tao.

Anthropogenic nutrient input from land and associated sedimentation are most likely leading to a decrease in Koh Tao’s nearshore reef hard coral cover (Figure 10). It is well documented that both of these stressors can lead to a reduction in coral recruitment, increased mortality and a shift towards algae dominance, which may favour the increased nutrient conditions or who can take advantage of the increased available substrate following coral death.

Figure 10: Photo of a nearshore degraded reef from Sairee Beach, Koh Tao

With coastal development steadily increasing on Koh Tao, anthropogenic pollution will continue to negatively affect many of the reefs surrounding the island. It is therefore imperative that action is taken immediately to at least try and stem some of this anthropogenic pollution. A simple and low cost initial step would be to fix the broken pipe of the main wastewater outlet at Site 1. This would at least transport the wastewater further offshore and into deeper water. The lack of motivation from local authorities not to fix this simple problem does not stem well for future conservation efforts.

It has been shown that at high levels of anthropogenic pollution the resilience of a coral reef is reduced. This can lead to increased bleaching events when the heat stress tolerance of corals is reduced. Koh Tao has already seen one major bleaching event in 2010, which reduced the islands hard coral cover, and so the importance of increasing the resilience of the reef through the local management of water quality around the island should not be understated. The nearshore coral reefs of Koh Tao offer vital protection in the face off storms and any further reduction in coral cover could eventually lead to greater economic costs in the future through a reduction in shoreline protection. In 2011 serious flooding occurred on Koh Tao, mainly attributed to land clearance. If the frequency and extremity of storms increase as a result of climate change, without the natural coastal protection of the coral reef, flooding events are also likely to increase, leading to a rise in damages and subsequent repair costs to coastline properties. Not to mention a decrease in tourist numbers of which Koh Tao's economy is entirely reliant upon.

Although the effects of sewage on human health is not the topic of this paper, there should be a serious concern to anyone bathing in the waters around the outlets at Site 1 and Site 2. Greater monitoring of bacterial pathogens detrimental to human health is recommended in the proximity of wastewater outlets at Sairee Beach and keeping bathers out of the water should be a priority if levels of pathogens are above recommended levels.

Conclusion

The level and pattern of $\delta^{15}N$ values found in this study would suggest land-based anthropogenic nutrient input at both Site 1 and Site 2 and is most likely in the form of untreated or primary treated sewage. Recommendations have been given to reduce and continue to monitor levels of anthropogenic nutrient input in to the coastal waters of Koh Tao.

This study helps to confirm that the marine alga *T. ornata* is a useful indicator in tracing anthropogenically derived nutrients from coastal areas that enter the marine ecosystem. This report suggests that due to its abundance around Koh Tao and the fact that it could be easily transported and deployed to areas for bioassay assessments that this alga would be a suitable species for pollution monitoring in the future and therefore should be considered in any future management strategies.

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