1. Introduction

1.1 - Overview

Harmful algae blooms (HABs) have established themselves as an area of primary concern in coastal waters throughout the world, as a result of their negative influence on local ecology, economics, and human health. Monitoring mechanisms have developed rapidly within a relatively short amount of time, but remain expensive and inaccesible to many developing countries which rely heavily on the sectors most affected – fisheries and tourism - and lack the equipment necessary to adequately treat Paralytic Shellfish Poisoning (PSP) (Walsh, 2008; Hartigan, 2006). Due to a lack of respirators, coupled with inadequate monitoring and public awareness, PSP has been reported to kill 6/100 of the afflicted in the Philippines, and in the worst cases 12/100 (Hartigan,2006). In the United States, the estimated average cost of a HAB is 49 million dollars – a conservative estimate if the bloom is sustained. The bulk of these costs are unrelated to monitoring: 45% = public health impacts, 37% = commercial fisheries, 13% = tourism (Walsh, 2008). Thus, the negative economic impacts of HABs accrue regardless of commitment to monitoring expenses. Of additional concern are the two economic generators primarily affected – tourism and fisheries, and their role in less diversified economies (Relox & Bajarias, 2003). Accordingly, it is clear that monetarily feasible HAB monitoring techniques must be explored (Furio, Gonzales, & Fukuyo, 2003; Vicente, Gaid, Dejarme, Roa & Avanza, 2003).

Figure 1. A fish kill episode in Bolinao, Philippines, occuring in 2002. The species affected were milkfish, and the losses valued at tens of millions in U.S. Dollars (IAEA, 2002).

Another cited concern is that red tide often occur in remote areas, and therefore conducting monitoring at the appropriate time is a challenge (Relox & Bajarias, 2003). This presents us with our task: Establishing a cost-effective method for monitoring algae, which is simple enough to be utilized anywhere, without the presence of specialists. A monitoring system that can be used by those communities which would be immediately impacted by a HAB.

1.2 – Red tide in Palawan

At present, monitoring of HABs in Palawan is confined to a monthly measurement of toxin levels within shellfish at specific sites, coupled with water quality samples collected at the same time by the Bureau of Fisheries and Aquatic Resources. The species of concern is *Pyrodinium bahamense*. There is no early warning system in place. The value of an early warning system is that it enables fisheries to be closed, ensuring the reliability of the product and eliminating human health risks. With caution and proper monitoring, the window of time between a bloom and sale of seafood should be large enough to eliminate any uncertainties. In the past, importers of Philippine seafood – for example Japan and Singapore in 1988, 1992, and 1993 - imposed bans on Philippine products during red tide outbreaks, for fear of contamination (Relox & Bajarias, 2003). Following a 1988 red tide outbreak in Manila, the overall price of Philippine seafood dropped 40% (Relox & Bajarias, 2003). Figure 2 displays the number of *P. bahamense* outbreaks (dark red shaded areas), as well as related PSP incidents (yellow circles) from 1983-2002 (Relox & Bajarias, 2003). This study was conducted by the Bureau of Fisheries and Aquatic Resources, the same agency responsible for current red tide monitoring in the Philippines. Within this 20 year period, there were 2,122 documented cases of PSP, and 117 deaths (Relox & Bajarias, 2003). The two red circles are the sites of the recorded HABs on the island of Palawan: Honda Bay and Malampaya Sound. These are the primary locations of study for this report. While ongoing monitoring by government authorities is in place, only six sites are examined at monthly intervals, and the results are unpublished.

Figure 2. A mapping of *P. bahamense* outbreaks in the Philippines between 1983-2002, based on a study conducted by the Bureau of Fisheries and Aquatic Resources. Yellow dots represent PSP incidents, darker red areas represent *P. Bahamense* blooms, and lighter red areas represent regions where fish kill occured as a result (Relox & Bajarias, 2003).

1.3 – Current monitoring methods

Monitoring methods currently in practice for HABs feature a wide variety of approaches to surveilling, based on either identifying the density and growth of specific phytoplankton species, or focusing on the harmful toxins. The most traditional method is light microscopy, wherein water samples are taken and analyzed for the density of potential toxic species. This method requires access to specialized equipment, is quite time-consuming, and most importantly, demands expertise in order to accurately identify the species. It is also difficult to utilize on-site, as samples must either be preserved using prepared substances such as lugol's solution – requiring elements that may not be available – or analyzed fresh. Thus, the accessibility of this method is quite limited. More specialized approaches, i.e species-specific characterization with a FLOWCAM, protein detection by immunofluorescence, or detection of nucleic acids, have proven to be effective, but are inaccesible due to high cost and inherent complexity (Anderson, 2008).

1.4 Optical monitoring

Increasingly, satellite-based optical imaging of waters is establishing itself as a reliable method for HAB monitoring, but it is still relatively ineffective in complex, coastal waters, where HABs are most common and have the biggest impact on human life (IOCCG, 2000). Utilizing the science behind this satellite-based imaging – the back-scattering of light – researchers have begun to experiment with utilizing digital cameras *in situ*, to monitor water quality. The feasability of adopting this approach to use as an early warning system for HABs is the focal point of this study.

This method is not expected to surpass established monitoring techniques in terms of its ability to identify phytoplankton – particularly down to a species level. The aim is to outline an approach to HAB monitoring that is accessible and effective: By duplicating the methodology of this study, with alterations for site-specific needs – e.g. different species, cameras, etc. – concerned individuals may be able to monitor the development of an algal bloom. This approach is one of accessibility over precision - adapting established satellite-based optical imaging techniques for use on the water by fishermen and coastal managers.

1.5 Ecophysiology of HABs

Due to the relatively crude nature of the *in situ* optical measurements, the coupling of an ecophysiological understanding of HAB occurances is estimated to improve reliability. The fundamental idea is that the optical data should provide sufficient information to verify the initiation of a bloom if coupled with an understanding of those environmental circumstances conducive to red tide. What this means is monitoring those factors which affect photosynthesis. The basic environmental parameters examined were temperature, salinity, and light intensity (Sellner, Doucette, & Kirkpatrick, 2003). While nutrients are a vital component of the process, there is no simple and inexpensive method available for monitoring them (Heisler, Glibert, Burkholder, Anderson, Cochlan, Dennison, Dortch, Gobler, Heil, Humphries, Lewitus, Magnien, Marshall, Sellner, Stockwell, Stoecker, & Suddleson, 2008; Anderson, Glibert, & Burkholder, 2004; Graneli, Weberg, & Saloman, 2008). As a result, the aim was to examine whether monitoring the 3 components mentioned, all of which can be done simply and cheaply, would prove sufficient. With respect to all 3 components, there is a certain range which favours the growth of *P. bahamense*.

1.6 Research frame

1.6.1 Aims

The aim of this research is to examine an alternative method for HAB monitoring, based primarily on the analysis of ocean colour derived from *in situ* digital images. In addition, the feasibility of estimating a bloom based on the study of basic environmental parameters – salinity, temperature, and light intensity – coupled with an understanding of *P. bahamense's* ecophysiological growth requirements, will be examined. Both approaches are cost-effective and do not require expertise, and the combination of the two seperate studies, if found effective, can be synthesized into a simple, standardized method. This report serves as an introduction and feasibility study to the approach:

Whether a wholistic examination of ecophysiology and crude digital camera measurements - combining information regarding growth conditions with an analysis ocean colour - could be sufficient for the monitoring of HABs.

The primary research questions are as follows:

- Can *in situ* digital camera-based monitoring serve as a reliable means for monitoring *P. bahamense* densities, and at an early enough interval that it could function as an early warning system? This question is the main focal point of the study, and hinges on the following: At what point will *P.bahamense* densities have a noticeable effect on overall RGB balance?
- Can monitoring salinity, temperature, and light intensity provide any indication of an impending bloom, based on the ecophysiological characteristics of *P. bahamense* or other HAB species*?*
- Can a digital camera function as a crude photometer, based on the cumulative brightness readings that result from a sum of the RGB values?
- Based on RGB values, can a stable baseline for categorizing waters around Palawan begin to be established?

A final primary aim, unrelated to a particular research question, is to begin establishing a standardized method for analyzing and categorizing RGB readings based on particular software and sampling techniques.

The secondary research questions are as follows:

- Do automatic and manual exposures produce different results in respect to RGB or overall brightness?
    - Past studies – particularly those most relevant - have primarily utilized automatic settings. As *in situ* optical monitoring has already proven effective for examining certain parameters, it is of value to explore how camera settings affect results.

- Does camera angle have an impact on RGB and overall brightness?
    - Begin to explore the question of how precise one must be when utilizing this method

1.6.2 Methods

Utilizing a small boat, a Nikon D60, and a Fujifilm Finepix S200 EXR, the RGB values of a number of water bodies surrounding Puerto Princesa City were examined. The focal point was Honda Bay, an area with a history of *P. bahamense* blooms, but other study sites included Puerto Princesa Bay, Malampaya Sound, the Iwahig River, the Embarcadero River, and the Bacungan River. Digital images were captured *in situ*, beneath the surface of the water, with the use of an aquarium as a waterproofing enclosure. The images were analyzed using ImageJ, free software, in order to derive their RGB values. These results were plotted using another free software, Triplot, in order to visually categorize and compare findings. Additional parameters monitored were temperature, salinity, light intensity, and depth. A thermometer was used to measure temperature, a hydrometer for salinity, a photometer for light intensity, and a secchi disk for water transparency depth. The findings of these additional parameters were cross-referenced with the ideal growth conditions of *P. bahamense*.

1.6.3 Limitations

Several key limitations were encountered over the course of the study. The first was the lack of certain equipment, primarily that which could be used to offer additional reliability to the research. As the research was motivated by the expensive nature of present algal monitoring technology, and carried out without financial support in an impoverished area, it was not possible to obtain a chlorophyll-a probe. This tool would have allowed for an accurate estimate of the algal presence within a sampled area, which could then have been compared to RGB findings. Attempts were made to collaborate with local government bodies in order to utilize the best equipment available. These attempts were useful in some respects, but ultimately failed to provide the tools desired. What was obtained through these collaborations was access to monthly *P. bahamense* surveys conducted in Honda Bay. The studies were carried out by the Bureau of Fisheries and Aquatic Resources, with the aid of the Provincial Agriculturalist's Office. Six sites were sampled, and light microscopy was utilized to search for the presence of *P. bahamens*e. No other data was collected from these surveys. Unfortunately, the results of these studies could only be witnessed within the office of the Bureau of Fisheries and Aquatic Resources, and thus cannot be sourced. As a result, more recent data regarding the presence of *P. bahamense* in Honda Bay cannot be provided, though the documents provide proof that the species remains present in the area. Further, water samples were taken personally for the purpose of species identification, but had decomposed by the time microsopes could be accessed. To summarize: The proper equipment and resources were not available for species identification. A final issue related to equipment was the inability to monitor the presence of nutrients, particularly nitrogen and phosphorus. These are basic components responsible for the development of HABs, and would have aided in the ecophysiological component of the study.

The next key limitation was due to weather, which inhibited data collection during the rainy season. Throughout the months of September and October it was difficult, and at times dangerous, to obtain data. On more than one occassion, fieldwork had to be cut short due to weather. As a result, the majority of fieldwork had to be delayed until the rainy season was over, and less data was obtained than had been desired.

An additional, crucial limitation was the inability to conduct multiple studies in the Malampaya Sound region. The body of water featured a unique RGB signature relative to the other bays studied, and has a history of red tide, but was quite far from Puerto Princesa City. A follow-up study, intended to be carried out the day after the first, was cut short due to illness.

The final key limitation was the lack of a *P. bahamense* bloom throughout the course of the study. Ultimately, this was by far the largest problem encountered, as without the development of the bloom it is impossible to form any solid conclusions regarding the use of an *in situ* digital camera-based monitoring system.

1.6.4 Hypothesis

It is expected that the RGB values derived from *in situ* digital images will be suitable for crude water quality monitoring and classification, as well as detection of an impending *P. bahamense* bloom. It is questionable whether the algal bloom will be detectable early enough to serve as a functional warning system, but operating in conjunction with the monitoring of basic environmental parameters should prove sufficient, and suitable for at-risk areas without access to more precise monitoring approaches. It is expected that the digital camera will not function well as a photometer due to its reliance on upwelling light and the relative complexity of coastal waters. The absorption and reflectance of light by various marine organisms and solids will affect the readings. In regards to camera settings, though previous studies have utilized automatic exposures, it is expected that the use of properly configured full manual settings will provide more reliable results. The angle at which the camera is held is also expected to affect the overall outcome, as the density of substances can vary with depth, as well as the overall intensity of the light. RGB readings gathered from various water bodies surrounding Puerto Princesa should be fairly consistent, and sufficient to distinguish them from one another. These readings should be coincide with expected RGB values, i.e rivers should feature higher red values than bays. Overall, the study should demonstrate the potential of exploring further the application of digital cameras *in situ* optical monitoring devices.

1.6.5 Organization and contents

This study is organized as follows: Following this introduction, a theoretical overview will be presented in order to put the issue into context, provide necessary terms, and expound the theoretical roots upon which the research is based. This will be supplemented by a review of relevant literature, in order to frame the issue and develop the necessary background knowledge. The literature review will begin with an in-depth look at *P. bahamense,* focusing on its behaviour, distribution, and ideal growth conditions. Next, a look at Ocean Colour satellite monitoring will be outlined, including how it has been used in the past, its limitations, and its theoretical foundations. Subsequently, digital camera monitoring applications will be explored in detail, providing the foundation for the method applied in this study. Finally, an in-depth look at phytoplankton growth conditions will be provided, which serves to explore the role of the environment in the development of blooms.

Next, the research methods section will serve to guide the reader through the research process, with the intention of enabling an interested party to reproduce the results. This will include the actual methods utilized to gather data, both in respect to *in situ* optical monitoring, as well as the gathering of additional environmental information. In addition, study sites will be discussed, as well as two key software programs that were used heavily throughout the process. The section will also include other miscellaneous matters, such as how *P. bahamense* were identified, how analysis was conducted, and the methods behind various secondary studies conducted.

Following this, the results section will serve to share the findings of the study. Results will appear in the following order:

1. A presentation of the results for RGB values, coupled with secchi disk findings
2. A comparison of automatic vs manual exposures, to determine whether there is a difference in their ability to accurately monitor water quality

3. An examination of the basic environmental parameters in key sites – sunlight, salinity, and temperature – and whether they fall within the optimal growth range of *P. bahamense*
4. A comparison of photometer readings with RGB brightness values, in order the explore the use of a digital camera as a light measuring tool
5. A camera angle test, in order to explore the degree to which variations in the method affect the results

This section will present the data, but will not analyze it.

Finally, the discussion section will follow up the results by exploring their significance, and relating the findings with the aims of the study. The subjects discussed will appear in the same order as the results, and the purpose of the section will be ultimately to explore the feasibility of this methodology as an early warning system for *P. bahamense.* As such, the aforementioned 5 sections will be followed by a sixth, dedicated to this subject. These discussions will be followed by recommendations for future study, and a conclusion.

2. Theoretical overview

The following serves to provide some necessary background information for the study, as well as its conceptual foundations. The reasoning behind the development of the method will be outlined, some key terms defined, and supporting literature presented.

2.1 Terms / Concepts:

A number of vital concepts and terms must be established before moving forward. First, it is necessary to develop a basic understanding of a HAB, and the other biological components of the study. HAB is a flexible acronym for 'harmful algae bloom', used to describe an aggregation of algae that has a detrimental effect on other species (Fogg, 2002). It is most commonly used to refer to toxic algae – which is the case in this study – but can also refer to a non-toxic bloom that causes harm due to its sheer density, or the creation of anoxic conditions due to oxygen depletion in bottom waters (Anderson, 2005). Red tide is a colloquial term for a HAB, and is commonly used in the Philippines and other locations. The focal species in this study is *Pyrodinium bahamense,* an armoured, chain-forming, bioluminescent dinoflagellate which produces saxitoxins, and is responsible for most HABs in the Philippines (Usup et. al, 1994). Dinoflagellate is the phylum to which *P. bahamense* belongs*,* and is one of the classes of the organisms responsible for HABs. They are a phytoplankton protist, both auto- and heterotrophic, with flagellum (Anderson, 2005). The aforementioned saxitoxins produced by *P. bahamense* are a toxin which affects sodium channels in the brain, leading to mild paralysis (Walsh, 2008). Saxitoxins are the cause of PSP, which is the direct concern for human health resulting from a HAB. PSP is an abbreviation for "paralytic shellfish poisoning", which can lead to death due to various neurotoxic effects and respiratory failure (Walsh, 2008). Saxitoxins accumulate in shellfish when exposed to a HAB, and it is in this way that they are transmitted to humans. PSP is responsible for the human deaths that occur due to HABs.

In respect to methodology, a number of other relevant terms must be explored. First, RGB is an abbreviation for red, green, and blue values - the way colour is organized in a digital image. These colours are the source of data for the *in situ* optical monitoring conducted here. *In situ* is a Latin term used in this case to describe an on-site study, rather than remote. For the purpose of this study, two forms of light must also be considered. The first is upwelling, which is light that is reflected off of a surface, and backscattered (IOCCG, 2000). It is this type of light that will be captured by the digital camera. The second type is downwelling light, which comes directly from the sun (IOCCG, 2000). It is this type of light which produces the photometer readings. The photometer's readings were measured in Lux, which is the standard unit for luminance measurement – an indication of light intensity. A lux unit represents one lumen per square meter (Thimijan & Heins, 1982). Another light measurement unit utilized in the study is micromoles. This term is a linguistic representation of the equation µmol m-2 s-1, which is micromoles per square metere per second. Micromoles are a unit used for measuring light intensity (Thimijan & Heins, 1982).

2.2 Theoretical background

Though not visible to the human eye, microscopic matter reflects light, and thus alters the optical properties of water. Every particle in the oceans will alter the RGB value captured in a digital image. Therefore, the early developmental stages of a HAB – if it can be identified – are available for optical analysis. In theory, the range of RGB values could prove sufficient to develop a classification system that can estimate the presence of specific substances, i.e. – dinoflagellates, sediment, and cyanobacteria – based on their unique colour and concentration. At present, the aim is simply to provide particular thresholds which indicate bloom conditions, bloom development, or the presence of a bloom. As there is no specific definition of the density required for algal division to become a "bloom", this method must be tested during the development of a toxic bloom in order to establish what RGB threshold accurately reflects the onset of shellfish contamination.

This methodology is expected to be relatively imprecise when compared with other detection methods, its purpose being an inexpensive, widely employable method. As a result, it should be most effective when coupled with an understanding of the ecophysiological behaviour of red tides, and ideally a specific species of concern. By understanding which environmental conditions are likely to produce a bloom, more precise results can be deduced from the *in situ* optical data.

2.3 Literature review

Within the Philippines there have been four recorded species responsible for a HAB: *Alexandrium, Cochlodinium polykrikoides, Prorocentrum minimum,* and *Pyrodinium bahamense* (Furio et. al, 2003; Azanza, Fukuyo, Yap, & Takayama, 2005). The latter species has had by far the most consistent and damaging presence, causing more than 40 HABs within a 20 year period, and occuring in 22 places throughout the country . Both of the recorded blooms on the island of Palawan were caused by *P. bahamense,* and thus this species is the focal point of the study (Relox & Bajarias, 2003). It is worth acknowleding, as a precursor to the literature review, the relatively dated nature of some of the key papers examined. This is the result of the absence of other relevant material, and thus, the studies discussed here are in fact the most recent pertinent publications available. The presence of a number of knowledge gaps which would have benefitted the study, particularly in regards to *P. bahamense,* serve to support this notion.

2.3.1 *Pyrodinium bahamense*

Pyrodinium bahamense is an armoured, chain-forming dinoflagellate, which is bioluminescent and characteristic of mangrove areas (Anderson, 1989; Usup, Kulis & Anderson, 1994). It features a fairly wide range of distribution, from the Western North Atlantic to Papua New Guinea and the Philippines, among other tropical regions (Phlips, Badylak, Bledsoe & Cichra, 2006; Okolodkov, 2005). *P. bahamense* produce saxitoxins, which accumulate within shellfish and may cause PSP within humans. PSP is the product of toxins impairing the sodium channels in an individual's brain, inhibiting its ability to control certain bodily functions and resulting in a mild form of paralysis. The primary concern is that with sufficient doses of saxitoxins, an individual may be unable to breathe without assistance (Hartigan,2006).

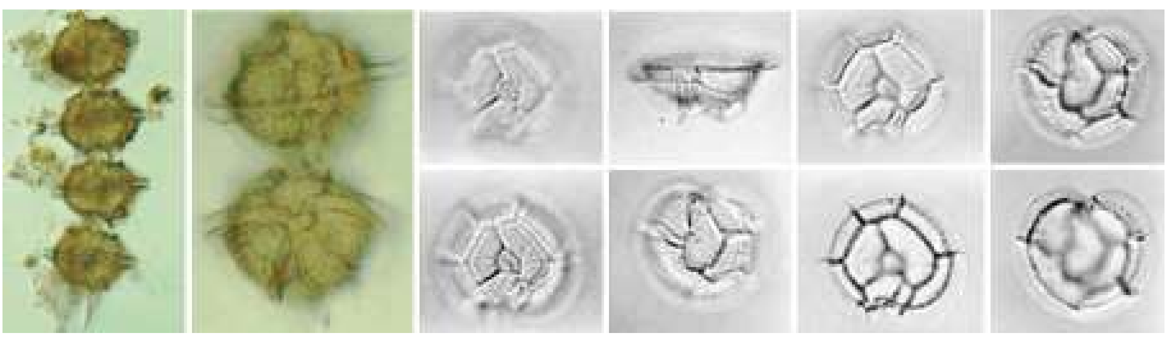


Figure 3. *Pyrodinium bahamense* photographed under a microscope (Yasuwo, 2000).

P. bahamense are a cyst producing species, and their sexuality is induced by nutrient limitation. This cyst stage helps to explain the sustained presence of *P. bahamense* in mangrove areas: A connection has been observed between the two, likely a result of the shelter that the estuarine trees offer. The *P. bahamense* cysts undergo a period of dormancy in which growth cannot occur, regardless of environmental conditions, and also a quiescence stage in which they wait for favourable condition. Studies have indicated that *P. bahamense* are sensitive to temperature, and will re-enter a dormancy stage if it is too cold. Another factor may be an internal clock, suggesting that seasonal blooms could be a very reliable occurance. Information on *P. bahamense* cysts is limited - i.e. no information on the impact of light on germination - but is vital for monitoring of the species. If blooms are indeed seasonal and dependant on an internal clock, it may not be necessary to monitor for *P. bahamense* at certain times of the year (Anderson, 1989).

The growth of *P. bahamense*, as with all photosynthetic organisms, is controlled by a few environmental parameters: Temperature, light, salinity, and availability of nutrients. Through cultured laboratory experiments, we have an idea how *P. bahamense* react to these parameters, and thus have an idea of what conditions are favourable for a bloom, as well as the production of toxins (Smayda, 1997). *P. bahamense* are able to grow between 20-38°Ce, but are morphologically altered beyond 30°Ce. Optimal growth occurs between 28-30°Ce. Temperature also has a significant inverse impact on the concentration of toxins: As temperature decreases, more toxins are produced. Going from the optimal growth temperature of 28°c to 22°Ce, the concentration of toxins increases threefold. In terms of salinity, growth is possible at 20 o/oo and above, optimal at 30 o/oo, and toxins are relatively stable between 24-36 o/oo. Going from 24 to 20 o/oo, the toxin concentration triples. Thus, as growth is inhibited, toxin production flourishes. Growth is possible under low light conditions and fairly steady between 50-150 micromoles, but a relationship between chlorophyll a and toxin content is evident. Under low light conditions, the *P. bahamense* focus on chlorophyll production – up to 40 micromoles - but at this point shift dramatically to toxin production, which peaks between 90-100. From the above we can gather the following:

- Toxin production generally increases as growth decreases
- Optimal growth occurs within a rather broad range of salinity and temperature
- Changes in light do not have a significant impact on growth
- All parameters have thresholds which lead to a dramatic increase in toxin production

Of particular importance to the implementation of this method is the growth rate of the species being monitored. Red tide species have generally been found to be slow growing – blooming despite this, as a result of low grazing pressure or aggregation (Smayda, 1997). The standard rate of a division is between 48-72 hours (Smayda, 1997). In the specific case of *P. bahamense*, cultured studies produced consistent reports: Even under optimal conditions, the maximum growth rate was between 0.2-0.4 divisions per day (Usup et. al, 1994). There is no clear definition of the density required for an algal aggregation to become a bloom, nor at what point their toxin concentrations will have a significant impact on shellfish species. The depth at which *P. bahamense* have been observed ranges from 0-9m below the surface (Maclean, 1977).

2.3.2 Satellite monitoring – Ocean Colour

In order to understand the foundations of optical sensing of HABs, it is necessary to review the ocean colour satellite-based approaches, and the physical principles that support them. The IOCCG (International Ocean Colour Co-ordinating Group) is the leading agency for optical monitoring of the world's oceans, sponsored by the NASA, NASDA, and ESA (IOCCG, 2000). The first sensor was launched in 1978, with the goal of determining phytoplankton densities. This remote sensing of phytoplankton was based on the isolation of certain wavelengths of sunlight, back-scattered from the various substances present in the ocean's waters. Each of the primary constituents of the ocean's waters – microscopic organisms, suspended inorganic material, and dissolved organic matter – have unique absorbance and reflectance characteristics, and thus, alter the colour of water. As stated by the IOCCG, if one group were to be identified as the primary agent responsible for variations in the ocean's colour, it would be phytoplankton (IOCCG, 2000). The highly pigmented phytoplankton dominate the reflectance of microscopic organisms.

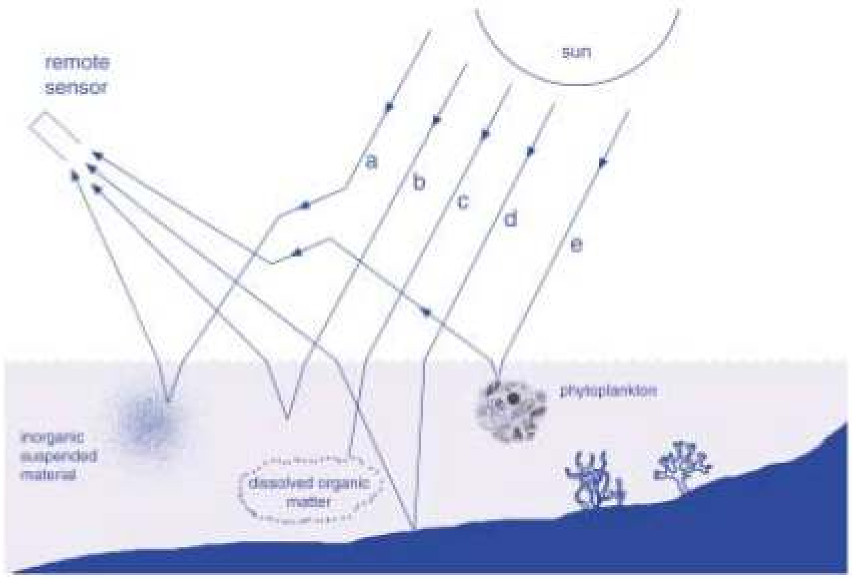


Figure 4. A visual represenation of how light is scattered off of the water's surface, and accordingly, how Ocean Color satellites capture the back-scattered light for analysis (IOCCG, 2000).

The data gathered by Ocean Colour satellites is then categorized into one of two cases used to describe the complexity of the waters. First introduced by Morel and Prieur in 1977 and refined by Gordon and Morel in 1983, this classification system is the standard for remote sensing. Case 1 waters are those where phytoplankton are the main colouring agent. These waters are typically found in the open ocean. Case 2 waters are more complex and coloured by multiple agents, the primary difference being the presence of inorganic substances. This is more typical of coastal waters, which can also be altered by reflectence from the sea floor. In terms of colour coding, phytoplankton represent a peak in green reflectance, dissolved organic matter a peak in yellow, and suspended inorganic material a peak in red (IOCCG, 2000).

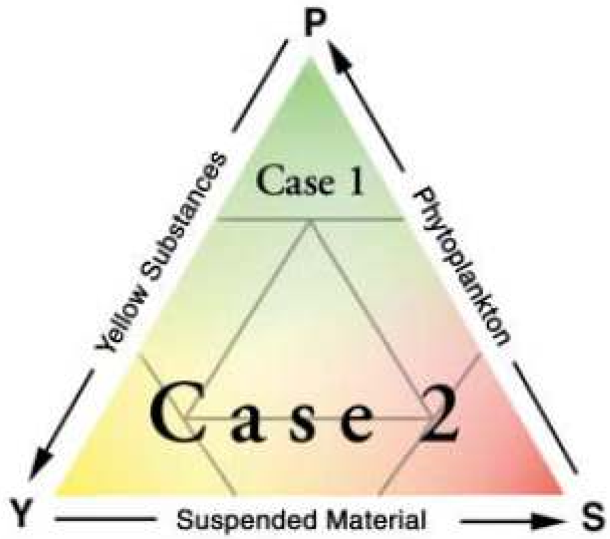


Figure 5. Ocean Color's system for categorizing waters based on their complexity. The colour of case 1 waters is green, as the water itself is dominated by algae. Case 2 waters are more difficult to categorize, as colours can vary due to the presence of suspended solids and yellow substances (IOCCG, 2000).

In studies conducted in the North Sea, Ocean Colour sensors have demonstrated that red tide's optical signature is very unique from what would otherwise occur. Their difficulty in monitoring is due to the complexity of case 2 waters and the issues this presents in differentiating red tide from suspended organic matter (IOCCG, 2000; Gitelson, Yacobi, Schalles, Runsquist, Han, Stark, & Etzion, 2000). It is for this reason that remote satellite systems are not suitable for monitoring of HABs in coastal areas.

2.3.3 *In situ* digital camera monitoring

Building on these concepts, a variety of pilot studies have been conducted for over a decade on the ability of standard digital cameras to measure specific criteria within the marine environment. Some advantage are that the proximity to the surface can ensure that the sea-floor does not colour the image, and additional parameters may be examined on-site. A simple and obvious advantage, is that near-surface samples can be examined first-hand. A 1997 report produced by Cullen et. al stressed the need for cost-effective methods of HAB monitoring, and identified optical methods as well suited for the task. A number of approaches were considered – buoyed radiometers, artificial illumination, fluorometers – with an emphasis on *in situ* measurement (Cullen, Ciotti, David & Lewis, 1997).

Based on developments between the years of 1994-1999, Maritime INTERREG published a report in 2001: *Feasability study on the use of digital cameras for water quality monitoring in the coastal zone.* Maritime INTERREG was an EU-funded Welsh/Irish collaboration, supported by the Irish Department of the Environment, the Department of Marine & Natural Resources, and the Marine Institute. This particular study was published by the Irish Marine Institute, and conducted by 6 individuals representing the National University of Ireland, Galway, and the University of Wales, Bangor. The study was motivated by the challenges satellite-based monitoring experience in coastal waters, due to their optical complexity. The aim of the study was to examine the feasibility of monitoring suspended particulate matter with a digital camera in order to measure water quality. The study had two distinct portions, testing the value of both aerial and *in situ* imagery. The aerial monitoring proved effective in monitoring coastal dynamics, such as river plumes, but was unreliable for measuring suspended sediment concentrations due to reflectance from the sea surface and the sky. These outlying optical contributants were eliminated using the *in situ* method, which succesfully monitored suspended sediment concentrations. The conclusion of the report is that traditional methods of suspended sediment measurement can be carried out using optical imaging *in situ* with a digital camera (Feighery, White, Bowers, Kelly, O'Riain, & Bowyer, 2001).

Following the results of this study, researchers from the University of Ireland, Galway, continued to explore the application of digital imaging for monitoring coastal waters. This involved refining the approach and establishing a method: Moving beyond the study of feasibility. Another study on water quality monitoring in Galway Bay was published in 2006, this time with the aim of developing an analytical approach using a camera's RGB numbers. A strong linear correlation was found with the red/blue camera output and salinity, which is consistent with the narrow band irradiance approach used by satellites. A relationship was also found between chlorophyll levels, and green/blue output. The results of this study serve to solidify – and begin to standardize – the notion that a digital camera can be effective in optical monitoring in much the same way as satellites, but can be used in complex coastal waters. Parameters that were found to be observable using this approach include salinity, chlorophyll concentration, and the presence of suspended matter (Godijin & White, 2006).

A subsequent study published in 2009 outlined the fundamentals of digital-camera based in situ water quality measurement: It described a method. The report states that digital cameras function as three-band radiometers, and thus, can function in the same capacity as satellite imaging. The reports from Galway Bay provide a reliable and comprehensive look at the value of *in situ* digital camera measurements for monitoring water quality, and their findings are supported by other independant reports. An overview of these previous successful applications of digital-camera-based optical monitoring are included in the report:

- Ocean surface currents
- River plume dynamics
- Underwater radiance distribution
- Marine suspended solids
- Dissolved organic matter
- Chlorophyll concentrations
- Salinity

The study moves on to establish the notion that a standard method is vital for the success of *in situ* optical monitoring, as different camera parameters are found to affect the RGB values captured in an image. The primary aim of the study was to establish whether or not results varied in different cameras, and whether the colour readings were indeed consistent with the results gathered by traditional three-band radiometers. In terms of the comparison with a three-band radiometer, it was imperative to find a correlation in order to establish the digital camera as a reliable tool for applying the optical monitoring principles previously utilized by ocean colour sensors. It was confirmed that digital cameras can be used as three-band radiometers. Further, comparing the readings of different cameras was necessary to determine whether reliable results could be derived consistently, or whether only certain cameras can be used. An underwater camera and a standard digital camera were used. The study found that if certain parameters are controlled, consistent readings are expected regardless of the camera. Of primary importance is that the "white balance" must remain fixed – otherwise the camera will automatically adjust the colours and an accurate RGB reading cannot be derived. The relationship between the RGB readings and water quality parameters are outlined as follows:

- Salinity – R/B
- Chlorophyll a – G/B
- Suspended solids – R/G

(Godijin, Dailloux, White, & Bowers, 2009)

In each case, the parameter is measured based on a ratio of the two colours, produced in the order listed, as would be done with a radiometer. The larger the ratio, the higher the concentration of the particular substance. For example, a site with an RGB of 3,10,8 is less saline and contains less chlorophyll than a site with an RGB of 3,10,6. This is because the decrease in blue pixels leads to an increased R/B ratio, as well as an increased G/B ratio. Additional pilot studies have been conducted on turbidity (Alsultan, Lim, MatJafri, Abdullah, & Bakar, 2004) and water quality monitoring (Mokhtar, 2008; White, Feighery, Bowers, O'Riain & Bowyer, 2005). The cumulative results of these studies serve as a foundation for the use of standard digital cameras as a tool for optical monitoring of HABs .

2.3.4 Phytoplankton growth conditions

Casting an eye forward to the methodology utilized in this study, it will become clear that it is vital for the environmental factors affecting dinoflagellate development to be taken into account. The primary challenge of *in situ* optical monitoring is determining which substance is responsible for the observed RGB values of the digital image. In this case, there is more than one optical property which can increase the red pixels. Thus, optical results can be clarified by measurements of those parameters which affect photosynthetic activity, as well as species growth conditions. The principle factors to consider are temperature, light, presence of nutrients, and salinity. Based on previous studies, but not explored here, is the notion that salinity can be measured using digital imaging. Hydrometers are effective, inexpensive, and easy to use, and so they were used in this case. Similarly, temperature can be monitored in parallel without any specific expertise or technical equipment. The last parameter, light, has been found to be less critical in regards to dinoflagellates, which have been described as a "shade" species (Falkowski & Owans, 1978; Domingues, Anselmo, Barbosa, Sommer, & Galvao, 2011). While light is absolutely pivotal for photosynthesis and sustained phytoplankton growth, the amount required is rather low and thus light conditions seldom sink below the minimum irradiance level (Falkowski & Owans, 1978). Other considerations to be taken into account are the weather patterns noted before the onset of a red tide. This would be a period of intense rainfall, followed by a brief interval of time in which nutrients are transported to coastal waters, and an initiation phase of intense irradiance (Smayda, 1997). While dinoflagellate species do not require high levels of irradiance, it may be that this intense irradiance causes the emergence of the dinoflagellates from their dormancy cyst stage. While no data is available in respect to *P. bahamense*, most dinoflagellates are able to germinate in low light conditions but require a longer time to do so (Anderson, 1989). Thus, waters are full of nutrients transported by rainfall, and a number of dinoflagellate species emerge from their cyst stages in ideal conditions for growth. As a relationship has been demonstrated between cysts and mangroves, it is likely that they are sheltered within them, and thus, high levels of irradiance need to be sustained long enough that the sun reaches an angle where the cysts are not blocked by the mangrove roots (Anderson, 1989). The high levels of irradiance do not need to be sustained beyond the bloom initiation stage. This pattern helps to eliminate a number of times where the weather conditions are unfavourable for bloom development, and thus, monitoring need not be as intensive.

Furthermore, it has been found that wind may have an even more dramatic impact on blooms. Studies in Papau New Guinea found that Northwesterly winds to the Southern coast – typical of monsoon season when *P. bahamense* blooms occur in the region – may in fact be the catalyst for the algal growth. When Southeasterly winds replaced them for a short time, the blooms decreased considerably (Maclean, 1977). The mixing associated with these winds may have been responsible for the nutrient enrichment required for the bloom.

Fundamentally, by synthesizing the relevant information regarding phytoplankton growth – from higher taxonomic levels to a particular species – it is possible to observe some trends in algal bloom developments. This information can be applied in order to make an educated guess about the occurance of a HAB.

3. Research Methods

3.1 Overview

Research for this study was conducted between the months of September and December of 2012 on the island of Palawan, in the Southwest of the Philippines. Two cameras were used for data gathering – a Nikon D60 DSLR as the primary camera, and a Fujifilm Finepix S200 EXR for the supplementary data. A thermometer, hydrometer, secchi disk, and photometer were utilized to measure other parameters or provide comparative data. There parameters were salinity, temperature, light intensity, and depth. A parallel, collaborative study unrelated to algal blooms, occuring between January and October of 2012, served to provide additional data in regards to RGB values of various waters in the area, as well as luminance values and secchi disk depth. This supplementary study was focused on Puerto Princesa Bay, a site with no reported history of algal blooms, and the Iwahig river which drains into it. The primary area of focus for this study was Honda Bay, located to the North of Puerto Princesa, Palawan's capital city. Additional research sites were Puerto Princesa Bay, Malampaya Sound, Embarcadero River, and Bacungan River. The primary question motivating the research is whether or not an *in situ* digital camera-based monitoring system may serve as a reliable means for monitoring *P. bahamense* densities, and at an early enough interval that it could function as an early warning system.

a)

b)

Figure 6. Overview of sites studied: a) Topographical look at the island of Palawan, and the three large bodies of water sampled. The Embarcadero river drains into Malampaya Sound, the Bacungan river drains into Honda Bay, and the Iwahig river drains into Puerto Princesa Bay. b) A topographical look at the Philippines, as a reference to where the island of Palwan is situated (Google, 2013).

3.2 In situ optical monitoring procedure

All measurements were taken from a small fishing boat. The depth at which measurements were taken needed always to exceed secchi disk depth, in order to avoid the optical properties of the seafloor from obscuring the results. The method was based on the developments of researchers in Galway Bay – with correspondance – but altered for specific needs, equipment limitations, and the particular aims of the study. In order to eliminate certain optical constituents which would obscure the results – atmosphere and sun reflectance off of the sea surface – a waterproof enclosure was constructed to submerge the camera while obtaining images. A re-enforced aquarium with a thickness of 3mm was utilized for this purpose. The base of the aquarium was placed just beneathe the sea surface with the rim remaining above water. The camera lens was held firmly against the base of the aquarium. The measurements were consistently taken in such a way that the boat did not obscure the passage of sunlight into the water. Photos were taken using two different exposure settings – both with automatic light compensation and full manual – in order to compare the results. One photo in each setting was taken at every station, and in both cases it was vital that white balance and ISO remained constant: Set at direct sunlight and 100, respectively. In full manual setting, the aperture was opened fully, at an f-stop value of 4.8, and the shutter speed was fixed at 1/100.

a) b) c)

Figure 7. Examples of photographs taken for in situ optical monitoring. a.) Honda Bay – November 4. b.) Puerto Princesa Bay – November 25. c.) Embarcadero River – November 11

3.3 Additional Parameters

A silicon photodiode photometer, Sekonic i-346, was used to gather light intensity readings for the primary purpose of examining their relationship with the camera's RGB values. The readings were gathered in Lux values. The photometer was sealed in a waterproof case and attached to a weight. It was used in continuous mode when monitoring, in order to observe fluctuations occuring due to wave action and ensure the consistency of the reading. Measurements were taken both above and below the water. Underwater readings were conducted at a depth of 1.5 metres, securing the weighted photometer to the boat to ensure consistency.

A secchi disk was used to determine whether the depth was sufficient to avoid capturing the seafloor in the images, and to examine the depth to which the camera is capable of gathering data. The depth to which the secchi disk disappears gives a rough estimate of the depth to which optical constituents are captured in an image. A basic thermometer and hydrometer were used to examine temperature and salinity, parameters of particular relevence to dinoflagellate growth.

3.4 Study Sites

The primary area of study – Honda Bay – was divided into its respective Barangay for the purpose of consistency in data gathering; specific sites were not necessary, as the aim was to develop a general classification of the waters. Barangay can be defined as townships. The bay, located to the North of Puerto Princesa city, was selected as a sample site due to historical accounts of red tide, and identification by local authorities as an at-risk area. Under national provisions for red tide monitoring, the Bureau of Fisheries and Aquatic Resources conduct monthly surveys of the bay using light microscopy to search for *P. bahamens*e. The results of these surveys are not available for sourcing, and thus the presence of the species, seasonal influence, and the frequency of blooms is unclear. Early attempts at data gathering in Honda Bay were disturbed by the weather, as they were undertaken during rainy reason. As a result, readings from September and October are few, and the number of sites examined on each day are irregular. Some results had to be discarded for the same reason. The Bacungan river drains into Honda Bay, and was studied in order to examine salinity levels as well as test RGB expectations. The river was generally too shallow to take measurement for secchi disk depth and light intensity.

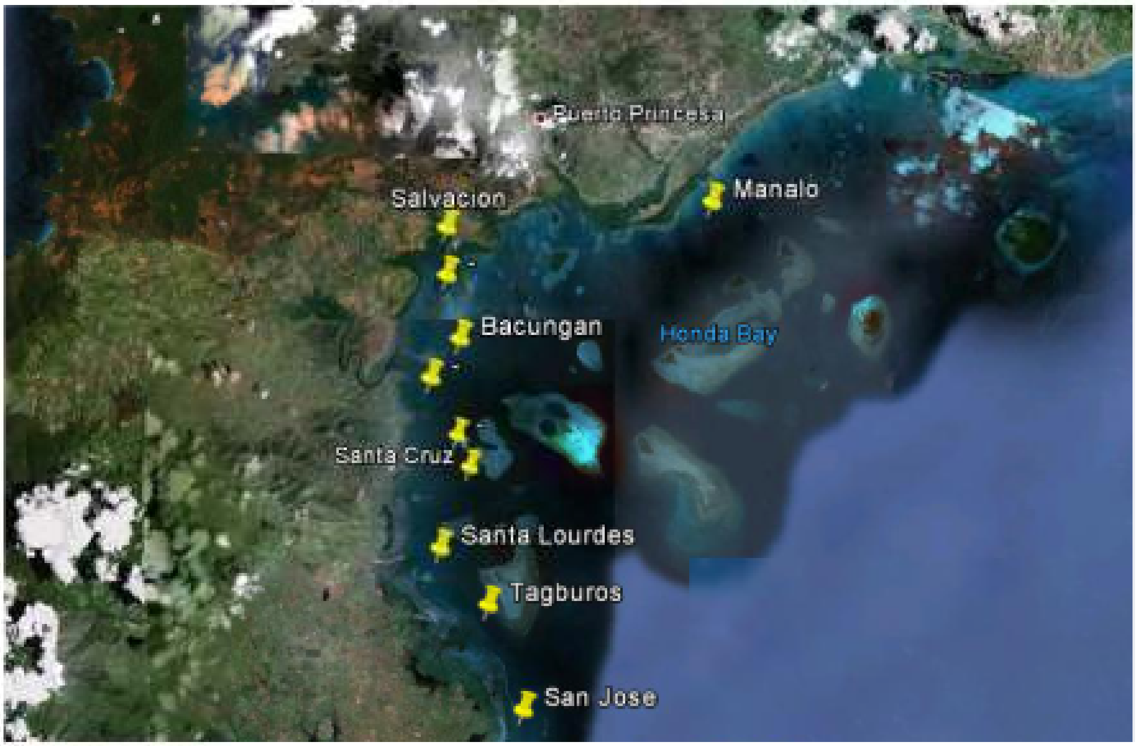


Figure 8. Overview of sample sites in Honda Bay. Labels indicate different Barangay sites, and provide an approximation of their Northern boundary (Google, 2013).

Puerto Princesa Bay has no recorded history of *P. bahamense* blooms, but was selected as a study site in order to compare RGB values and begin to establish a general classification of waters in the area. Samples were collected at random within the bay. The Iwahig river drains into Puerto Princesa bay, and further studies were carried out in this location.

Figure 9. Overview of Puerto Princesa Bay and the Iwahig river which drains into it (Google, 2013).

The final location studied was Malampaya Sound, in the North of Palawan. This area and Honda Bay are the only water bodies on Palawan to feature a reported *P. bahamense* bloom. It is also a prominent fishing grounds as well as shellfish harvesting area, making the potential losses and impacts on human health significant. Due to the distance of this site from Puerto Princesa City, only one study could be undertaken.

a)

b)

Figure 10. GPS coordinates of sample sites from the Malampaya region. a) Malampaya Sound. b) Embarcadero River (Google, 2013).

3.5 Software

In order to analyze the data from the field, two free windows-based software were utilized: ImageJ and Triplot. The selection criteria was based on three concepts: Cost, ease of use, and applicability. It was vital that this portion of the study remained consistent with the initial aims and did not become expensive or overly complex, while still providing accurate data analysis. ImageJ served to derive the RGB values from the *in situ* measurements, and to experiment with manipulating images in order to isolate certain wavelengths. The range of possible RGB values extend from 0 – 255, and are counted in pixels. The RGB values derived from ImageJ were then placed in Triplot. The Triplot software was used to graphically display the findings, and acted as the foundation for a visual categorization of RGB findings.

Image J is a free, open source image processing software with a multitude of applications. For the purpose of this study it was used to derive RGB values, which is a simple process (pictured in figure 11): Once an image is opened, RGB values can be derived by accessing the “Plugins” scroll-down menu, hovering over the subcategory of “Analyze”, and selecting “Measure RGB” (Rasband, 2002). This data was taken and input into Triplot for visual comparison and analysis.

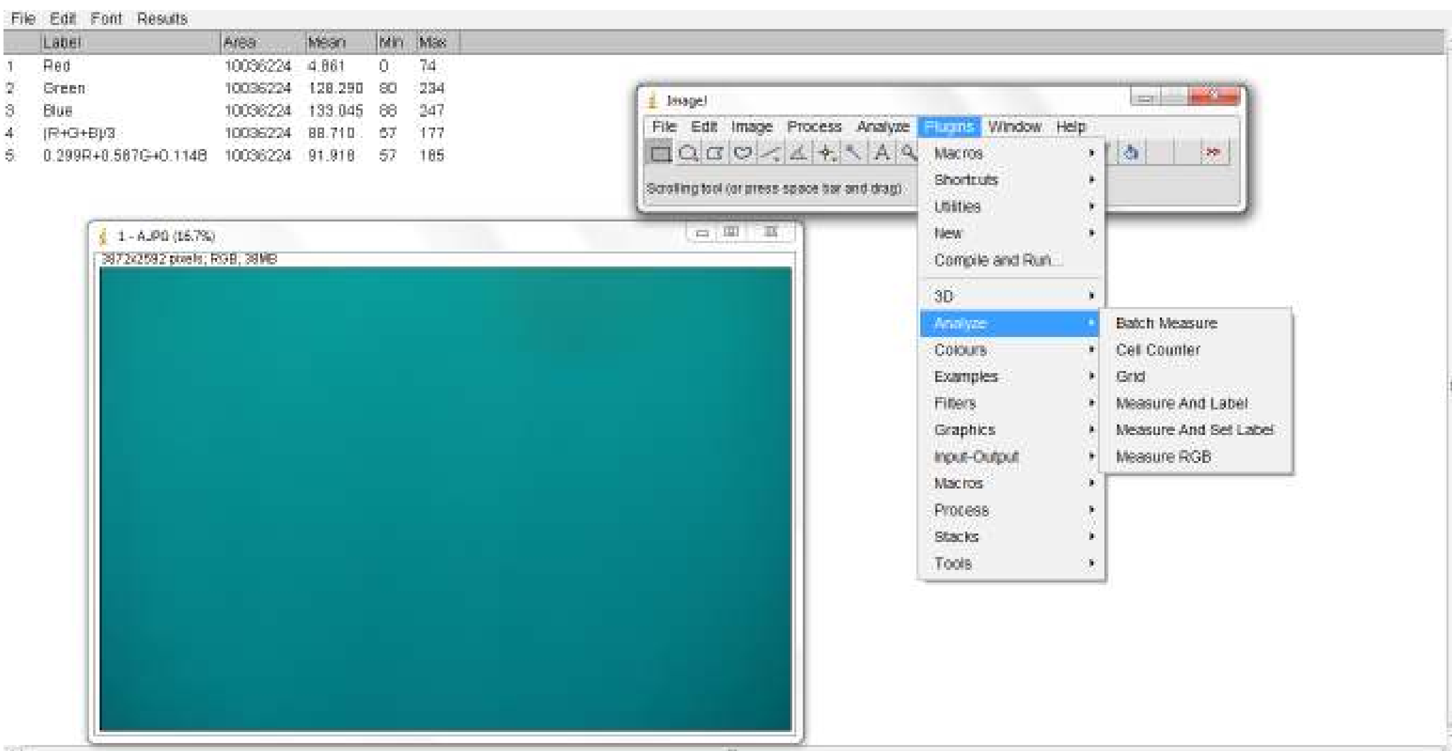
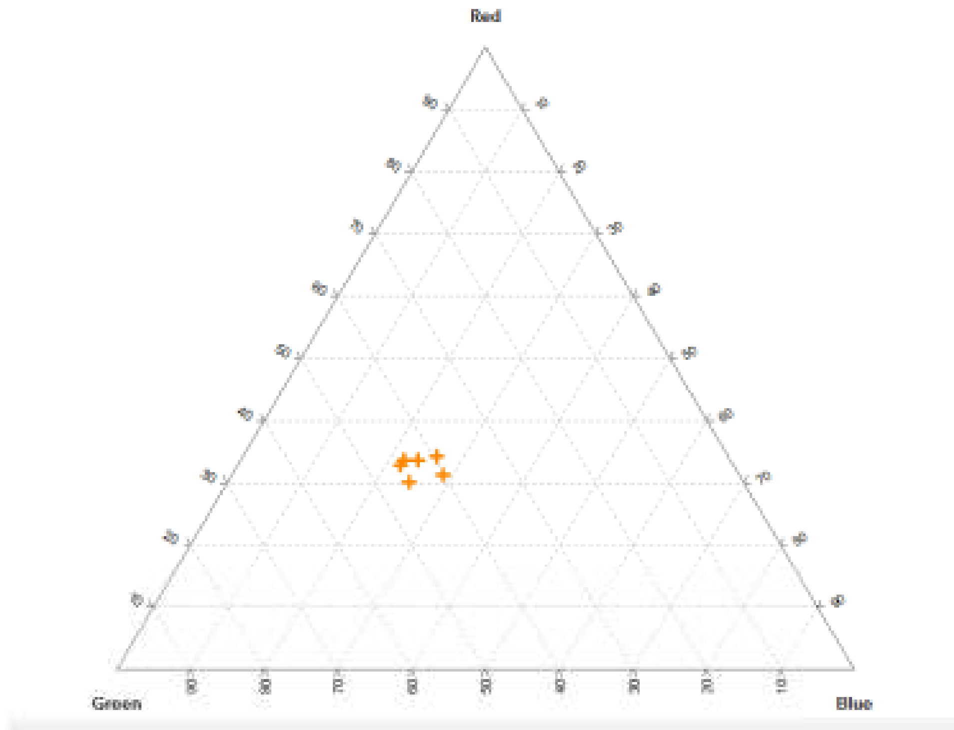


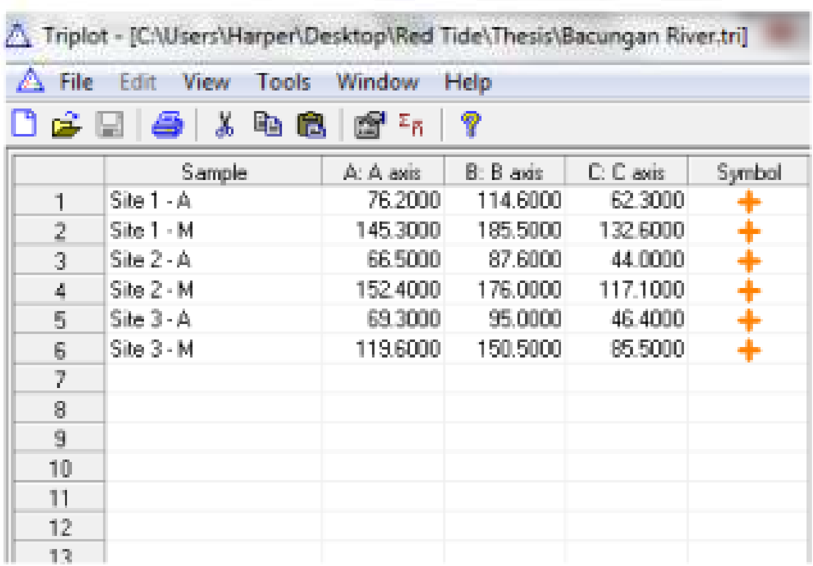
Figure 11. Outline of ImageJ being used to extract RGB values from a digital image. The image being examined is pictured in the bottom left corner, and the RGB values, as well as brightness, displayed in the top-left. The top right displays the process required to derive the RGB values: Plugins – Analyze – Measure RGB (Rasband, 2002) .

Triplot is a free trilinear-diagram plotting software, which serves well as a means for visually displaying the RGB data derived from digital images (Thompson, 2008). The A axis, the top point on the diagram, represents red pixels, the B axis to the bottom-left represents green, and the C axis to the bottom-right represents blue. The points each represent one image, and situate themselves according to which colours were predominant in its composition. Each axis ranges from 0-100, representing what percent of the total RGB was comprised of each colour. If the RGB classification system proves fruitful and further referential data can be compiled, the triplot graph can be subdivided and organized in such a fashion that each point's position on the graph gives specific information regarding the water's quality and contents. At present, the red, green, and blue corners of the graph can be understood to represent the primary substances which contribute to their respective colour in the oceans: Suspended sediment for red, algae for green, and a lack of substances for blue. In order to establish the expected position of a point for a given body of water, and further, to determine what degree of change is significant for each colour, it is necessary first to develop a baseline. For example, the highest concentration of red pixels managed to just exceed 40 on the triplot graph, whereas nearly all images exceeded 40 in respect to green pixels, topping out at 60. What this tells us is that what would be considered a significant presence of red pixels is different from a significant presence with respect to green pixels. This was a primary focus of the study. As a result, the points on figure 12, taken from Bacungan river, indicate significant presence of suspended sediment

a)



b)



	Sample	A: A axis	B: B axis	C: C axis	Symbol
1	Site 1 - A	76.2000	114.6000	62.3000	+
2	Site 1 - M	145.3000	185.5000	132.6000	+
3	Site 2 - A	66.5000	87.6000	44.0000	+
4	Site 2 - M	152.4000	176.0000	117.1000	+
5	Site 3 - A	69.3000	95.0000	46.4000	+
6	Site 3 - M	119.6000	150.5000	85.5000	+
7					
8					
9					
10					
11					
12					
13					

Figure 12. a) An example of RGB values plotted on Triplot (sample taken from Bacungan River). b) Triplot's data input menu (Thompson, 2008).

3.6 – Identification of Pyrodinium bahamense

In order to correlate RGB readings with the actual density of *P. bahamense* present, a number of monitoring approaches were considered. Access to materials proved problematic, as reliable tools such as a chlorophyll-a probe could not be attained. Though ultimately no effective identification method was established for *P. bahamense*, available resources were applied in an attempt to do so. During a September 15th survey of Honda Bay, 13 water samples were collected manually, by diving, from a depth of approximately 3m. The samples were kept in small glass vials, which were kept in an icebox in an attempt to preserve them. On this day, visual sightings of a reddish, chain-forming dinoflagellate were observed in the bay. Unfortunately, access to light microscopes was restricted, and thus the samples could not be examined fresh. Ultimately, despite attempts to preserve the samples, the organisms within had decomposed over the 2 days between extraction and examination. Attempts to establish partnerships between local government authorities – the Bureau of Fisheries and Aquatic Resource and the Provinicial Agriculturalist's Office – were beneficial, but did not lead to a method for monitoring *P. bahamense* densities. In the end, all that could be ascertained with confidence, through government surveys, is the sustained presence of *P. bahamense* in Honda Bay.

3.7 – Environmental parameter analysis

The additional environmental parameters gathered were filtered into one of two categories, depending on whether or not they fit the ideal growth requirement of *P. bahamense*. Light intensity readings, gathered in Lux, were converted into micromoles per square meter per second, in order to compare them with a previous study on cultured *P. bahamense* growth (Thimijan & Heins, 1982; Usup, et al. 1994). The growth conditions selected were rather conservative, excluding favourable growth conditions and including only those that are ideal. These conditions were restricted to temperatures between 28-30ºCe, a salinity range of greater than 30 o/oo, and a light intensity of greater than 3500 lux.

3.8 Photometer vs RGB comparison

At each site, a photometer was used, both above, and 1.5m below water, to gather reliable readings of light intensity. This information was cross-referenced with the RGB values captured in each digital image, in order to determine whether an increase in light intensity, according to the photometer's readings, correlated with an increase in overall RGB brightness.

3.9 Camera angle test

The final consideration taken into account was an attempt to address a question of reliability: Would the results be consistent if the camera was tilted at different angles? This was essentially the only factor varying from one station to the next, due to wave action and human error. On the final day of sampling, at Puerto Bay, two manual photos were taken at each station from significantly differently angles. The RGB results from each station, along with overall brightness, were compared in order to ascertain how important it was for the camera to be held at a fixed angle.

4. Results

4.1 RGB values / Secchi disk depth

A cumulative plotting of all RGB values derived from the 6 different research sites (Figure 13) shows a tendency for points to cluster in a general area, producing a unique RGB signature for each body of water. The relative consistency of RGB values – despite some measurements being taken during rainy season – indicate a certain consistency in the water's contents. All sites featured a tendancy towards green pixels as the dominant colour, which focused in the 50-65% range when plotted. This is consistent with Ocean Colour's statement that algae is the substance most responsible for waters' colour (IOCCG, 2000). The most variable colour was red, which was distributed fairly evenly between 0-40%. This indicates that the presence of suspended solids varies widely depending on the body of water. Blue pixels, indicative of a lack of substances, occured primarily between 20-45%.

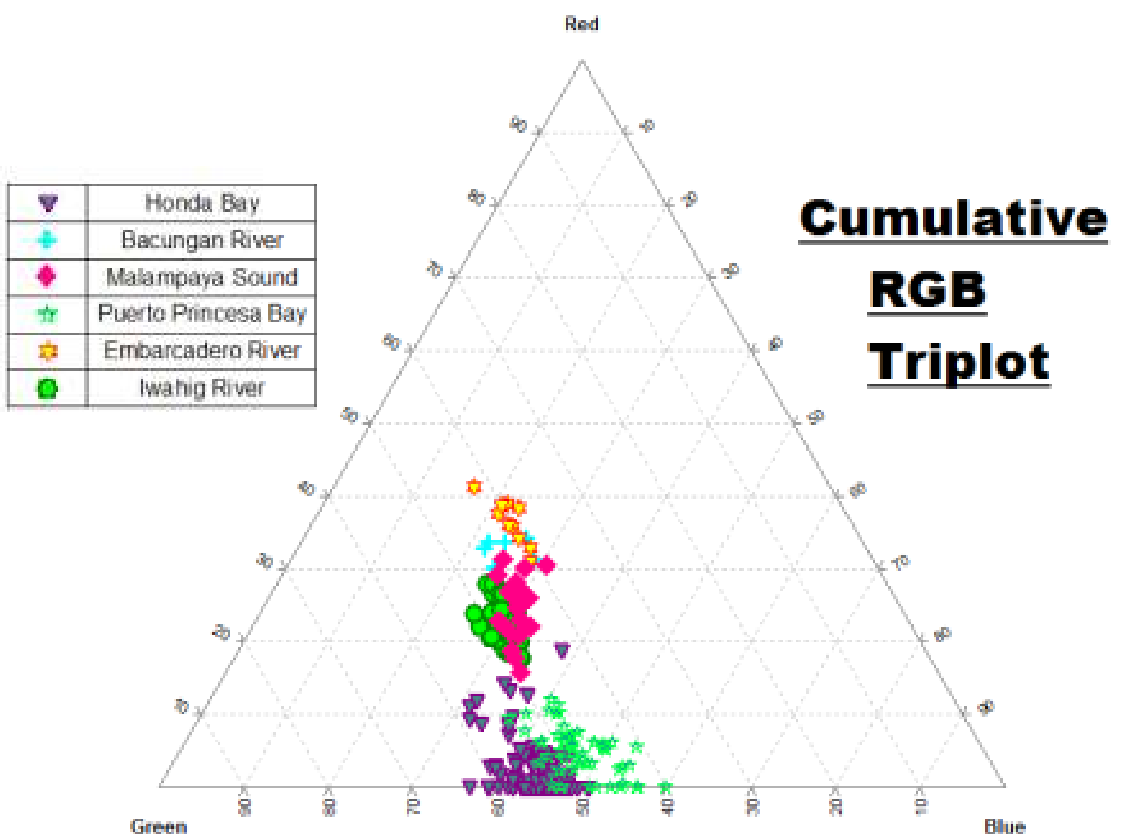


Figure 13. Summative plotting of the RGB values derived from all images.

The images taken in the Embarcadero river feature the highest total red values – exceeding 40% - indicating a large presence of suspended sediment, as would be expected in a river. In general, the rivers are shown to have higher red values than the bays, with the exception of Malampaya Sound, which had comparable red pixel values to the Iwahig river. This would suggest that Malampaya Sound has a higher standing sediment presence than Honda and Puerto Princesa Bay. Puerto Princesa Bay displayed the lowest green (less than 50%, predominantly) and the highest blue totals (up to 60%). An increase in either green or red pixels is expected to correspond with a decrease in blue pixels, unless overall brightness increases, and vice versa. The increase in blue pixels indicates a lack of substances – in essence, there is nothing to reflect and colour the sunlight, so it appears blue. Accordingly, coupled with low levels of red pixels, the results suggest that Puerto Princesa Bay is the body of water most free of substances. Applying the same principles, Honda Bay appears to be the body of water with the most photosynthetic activity, due to the number of green pixels (up to 60%). It also lacks in red pixels (0-10%, predominantly), indicating a low presence of the suspended sediment which could impede the photosynthetic process. The waters of Malampaya Sound feature lower blue pixels (30-40%) than either of the two bays, but display similar green pixels as Puerto Princesa Bay (40-50%). The aforementioned increase in red pixels accounts for the resulting lack of blue. This indicates that Malampaya Sound is the most optically complex of the three water bodies, and thus, contains the greatest variety of substances. This is supported by the relatively shallow depth at which the secchi disk disappears, at an average of 2.1 metres.

Interestingly, the Iwahig river displays a very similar RGB signature to the Malampaya Sound region. The Iwahig river features a lower number of red pixels (15-30%) compared to the other two rivers, indicating a lower presence of suspended solids. This could provide a clue as to the reason for less photosynthetic activity in Puerto Princesa Bay when compared to Honda Bay – the Iwahig river may provide less nutrients than the Bacungan. The Bacungan and Embarcadero rivers feature rather similar RGB signatures. Unsurprisingly, the body of water with the highest number of red pixels is fed by the river with the highest number of red pixels. Further, the level of green pixels in all rivers are relatively high (40-50%), and similar to Malampaya Sound and Puerto Princesa Bay. This indicates, when coupled with the high presence of red pixels, that all of the rivers contain a good deal of algae as well as suspended sediment, and are rather complex bodies of water.

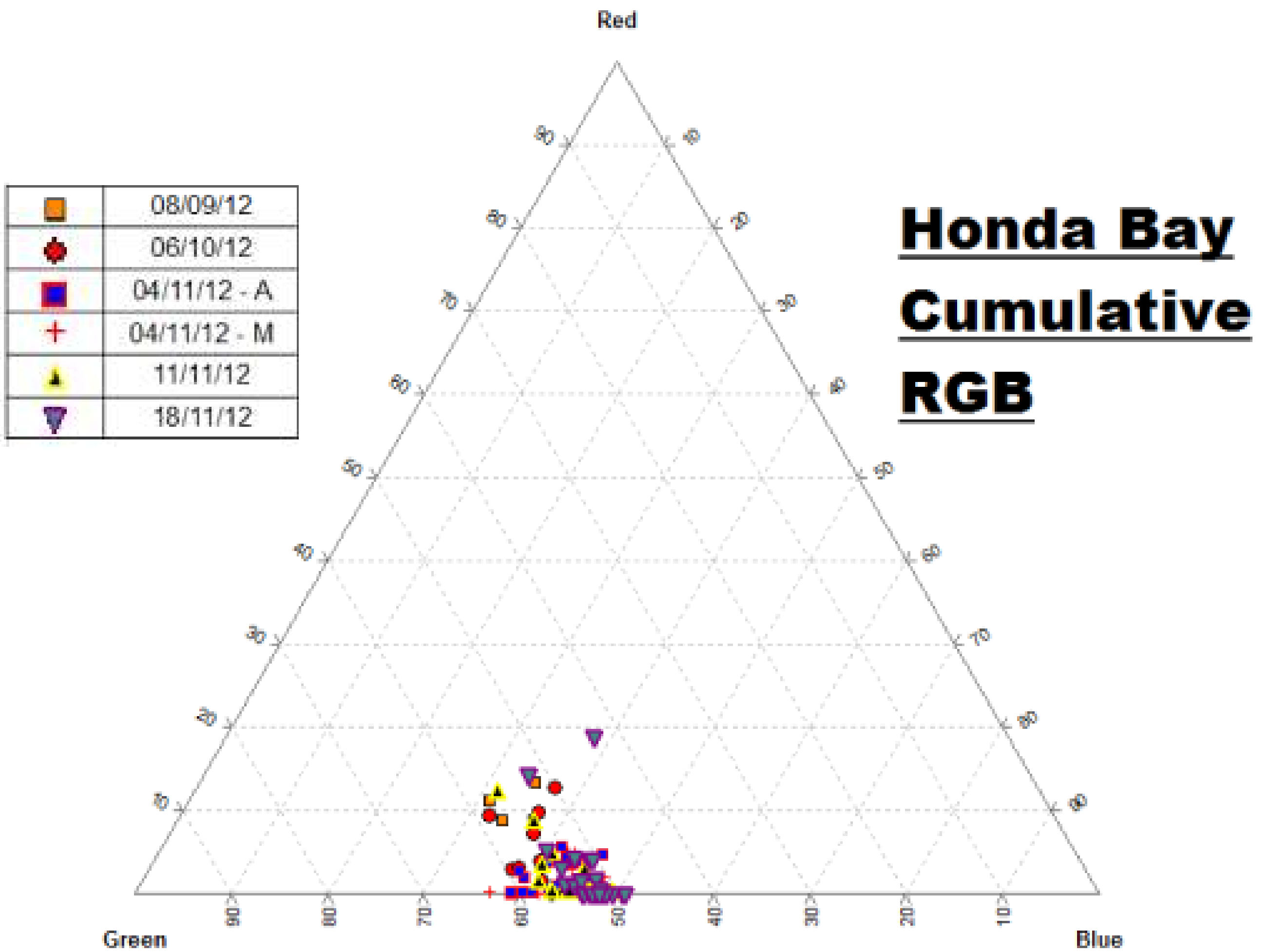


Figure 14. A cumulative plotting of all RGB values derived from images in the Honda Bay region. Each point represents one image.

Of note in the Honda Bay region is a heavy clustering of points lacking in red pixels, offset by several outliers from a number of different dates. These points can be explained in two ways:

1.) Influence of weather conditions; 2.) Distance from a river. They do not reflect an inconsistency in the RGB signature of the bay so much as an inconsistency in sampling and an organizational failure. In the case of the orange squares and red circles, these samples were collected on days where storm conditions forced an end to the study. Thus, it is likely that the increased wave action before the storm was responsible for the increase in red pixels, due to the stirring up of sediments. In the case of the yellow and purple triangles, samples were taken near the mouth of the Bacungan river but were not organized as distinct from the other stations. They did not fit into either the Bacungan river sample either, and thus can be considered outliers. For these reasons, it is estimated that, were further data to be gathered and organized appropriately, a more consistent RGB signature would emerge.

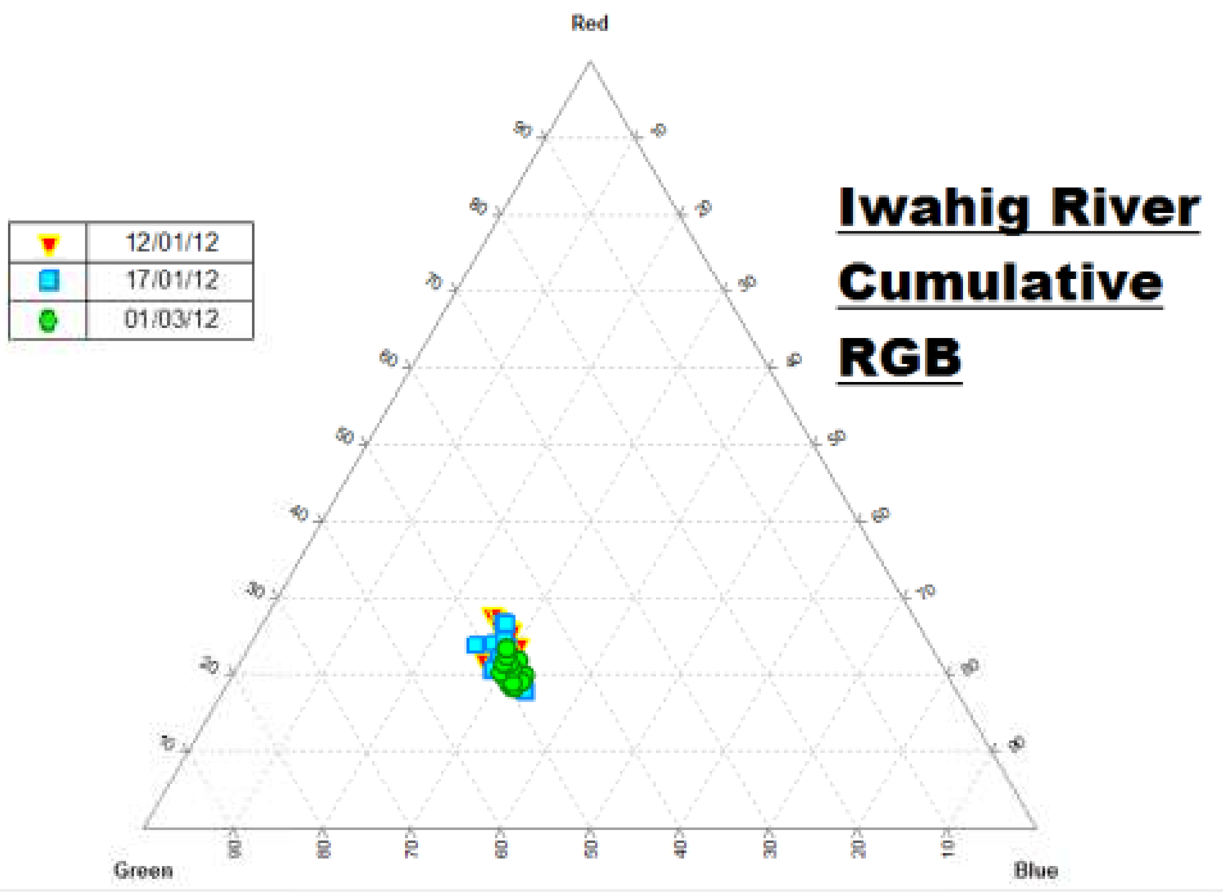


Figure 15. A cumulative plotting of all RGB values derived from images taken in the Iwahig River. Each point represents one image.

The results from the Iwahig river display a very consistent colour balance. Three dates were examined, with very little deviation. Green was the dominant colour, contributing 45-50% of the overall mix. Red values were the lowest, between 20-30%, and blue contributed 25-35%.

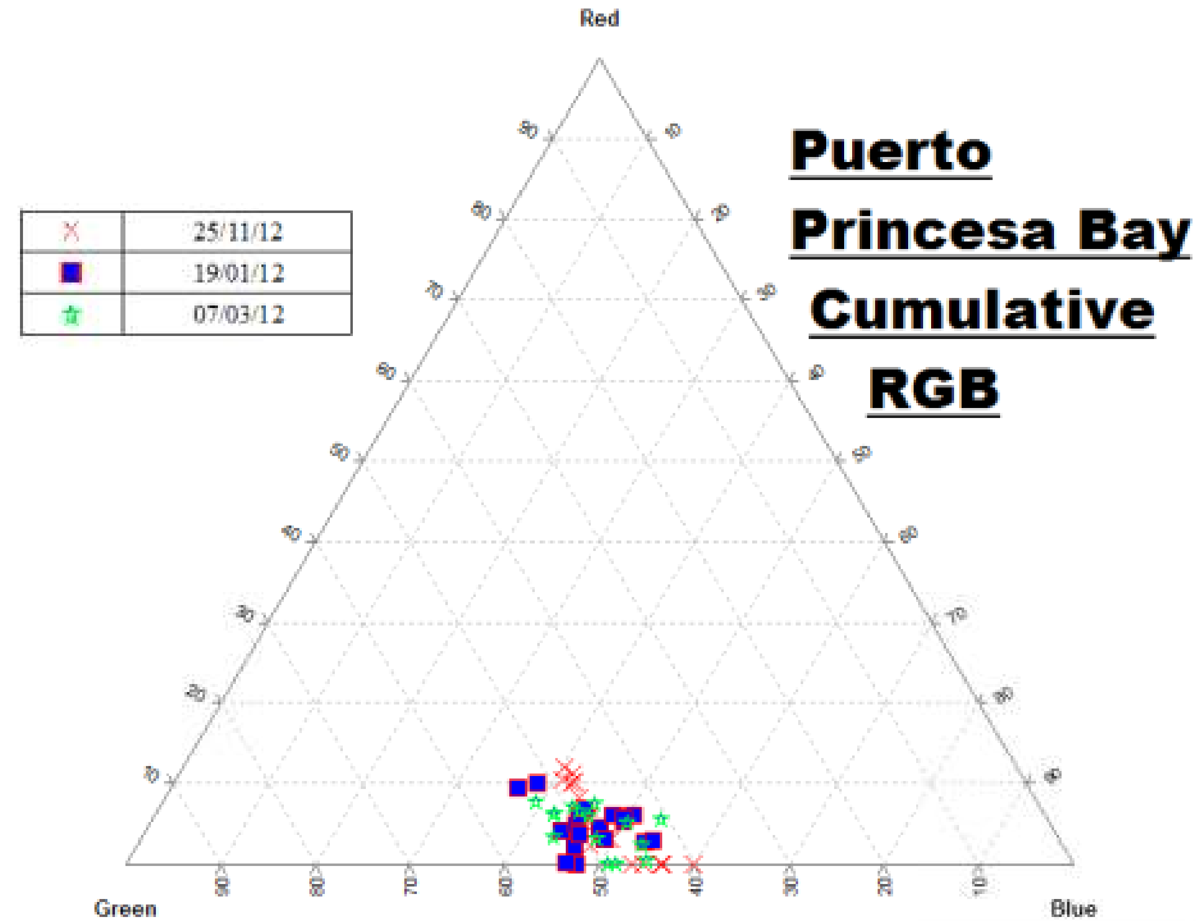


Figure 16. A cumulative plotting of all RGB values derived from images in the Puerto Princesa Bay region. Each point represents one image.

As with Honda Bay, the results from Puerto Princesa Bay appear less consistent than they should due to a failure to organize the bay. Red values increased considerably with vicinity to the Iwahig river, which is not accounted for in the results. Despite featuring lower green values than any other site, the colour's contribution yet ranges from 40-50% of the overall balance. Blue pixels are the most responsible for colouring in Puerto Princesa Bay, ranging fairly evenly between 40-60%. Red pixels are clustered between 0-10%.

Finally, the secchi disk results offer some insight into the depth at which a digital image may gather information. Anything beyond this range would not affect the RGB balance, and thus, would not be monitored.

Table 1. An average of the secchi disk depth for each site studied. Disappear decribes the depth at which the secchi disk is no longer visible, and appear when it returns.

Secchi Disk Depth

Location	Average Disappear	Average Appear
Honda Bay	7.7m	7.4m
Puerto Princesa Bay	12.6m	12m
Malampaya Sound	2.1m	1.7m
Embarcadero River	1m	0.7m
Bacungan River	N/A – Too shallow	N/A – Too shallow
Iwahig River	N/A	N/A

What can be noted is a correlation between the number of blue pixels and the secchi disk depth, confirming the aforementioned notion that the number of blue pixels is representative of water clarity, and thus, a lack of substances. The more blue pixels, the lower the depth in which the secchi disk is visible.

4.2 Automatic vs manual exposure

A comparison of manual and automatic exposures, taken from 74 stations, revealed only slight deviations in regards to colour balance results. What was noted was a slight tendency for automatic exposure to fluctuate less than manual exposures in terms of overall brightness. This was expected, and it can be inferred that the camera automatically reduced the brightness for a more balanced image. No colour showed a signficant imbalance, and thus both exposures seem equally reliable in terms of RGB analysis. In other words: Though overall brightness was quite different, the brightness of colours changed in proportion to one another in both exposures, so that the overall balance was quite similar and consistent. The range of brightness for pixels is between 0-255. In terms of overall brightness, the automatic exposure exceeded 100 at only one station, by one pixel. Manual exposure, on the other hand, exceeded 100 at 22 stations by a sizable margin.

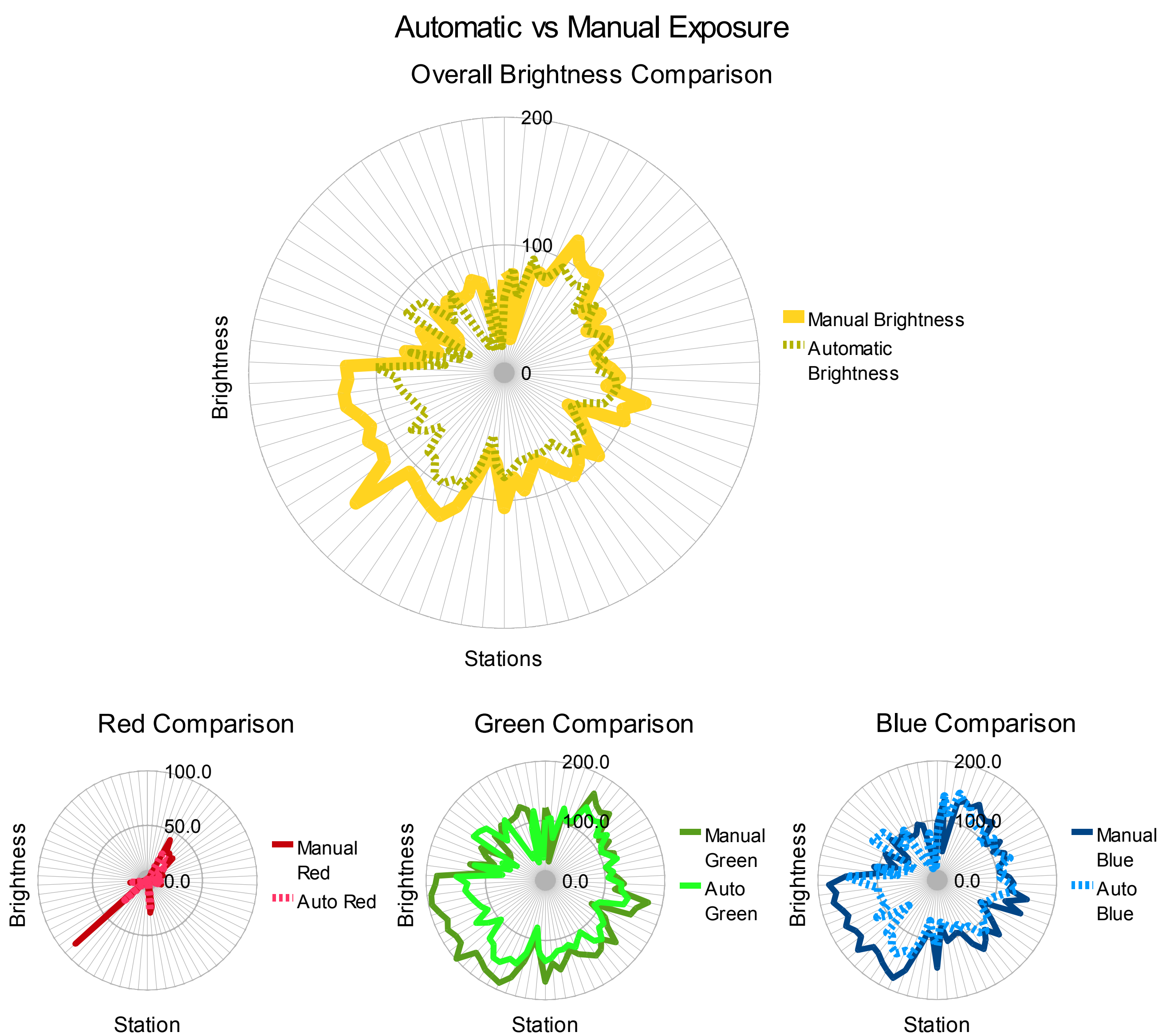


Figure 17. A comparison of the RGB results of two images gathered at the same station, but with two different exposure settings. Automatic exposures featured fixed white balance and ISO values, but the aperture and shutter speed varied. Manual photos were set at a shutter speed of 1/100, and with aperture fully open – f/4.8. The white balance was set for direct sunlight, and the ISO at 100 in both cases.

Utilizing figure 17 as a visual example, the limitations and value of automatic exposures are on display. Noting the shape of the pattern that emerges in green and blue values, the synchronicity between the two colours is clear, thus producing a reliable RGB balance. On the other hand, the tendency towards a more moderate brightness can be noted in contrast to the results from the manual exposure.

4.3 Environmental parameters

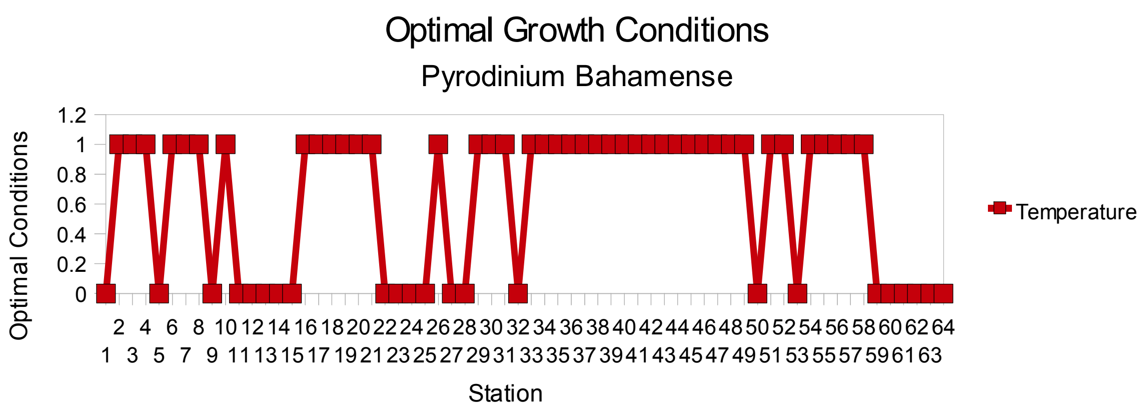
The environmental parameters examined proved to predominantly favour *P. bahamense* growth. In regards to light intensity, at a depth of 1.5m, all stations examined were within the optimal growth requirements, and the same can be said of surface salinity with only one exception. Temperature was the only parameter which was not consistently within the optimal range, with 23 out of a total of 64 stations proving to be too warm. With this said, the results were interpreted quite literally, and of these 23 sites, 22 were exactly 31°Ce. Thus, the difference may be too small to dramatically impact the growth of *P. bahamense,* particularly considering their ability to vertically migrate. Further, measurements were taken at the surface, and it is possible that temperatures would fall to within the optimal range within a metre or two. Thus, the standing conditions in both Honda Bay and Puerto Princesa Bay, in respect to the three parameters monitored, appear to fall within the optimal growth range for *P. bahamens*e.

Table 2. An average of the salinity, temperature, and light intensity readings taken in Honda Bay and Puerto Princesa Bay, as well as the optimal conditions for both growth and toxin production for *P. bahamense.*

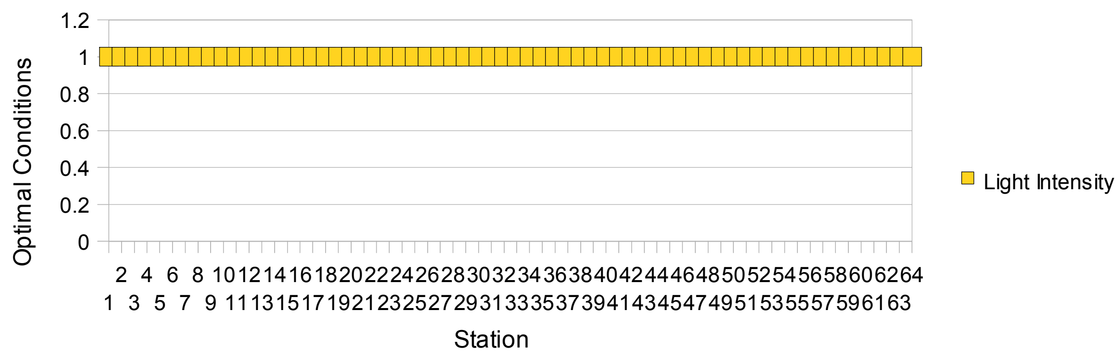
Monitored Conditions			
Location	Salinity	Temperature	Light Intensity
Honda Bay	34.9 o/oo	30.4°Ce	32,710 lux
Puerto Princesa Bay	32 o/oo	30.4°Ce	42,250 lux

Optimal Conditions		
Salinity	Growth = 30o/oo <	Toxin Production = 20-24 o/oo
Temperature	= 28-30°Ce	= 22-24°Ce
Light Intensity	= 3,530 lux	= 3,530 lux

a)



b)



c)

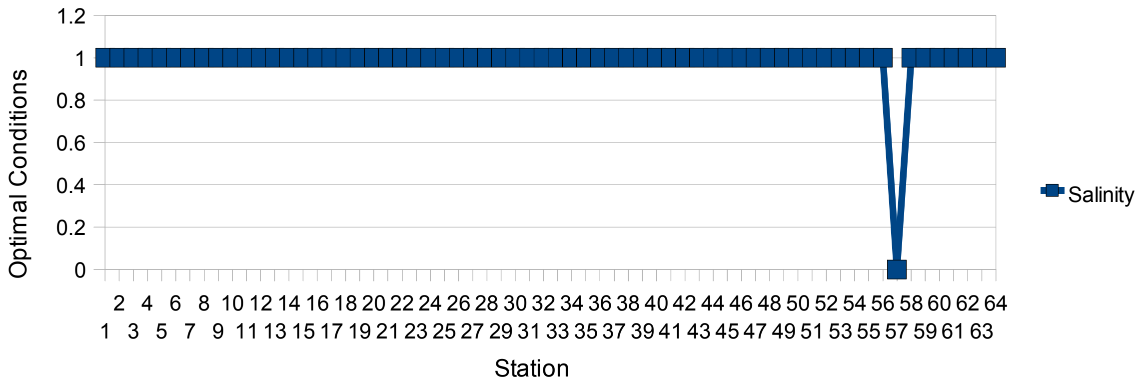


Figure 18. The cumulative results of an examination of environmental conditions – spanning two months - and whether or not they satisfy the optimal growth requirements of *P. bahamense*. 0 indicates unfavourable conditions, and 1 represents optimal conditions. a) Temperature; b) Light intensity; c) Salinity

In regards to optimal conditions for toxin development, only light intensity was consistently within the favourable range. In this case, the results were quite uniform – light intensity was always within the optimal range, whereas salinity and temperature were never within the optimal range.

4.4 Photometer vs RGB comparison

The coefficient of determination in the linear regression analysis was 0.01 (Figure 19), indicating a very weak relationship between RGB brightness and the photometer light intensity readings. In general, the RGB readings displayed little consistency in respect to their connection with the photometer's results, or what we can understand in this case as an accurate indication of actual light intensity. Photometer readings were taken above and below water, to examine what impact the water itself was having on the readings. In general, changes in surface brightness were reflected in the underwater measurements (Figure 20), but a number of deviations were also present. Thus, we can gather that the water does have an impact on underwater brightness. The coefficient of determination in the linear regression analysis of submerged and surface photometer readings was 0.34 – a stronger relationship than between the submerged photometer and RGB, but rather weak overall. The discrepancy can be explained by fairly stable surface readings, generally clustering around 120,000 lux, offset by fluctuating submerged values. But, ultimately, the noted lack of consistency between surface and submerged readings does not account for the lack of a relationship between the digital camera results and those of the photometer.

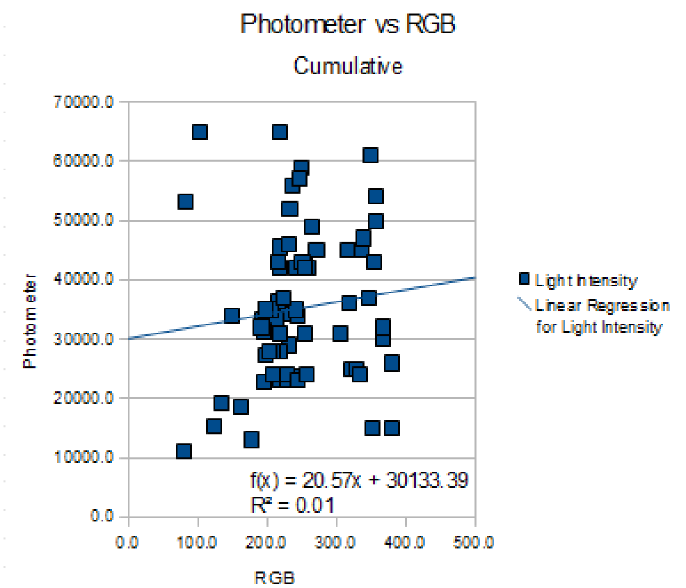


Figure 19: The cumulative data from 4 days, examining whether an increase in RGB brightness captured in a digital image correlates with the light intensity readings of a photometer

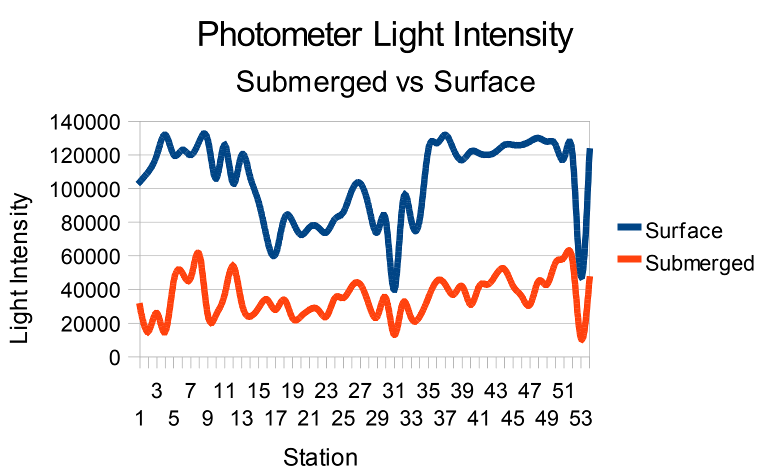


Figure 20. A comparison of photometer readings taken from a metre above the water's surface and 1.5 metres below.

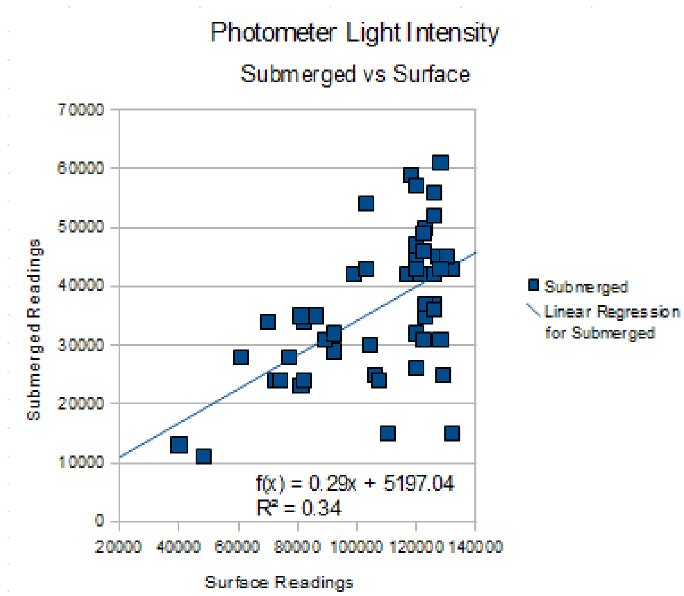


Figure 21. A scatterplot to demonstrate the linear regression of submerged vs. surface photometer readings.

4.5 Camera angle test

Comparing 2 sets of 20 images, with each pair featuring images captured at significantly different angles, the overall RGB values proved to be quite similar overall. Mild deviations occur throughout, but primarily they were not drastic enough to suggest camera angle has a severe impact on the outcome, and thus, the overall reliability of the data captured. Further, due to the relative consistency of the overall results, the deviations observed may be the result of changing light conditions rather than camera angle. Between stations 12 and 17, where the greatest discrepancies were observed, the average difference between image A and image B's overall brightness was 3%. Further, the colours shift in proportion to one another, which is to say that differences in angle did not favour one colour over another. With this said, the sample size was small, and the discrepancies observed do seem significant enough to suggest that consistency in camera angle would benefit future studies. Further, substances may differ with depth, another reason consistency is important despite the results.

Table 3. The RGB values from those stations featuring the most significant differences between each pair of pictures.

Camera Angle Test

Station	Red A	Red B	Green A	Green B	Blue A	Blue B	Brightness A	Brightness B
4	15.2	8.1	124	109.4	117.6	105.5	85.5	74.3
7	15.9	13.3	132.9	123.5	126.2	121.8	91.6	86.2
12	33.7	29.1	156.1	154.7	138	134.1	109.1	105.9
13	37.4	31.8	158.8	146.4	138.9	127.8	111.7	101.9
14	32.4	30.9	156.1	152.4	135.8	131.9	108.1	105.1
15	33.4	42.4	155.3	167.1	130.8	145.1	106.6	118.3
16	0	0	129.8	104.8	155.8	132.2	95.2	79
17	0	0	112.1	108.2	145.1	140.5	85.7	82.9

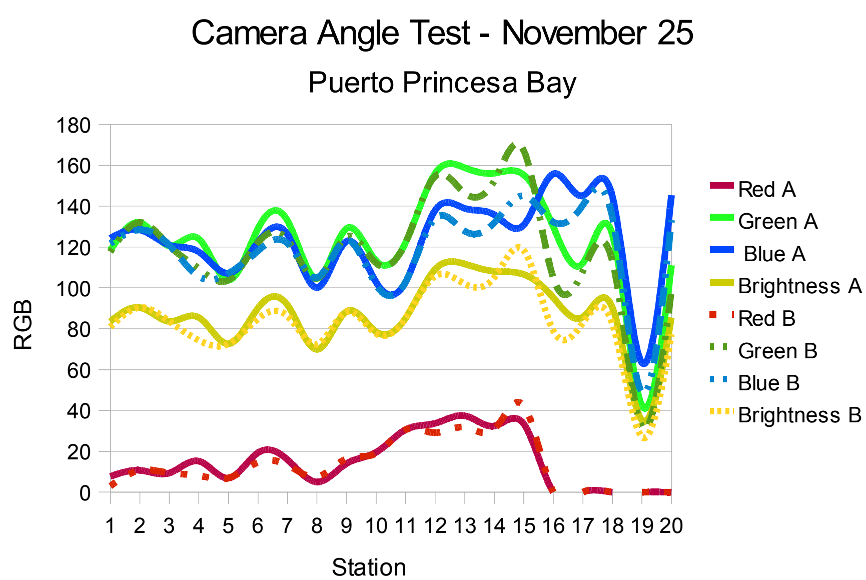


Figure 22. A comparison of the RGB values of 2 sets of 20 images, taken at the same time but from different angles

5. Discussion

5.1 RGB results

The results obtained by deriving RGB values from digital images can be interpreted in a number of ways. The first thing to note is that in all of the locations where multiple studies were conducted, a pattern emerged. On average, green pixels were the highest, suggesting that, in general, algae are the predominant colouring agent. This was true for both rivers and bays, with surprisingly uniform results in respect to green pixels. All the water bodies featured near-idential green values (40-50%), with the exception of Honda Bay's, which were higher (50-60%). This can be interpreted one of two ways: 1) Algae are quite prominent in the areas studied, or 2) Their reflective tendencies are strong. As algae are absorbent as well as reflective, it is more likely that the green pixels are an underrepresentation of actual algal presence. In the same vein, the results for red pixels, due to the highly reflective nature of suspended sediment, are likley an overrepresenation of their presence within the waters.

The following serves as a speculative application of how the information derived from the RGB readings can be applied. This information cannot be confirmed, due to the inability to procure the equipment necessary to gather reliable data on water's contents, but gives an idea for future RGB analysis. What the RGB results suggest is that Honda Bay is the most photosynthetically active body of water, due to high levels of green pixels, which, despite the absence of *P. bahamense*, may serve as an indication that it is the site most likely to support a bloom. If the results were to be analyzed in isolation, without any awareness of past HAB events in the area, the RGB values may serve as reliable clues as to which regions are prone to a red tide. As Puerto Princesa Bay appears to have the lowest photosynthetic activity, due to a lack of green pixels, we may be able to infer that it is not a likely location for algal development. This could be due to a lack of nutrient input, which is supported by the lower levels of red pixels in the Iwahig River when compared with the Bacungan and Embarcadero.

This is of course an oversimplification, as there are a number of factors which could explain the lack of an algal presence, including transport mechanisms, but it does begin to explore how results from *in situ* optical monitoring can be applied. Much more study must be done before this can be utilized with reliability, but it does offer guidelines for monitoring in regions where resources are limited. Further, the waters of Malampaya Sound also appear to feature lower levels of photosynthesis compared to Honda Bay. This may be due to the high levels of suspended sediment, as indicated by the red pixels. In essence, it suffers from the opposite problem of Puerto Princesa Bay, in that river input impedes algal development. Realistically, the optical complexity and lack of clarity witnessed in Malampaya Sound is likely more indicative of passing weather conditions than a stable state.

One element of particular note for the application of RGB values to the monitoring of *P. bahamense* is the stability of the water bodies in respect to their red pixel totals. As this colour is the one which would indicate their presence, it is rather important that it does not fluctuate too much. In the case of Malampaya Sound, one day of monitoring produced a wide range of fluctuation, as red pixels ranged from 15-30% of the total RGB values. Comparing these values to the locations of each site, as marked by a GPS, does not provide any consistency in respect to what causes the change – for example, proximity to the river mouth does not seem to be the primary factor. Thus, though Malampaya Sound's results are limited by the lack of a follow-up study, the seemingly random changes in red pixel concentration appears problematic for the application of *in situ* optical monitoring for *P. bahamense*. A number of factors could explain the fluctuations in red pixels in this site, including the presence of *P. bahamense* themselves. Accordingly, the results are inconclusive, but suggest that the system may not work for all locations.

Honda Bay, on the other hand, featured quite stable red pixel values. Though some outliers were present, they were few, and are considered research error rather than changes in the physical environment. One issue that is introduced in the case of Honda Bay is that red pixels increase with proximity to a river. Accordingly, going forward it will be necessary to be more thorough in organizing the research sites, so that each may be analyzed seperately. It is expected that a certain consistency should arise, where near-river sites feature higher, but relatively constant red pixel values in constrast to other sites. It is expected that red pixels will rise with proximity to rivers in most cases, and thus this is a principle that should be applied generally. Aside from the few outliers noted, the red pixel values in Honda Bay appeared stable enough that a threshold could be established for the monitoring of *P. bahamense*. In this case, based on limited results, an appropriate threshold appears to be 5%: If the presence of red pixels in the overall RGB balance exceeds this percentage, it is suspicious. Weather conditions must also be factored into this analysis. It is also worth noting that later studies, taken outside of rainy season, routinely found red pixel values of less than 1 on the scale of 0-255. If this continues to be the case, monitoring of *P. bahamense* should be even more effective, as even the slightest changes in water's red colour would be notable. This is encouraging, considering the remaining question of the density at which *P. bahamense* would be able to induce a notable change on ocean colour. If the red pixel values are routinely 0, a change may be easy to notice.

A question that must be considered in respect to RGB acting as an indicator of water quality, is how accurate colour indicators can be when considering density and depth. For example, a surplus of suspended sediment, which are very reflective, may tilt the RGB in favour of red pixels, which would indicate a lack of algae that may not actually be the case. Fundamentally, the substance closer to the surface, or more reflective, would appear more prominently in the camera's results, impairing the RGB values from accurately producing a complete picture of water contents. This could be the case with Malampaya Sound, as the secchi disk disappeared at an average of 2.1 metres. This means anything beyond that depth would not be monitored. It should be noted that in rivers, which feature the highest suspended solid densities, the RGB balance was not radically shifted. Thus, results appear to suggest that, despite being the most reflective substance, suspended sediments do not overwhelm the other optical constituents and create an overly unbalanced representation of water contents. Going forward, it may be possible to roughly calculate densities utilizing RGB values, combined with what we know of the reflective behaviour of the substances present.

5.2 Automatic vs Manual Exposure

Overall, manual and automatic exposures did not produce starkly different RGB values, but some deviations were found. When comparing the overall brightness of the automatic and manual images, the automatic images tend to be more moderate, fluctuating less than the manual brightness. Specifically, automatic exposures tended to be less bright than manual exposures. This is consistent with expectations: The automatic exposure appears to be compensating for increases in brightness, in order to preserve contrast within the photograph. For standard photography this is a useful mechanism, but in this case it impairs the results. It is primarily for this reason that the manual exposure appears to be the better of the two, as it does not interfere with the information gathered by the lens.

In general, this moderation of brightness did not affect the balance of the RGB pixels, and thus has little consequence in respect to colour. As a result, previous studies utilizing automatic exposure settings to monitor RGB values are not discredited in any way (Godijin & White, 2006; Godijin et. al, 2009; Feighery et. al, 2001). But, moving forward, it is difficult to conceive of a reason why automatic exposure settings should provide more reliable results than manual exposure settings, and so it is recommended that manual settings be utilized for future studies. In this way, less mechanical adjustments interfere with the camera's reading of raw optical data from the environment. A number of different manual settings were explored before arriving on one which appeared suitable for getting reliable colour readings in different extremes. Despite using manual settings in a variety of light conditions, utilizing the aforementioned settings proved moderate enough that images were never too dark or too bright. These settings are:

- White balance: Direct sunlight
- ISO: 100
- F-stop / Aperture: 4.8
- Shutter speed: 1/100

5.3 Ecophysiological Monitoring

In the case of *P. bahamense*, it was found that monitoring light intensity and salinity would provide little information for estimating the development of an algal bloom. Despite taking measurements in many different light conditions, the intensity consistently exceeded the minimum growth requirements substantially, and as a result, it seems likely that variations in light intensity are seldom a limiting factor for *P. bahamense* growth. Due to dinoflagellate's ability to migrate to ideal light conditions, it appears unlikely that they would ever be less than suitable in the areas studied. Studies of the species in Papua New Guinea found them at a variety of depths, from 0-9m (Maclean, 1977). What remains to be examined is the point at which *P. bahamense* suffer from photoinhibition, but it appears likely that the species would favour a lower intensity than found at 1.5m in the clear waters surrounding Palawan. This could yet prove to be a reliable mechanism for monitoring the species, depending on their ability to photoacclimate and adjust their depth. Another pivotal piece of information which may yet be gathered from the monitoring of light intensity is an estimate on the extent to which toxins will be produced. *P. bahamense* shift from the production of chlorophyll to the production of toxins as light levels increase (Usup et. al, 1994). Again, in this case the light levels far exceeded those which are ideal for toxin production, and it is likely that density is far more important, but this is an angle that remains to be explored, and could be a factor for other species.

Results for temperature on the other hand suggest that this parameter may be worth monitoring. It is vital to note that *P. bahamense* are in fact able to grow beyond 30°Ce, but are morphologically altered by the higher temperature. While the degree to which these morphological changes would impair their ability to form a bloom is unknown, it could prove to be the one environmental parameter that could provide some estimation regarding *P. bahamense* development. In order to explore this concept further, it would be useful to compile data on temperature alongside the occurance of *P. bahamense* blooms. But, ultimately, due to their ability to relocate, and the fact that temperature readings were taken at the surface, where it is warmest, it is unlikely that any of the studied parameters would fall outside of the optimal range consistently enough to be a factor in *P. bahamense* development.

As alluded to earlier in respect to toxin production, though light intensity is yet within the optimal range, salinity and temperature were never favourable, and thus, examining this angle may indicate at which point a bloom will become harmful. A decrease from the optimal growth temperature of 28 to 22°Ce leads to a threefold increase in toxin production. The same can be said in respect to salinity, moving from 24 o/oo to 20 o/oo (Usup et. al, 1994). The impact of temperature and salinity on toxin production is quite drastic, and thus, it is worth speculating how much of a role it plays in fish kill episodes and the contamination of shellfish. In short, would a *P. bahamens*e bloom be toxic enough to cause harm if these conditions are absent?

What can be gathered from this is that conditions are generally favourable for growth of *P. bahamense*, and thus some other factor is likely limiting the formation of a bloom. This could indicate a lack of nutrient input, or perhaps cyst stages that are seasonal. Thus, the indirect benefit of monitoring these basic environmental parameters is that they will at least help in narrowing down an understanding of the specific circumstances which lead to a bloom in different places. As a number of ecological interactions underpin a HAB, varying based on species and location, it may be wise to continue to explore these basic environmental conditions for this reason.

Finally, while monitoring of light intensity did not prove to be a useful estimation tool for *P. bahamense*, this does not necessarily indicate similar results for other species. Dinoflagellates are known to be a shade species, and thus it is likely that other dinoflagellates will thrive under similar low-light conditions, but this does not necessarily apply to other phytoplankton (Falkowski & Owans, 1978).

Overall, for the intended purposes, the study of basic environmental parameters,coupled with an understanding of the ecophysiology of *P. bahamense*, has not served to be an effective method for estimating an algal bloom. What was not explored in this study is a weather-based estimation system. As a *P. bahamense* bloom was not encountered, it was not possible to make observations to this end. Further research should examine the impact of wind, which has been found to be equally as important as rainfall in *P. bahamense* studies conducted in Papua New Guinea (Maclean, 1977). Not only could weather patterns explain the mixing that provides nutrients, but they may account for changes in temperature and freshwater influx – two factors which could produce conditions favourable to toxin production.

5.4 Using RGB to Measure Light Intensity

While the results rather decisively indicate that a digital camera does not serve well as a crude photometer, the findings may serve to influence further refinement of *in situ* optical monitoring techniques. The discrepancy between brightness as captured by the digital camera versus the photometer is estimated to be the product of one examining upwelling light, and the other downwelling. Indeed, it is unsurprising that the results were quite different. The digital camera's image is created entirely by light which is reflected from the water and the substances within it – upwelling light. The photometer on the other hand, submerged under the water and facing upwards, gathers its readings from downwelling light coming directly from the sun.

The key is understanding that the substances in the water both reflect and absorb light, and thus the brightness of an image will vary depending on the contents of the water. For example, algae are quite absorbent and may lead to a brightness, based on RGB values, that appears lower than it actually is. At present, a lack of further examination inhibits an understanding of the degree to which this is the case, but it does suggest that a comparison of a digital image with photometer results may provide further information regarding what substances are present in a body of water. For example, suspended solids are quite reflective, whereas *P. bahamense* absorb light. Accordingly, an increase in suspended solids should be accompanied by a rise in overall RGB brightness. As both substances appear red, if a spike in red pixels is not accompanied by a significant increase in overall brightness, it may serve to indicate a red tide. Fundamentally, by constrasting changes in brightness as perceived by a photometer with the overall brightness of RGB values, it may be possible to deduce further information regarding substances within a body of water.

Further, the discrepancy between the submerged and surface photometer readings support this notion, and may provide further information. Though the photometer was indeed capturing downwelling light, it too was affected by the contents of the water. For this reason, submerged readings changed despite consistent light intensity at the surface. Functioning inversely, relative to the camera, suspended solids would lead to decreased light intensity readings with the photometer, as light would be reflected back to the surface before it could reach the device. In this way, contrasting surface with submerged photometer readings could serve as a reliable starting point for an examination of water contents based on light intensity and RGB brightness. If surface photometer readings remain constant between stations but submerged readings decrease, it can be deduced that something in the water is obstructing the light before it can reach the photometer. Taking this one step further, the submerged results can be compared with the RGB brightness, as well as the colour balance. If a rise in red pixels is observed, but not a significant increase in overall brightness, this may serve as an indication of *P. bahamense* . Further studies must be conducted to explore the feasibility of applying this approach.

5.5 Camera Angle

Overall, while the differences in RGB values were rather negligable, the observed discrepancies do suggest a certain consistency would best serve the overall reliability of the data. Due to the relatively crude waterproofing mechanisms utilized in this study (an aquarium held manually) , it was at times difficult to maintain a constant angle due to light and weather conditions. This is particularly true in respect to choppier waters.

The observed differences in RGB brightness due to angle can likely be accounted for in three ways. The first is that different depths of the water column feature varying densities of the substances responsible for its colour. For example, as light intensity varies, phytoplankton may situate themselves at the optimal depth for their photosynthetic requirements based on their chlorophyll levels. Further, the mixing of waters may also account for an uneven distribution of suspended solids or marine detritus. The second reason is the angle of the camera relative to the angle of the sun, as this will affect how light is reflected. Thirdly, how sharply the angle is tilted towards the surface, as brightness will increase the further the lens is tilted away from the depths. This should only make a difference if the angles are radically different from one station to the next. With these factors in mind, the most sensible approach is simply to position the camera straight down at all times, and ensure the boat is positioned in such a way that its shadow is not cast over the image. Overall, establishing a consistency in the method is advisable.

5.6 Feasibility of HAB early warning system

Summatively, due to a lack of *P. bahamense* activity during the period of study, it is difficult to form any firm impression on the effectiveness of using *in situ* digital camera monitoring as an early warning system. What can be ascertained with relative confidence is that *P. bahamense* will affect the colour balance to some extent – the only question which remains is how much, and at which phase of bloom development. The secchi disk results, coupled with the maximum depth at which *P. bahamense* have been observed, gives relative confidence that blooms will occur within a visible depth (Maclean, 1977). As the method is intended to function as an early warning system, it is vital that changes in the RGB balance be observed before the toxins have an affect on local fisheries or aquaculture. Thus, the feasibility of the method, applied in its simplest form, i.e. based primarily on RGB data, hinges on the stability of RGB values within waters, and the density at which *P. bahamens*e will shift the colour balance. More data is required in both respects, but in the case of Honda Bay it appears that RGB values may be stable enough for the method to work.

One aspect of *P. bahamens*e's ecophysiology which lends confidence to the system is their slow rate of division (Smayda, 1997). With the exception of a few outlying results, the red values were relatively constant in Honda Bay. These outliers were taken during stormy conditions, and thus the increase in red pixels is suspected to be the result of wave action. Noting the impact of storm conditions, it appears possible to establish a baseline for expected red pixels within the region. If this baseline is exceeded, the development of a red tide should be considered. Due to *P. bahamense*'s slow rate of division, another few days of follow-up study may be possible before the algal bloom has any negative impacts within the region. If a certain degree of consistency and reliability can be established with the method, this will allow for a taking of preliminary readings and a follow-up, with enough time remaining that authorities may be contacted to conduct a thorough study. In this way, the likelihood of discovering a bloom is increased, as the authorities - in this case, BFAR - only conduct monthly surveys. The slow growth rate is vital, as it should give enough time to verify preliminary findings.

The biggest issue which could impair the use of RGB values to detect red tide species is the presence of suspended solids, which reflect red wavelength light and thus tilt the RGB values in the same direction as red tide species. While data was gathered over a relatively short period of time, each body of water studied on numerous occassions displayed a unique RGB signature. Thus, a stable baseline was established. Suspended solids were seldom present in Honda Bay, and thus red values were more often than not around 0. The application of this methodology to other locations may depend on the relative stability of its waters. *In situ* optical monitoring in areas with fluctuating suspended solid concentrations was considered, and there may be ways to differentiate between suspended solids and dinoflagellates using only the captured images. As dinoflagellates and suspended solids have a different relationship with light in terms of reflectance and absorbance, the total intensity captured by an image, when compared with photometer readings, may indicate which substance is responsible for an increase in water's red values. Further, suspended solids are expected to increase overall brightness to a greater extent than *P. bahamense*, as they are not absorbant. This is an area that remains to be explored. It is expected that suspended solids will more often than not account for an increase in red pixels, and thus a means of differentiation must be utilized. Fortunately, water's turbidity has been shown to have a significant impact on dinoflagellate species in a number of ways which do not favour bloom development. The most apparent effect is the physical damage the suspended solids incur on the dinoflagellates, perhaps most notably resulting in the loss of flagellum or cellular disintegration (May, Koseff, Lucas, Cloern, & Schoellhamer, 2003). As dinoflagellates rely on their motility to obtain nutrients and move into areas with ideal light conditions, the loss of flagellum greatly decreases the likelihood of a bloom. The loss of flagellum also leads to decreased aggregation and photoaxis (May et. al, 2003). With the impairment of all these functions pivotal to bloom development, we may gather that when high concentrations of suspended solids are present, the risk of an algal bloom decreases considerably. Thus, a noticeable increase in red content can either be explained as suspended solids or *P. bahamense*, but likely not a combination of the two.

In summary, it is inadvisable that the method be considered reliable at present, but the results are encouraging enough to suggest further studies be conducted. Further, as long as the unproven nature of the method is ackowledged, there is no reason why it should not be applied in areas where no other early warning system is available. The people responsible for conducting the monitoring would either be coastal managers, or fishermen. The intention had been to utilize labour forces that are already on the water, as they would be in the best position to detect a bloom. Rewards could be offered as incentive. Furthermore, aquaculture sites would have the option to protect their harvests by conducting daily tests. The amount of time and effort required to apply the methodology would be minimal, and the results could contribute to the refinement of the system.

5.7 Recommendations

Going forward, the results of this study provide a number of points from which to branch off and explore further. In respect to establishing the reliability of water-body classification based on RGB data, the most immediate follow-up study should involve a similar survey of water bodies, coupled with an appropriate probe to reliably gather information on water's contents for comparison. This should include readings of chlorophyll-a to establish algal densities, as well as turbidity to examine suspended solids. If RGB readings can be examined alongside an accurate reading of water contents, it will be possible to understand exactly what the colours tell us. In this way, rather than gathering a rough estimate of algal presence, it will be possible to discern just what density of algae is necessary for the 50-65% colour dominance observed. Furthermore, more bodies of water are needed to understand what the colours really tell us of the six that were examined, i.e. whether their colours are typical or not. Building on this concept, it may eventually be possible to classify bodies of water based on where they are situated on the Triplot graph. Most importantly, as discussed earlier, the primary uncertainty in respect to utilizing RGB for water quality monitoring is that some substances are more reflective than others, and depth and density may create a bias in results. The extent to which this is an issue should be explored. Particularly in waters with very low visibility, water contents should be examined below secchi disk depth to examine if there is a discrepancy between what the image indicates versus actual water contents . What this would tell us, fundamentally, is whether or not RGB values are more often than not indicative of overall water quality, or whether conditions vary with depth to the extent that only the secchi disk range can be monitored. Further, it must be examined how much the reflective nature of suspended sediments skew the RGB balance.

Most relevant for this study would be a follow-up in an area with consistent *P. bahamense* activity. The body of water should be photographed extensively in the absence of a bloom, in order to determine if a consistent RGB balance is present. This study, ideally, would be conducted alongside a tested monitoring mechanism for *P. bahamense* - e.g. light microscopy, a FLOWCAM, or any proven detection method - in order to accurately ascertain the density of the species. It is expected that a consistent RGB signature will present itself. The colour balance should be monitored consistently, paying particular attention to red pixels. As a bloom initiates, the density of *P. bahamense* should be compared with the expected rise in red pixels. The primary aim would be to examine the density at which the bloom will alter the RGB balance. Another parameter which should be examined is temperature, in order to determine whether or not it does have an impact on bloom development. As noted in this study, surface temperatures exceeded the optimal growth range fairly regularly, and of the parameters studied it is the most likely to have an influence on *P. bahamense* growth. But, as readings were taken at the surface, and were just slightly outside of the optimal conditions, more study must be done on whether it has an observable impact. If a link does present itself, it will be a great help in estimating future blooms, and differentiating between suspended sediment and *P. bahamense.*

Future *in situ* optical monitoring studies utilizing digital cameras should apply manual camera settings, in order to obtain the most reliable results possible. This includes all applications of digital cameras, not just the study of HABs.

The second element necessary for the succesful implementation of this approach – as vital as the consistent use of the method – is communication. This is an early warning system, not a precise *P. bahamense* monitoring device. During early stages of research, the operational application of the system on the island of Palawan was explored. The results will be discussed here, as a launching point for future endeavours. At present, given the lack of an alternative, it is feasible to begin applying this system to monitor HABs, but it must be utilized appropriately. In short, its limitations must be acknowledged: Based on current results, it is only capable of providing a warning. This system is a conduit of the practical application of the precautionary principle. It is not precise, and thus, communication between those undertaking the monitoring and the concerned parties is vital. In the Philippines, this primarily means BFAR – responsible for measuring shellfish toxicity – fishermen, and aquaculture practitioners. The early warning system will detect the increased presence of potentially harmful species before BFAR, and can warn shellfish harvesters accordingly. Keeping in mind the slow division rates of *P. bahamense* , a cautionary approach, coupled with a response by BFAR, would help reduce the risk of PSP, and provide confidence to importers of Philippine seafood. Once a certain rise in pixels is observed, BFAR can be notified and can focus their monitoring efforts, while those involved with the potentially affected seafood have the option to take precautions. If the system proves itself reliable, or can be supported by future studies, this should ideally be coupled with a ban on the sale of shellfish, enforced by fines, until BFAR can establish reliable toxicity levels. The benefit is that BFAR and the Provincial Agriculturalist need no longer conduct monthly surveys, but can act responsively. This cautionary approach incurs few additional costs, and may save millions in the case of a HAB.

5.8 Conclusions

Summatively, this study functions well as a pilot: Providing enough encouraging results to suggest further study, eliminating some less useful elements of the methodology, refining the approach, and indicating directions for further research. Going forward, the presence of HABs will continue to be an issue, and this methodology has the potential for widespread application. It is vital to note that this was the primary motivation behind the research: Utilizing available technology in novel ways, to overcome high costs and technical limitations. This is an approach that shows much potential in all fields: The unintended application of existing technologies. The classification of water bodies through the use of RGB values appears to be feasible, based on the relative stability, and uniqueness of colour balance found. Manual exposures are recommended for future *in situ* optical monitoring studies, regardless of the parameter being examined. Further monitoring of basic environmental parameters and their relationships with HABs is likely to be beneficial, but at present it is an unproven method for estimating algal blooms. In conclusion, the field of *in situ* optical monitoring with the use of digital cameras has shown promise, and monitoring of HABs appears to be a feasible application.

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