UNIVERSITI PUTRA MALAYSIA

POTENTIAL MARINE PHYTOPLANKTON AS LIVE FEED IN BINTULU AND MUKAH, SARAWAK

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FACULTY OF AGRICULTURAL AND FORESTRY SCIENCES

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Potential Marine Phytoplankton as Live Feed in Bintulu and Mukah, Sarawak

By

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A Project Report Submitted in Partial Fulfilment of the Requirement for the Degree of Bachelor of Science (Honors) Aquaculture in the Faculty of Agricultural and Forestry Sciences Universiti Putra Malaysia Bintulu Sarawak Campus

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ABSTRACT

Globally, marine phytoplankton are the primary producer in the ocean which is vital to the ecosystem as they feed a huge range of oceanic animals from tiny zooplankton to enormous whale. They are tiny individuals that convert solar energy into lipid, protein, carbohydrate, vitamin and pigments. Which are important nutrients that are needed by larvae and zooplankton. But there are limited documentation of marine phytoplankton that can be used as live feed, therefore the study is to identify those "potential" one. In order to provide more choice to the farmers when they carry out aquaculture in Bintulu and Mukah area. In this study, 40 individuals from Bintulu and Mukah coastal areas are identified until genus and some until species. In order to identified the marine phytoplankton species in both sites, study is carried out twice a month to represent the population. Sample collected are preserved in the lab, and single-cell isolation is practiced. The individual isolated is taken photo and identified with the reference of some books, online data bases and posters. Those genus or species which has the potential to be used as live feed are described base on their morphology or nutrient content. 19 genera were found at both site and is found out that they have the potential to be used as live feed, according to their nutrient content and morphology. Meanwhile, *Leptocylindrus* sp. and *Ceratium* sp. found is stated as live feed and harmful algae respectively. For this investigation, further identification of species for a wider range of location is needed to reach a significant result. In order to provide more choice of species that can be used as live feed in the future aquaculture sector.

ABSTRAK

Di peringkat global, fitoplankton marin adalah pengeluar utama di lautan yang penting kepada ekosistem kerana ia memberi makan kepada pelbagai jenis haiwan lautan daripada zooplankton kecil kepada ikan paus yang besar. Mereka adalah individu kecil yang menukar tenaga suria kepada lipid, protein, karbohidrat, vitamin dan pigmen. Yang merupakan nutrien penting yang diperlukan oleh larva dan zooplankton. Tetapi terdapat dokumentasi terhad fitoplankton marin yang boleh digunakan sebagai makanan hidup, oleh itu kajian ini adalah untuk mengenal pasti "berpotensi" itu. Bagi memberi lebih banyak pilihan kepada penternak apabila mereka menjalankan aktiviti akuakultur di kawasan Bintulu dan Mukah. Dalam kajian ini, 40 individu dari kawasan pantai Bintulu dan Mukah dikenal pasti sehingga genus dan beberapa sehingga spesies. Bagi mengenal pasti spesies fitoplankton marin di kedua-dua tapak, kajian dijalankan dua kali sebulan untuk mewakili populasi. Sampel yang dikumpul disimpan di makmal, dan pengasingan sel tunggal diamalkan. Individu yang diasingkan diambil gambar dan dikenal pasti dengan rujukan beberapa buku, pangkalan data dalam talian dan poster. Genus atau spesies yang berpotensi untuk digunakan sebagai makanan hidup diterangkan berdasarkan morfologi atau kandungan nutriennya. 19 genera ditemui di kedua-dua tapak dan didapati ia berpotensi untuk digunakan sebagai makanan hidup, mengikut kandungan nutrien dan morfologinya. Manakala, Leptocylindrus sp. dan Ceratium sp. didapati dinyatakan sebagai makanan hidup dan alga berbahaya masing-masing. Untuk penyiasatan ini, pengenalpastian lanjut spesies untuk julat lokasi yang lebih luas diperlukan untuk mencapai hasil yang ketara. Bagi menyediakan lebih banyak pilihan spesies yang boleh digunakan sebagai makanan hidup dalam sektor akuakultur akan datang.

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APPROVAL SHEETS

I certify that this research project entitled "POTENTIAL MARINE PHYTOPLANKTON AS LIVE FEED IN BINTULU AND MUKAH, SARAWAK" has been examined and approved as a partial fulfilment of the requirement for the degree of Bachelor of Science in Aquaculture with Honours in the Faculty of Agricultural and Forestry Science, Universiti Putra Malaysia Bintulu Sarawak Campus.

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1.0 INTRODUCTION

1.1 Background

Sarawak is the largest state in Malaysia located northwest of Borneo Island. Bintulu is one of the towns located in-between Miri and Sibu, it has a wide seaside making it strategic to carry out research related to marine microalgae compared with Kapit and Belaga which are located far from the sea. Bintulu's average temperature is around 26.8°C, providing phytoplankton with a suitable environment and temperature for growth (Demies *et al.* 2019). For Mukah, it is located just 148km away from Bintulu, similar to Bintulu the data show at "Tide-forecast" stated that sea temperature is also fluctuating between 25 - 29°C. Throughout the year there is also significant sunlight allowing them to bloom the whole year. In Bintulu and Mukah phytoplankton can be collected throughout the year.

Hatcheries provide seed for aquaculture and some commercial fisheries. A hatchery is where many species of fish and shellfish start their lives. A hatchery is a hybrid of a laboratory and a farm where fish and shellfish are produced, hatched, and cared for. They remain in the hatchery until they grow large enough to be moved to a fish or shellfish farm or released into the wild as part of a stock enhancement programme (Fisheries, 2021). In this controlled environment, the survival rate of the larvae can be increased compared with open space. According to research, shrimp larvae fed only cultured phytoplankton moulted to juveniles with higher survival rates (Hamasaki *et al.* 2020).

There are more than 25000 species of phytoplankton in the world and more are yet to be discovered. In total of 9 taxonomic groups: Diatoms, Dinoflagellate, Crytophytes, Coccolithophores, Chlorophytes, Euglenophytes, Prasinophytes, Cyanophytes, and small flagellates (Eker-Develi *et* *al*. 2012). Phytoplankton is tiny algae that can produce their own food via photosynthesis, similar to most plants on land. They live in the photic zone, where there is sufficient light for photosynthesis. They are the main producers in aquatic habitats like seas, rivers, lakes, ponds and estuaries. (Shoaib 2022). Some of the species contain toxin for example, toxic cyanophytes. Eutrophication is one of the factors that causes this high abundancy, resulting in affecting the ecosystem and human health (Skulberg *et al.* 1995).

Phytoplankton can be produced and applied in many fields example pharmaceuticals, biofuel, pigment, supplements and also live feed in aquaculture. In the last few decades, many microalgae species were discovered, but only less than 20 have gained widespread use in the aquaculture field (Das *et al.* 2012). Soedibya *et al.* (2021) stated that phytoplankton can be used as live feed during fish larvae culture. Live feed is a kind of food that is fed to another organism in its live form. In hatcheries, live feed is an important type of food. Fish larvae, shrimp, and crustacean larvae or fry feed on live feed to get the nutrients they need. Ma *et al.* (2012) mentioned that understanding the anatomical and functional changes of the larval digestive system is important for choosing an optimal diet to optimise fish survival throughout larval rearing. High fish mortality is common in aquaculture during the early stage, which correlates with the period of initial external feeding. Food consumption by first-feeding larvae impacts subsequent fish survival and growth; hence, the timing of food introduction is important (Ma *et al.* 2012). In hatcheries, live feed is used to feed larvae because their digestive systems have not fully developed during the larval stage (Dabrowski & Rusiecki 1983). Phytoplankton in larvae culture plays an important role, but there are very few phytoplankton species that are used and cultured for aquaculture purposes. But there are still very limited studies on marine phytoplankton species that can be used as live feed, which can provide a lot of essential nutrients to the culture. Thus, the objective of this study was to isolate, identify, and document the potential marine phytoplankton species that can be used as live feed in Bintulu and Mukah, Sarawak. In order to provide more choice for the farmer to choose species of phytoplankton that are most suitable for their culture.

2.0 LITERATURE REVIEW

2.1 Marine Phytoplankton

Phytoplankton is the primary producer of the pelagic food web in the water column, it has an important role in the aquatic ecosystems. They have two main classes, which are diatoms and dinoflagellate and they can be found in both fresh and saltwater and are commonly referred to as microalgae. They have a variety of habitats like lakes, estuaries, freshwater springs, lagoons, open oceans and so on. They are widely distributed around the world, there are approximately 25000 known species of phytoplankton. The size of them ranges from Cyanobacteria the smallest cell volume of 0.1 μm^3 to the largest Diatom more than 108 μm^3. The most common species used in the aquaculture includes *Chlorella, Tetraselmis, Isochrysis, Skeletonema, Thallssiosira, Nannochloropsis* and Phaeodactylum. Phytoplanktons are used in all stages of bivalve molluscs, abalone, crustacean, some fish and zooplankton in aquaculture (Das *et al.* 2012). Therefore there is no doubt phytoplankton are cultured as live feed in aquaculture. There are several types of phytoplankton culture as live feed for molluscs and fry in the hatcheries, for example, dinoflagellate and diatoms. Previous research states that there are 2 species of microalgae commonly used. Example: *Isochrysis* and *Tetraselmis* are used in bivalve hatcheries (Creswell 2010).

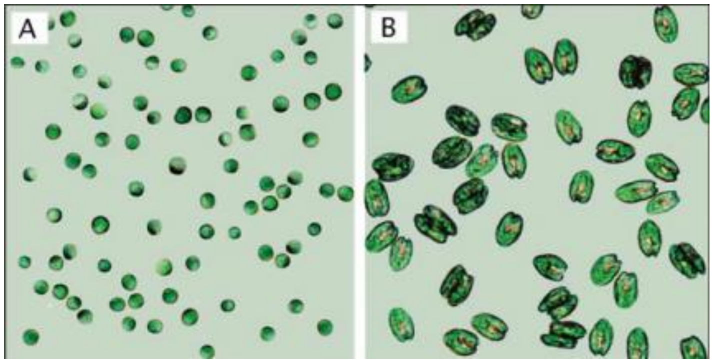


Figure 2.1: Micrographs of microalgae used in bivalve hatcheries. (A) *Isochrysis* sp. *and* (B) *Tetraselmis* sp

Study shows that in Kuala Sibuti and Kuala Nyalau (Estuaries) in Sarawak, there is a total of 46 phytoplankton species collected and their mean density in these two estuaries is 147000 cells per litre. The highest composition of species found there is Diatom, flowing by Dinoflagellate, Cyanophyceae, and Chlorophyceae (Saifullah *et al*. 2014). .Phytoplankton plays important role as live feed, however there are toxic species that are not suitable to be used as live feed. For example a genus named Anabaena is the most common genus that contain toxins. Research in Norwegian Inland water shows the abundancy is at around 84%. They have toxic producing strain and dominating in 50% of natural blooms (Skulberg *et al*. 1995). Those phytoplankton that can produce toxins will be excluded as a live feed in aquaculture.

2.2 Aquaculture

Aquaculture is an industry that carries out the breeding, rearing, and harvesting of fish, shellfish, algae, and so on in the water environment. According to the Food and Agriculture Organization of the United Nations, it is stated that in 2018, global aquaculture fish output reached 82.1 million metric tonnes, 32.4 million metric tonnes of aquatic algae, and 26 000 metric tonnes of decorative seashells and pearls, bringing the total to an all-time high of 114.5 million metric tonnes. The National Oceanic and Atmospheric Administration (2022) says that the present world population exceeds 7 billion people and is anticipated to exceed 10 billion by 2050. Traditional land-based agriculture consumes more than half of all developed land and more than 70% of the world's freshwater resources. Despite the fact that the ocean covers more than 70% of the Earth's surface, fisheries and a modest marine aquaculture industry contribute just 2% of the world's food supply. Fish farming in the ocean has the potential to significantly lessen demand on scarce land-based resources. Aquaculture is more effective than beef, pigs, and poultry at converting feed into protein for human consumption. Das *et al.* (2012) mentioned that larval rearing is one of the most risky phases of aquaculture, yet it might also be one of the most profitable. To overcome the risk of high mortality during this stage of culture, special planning and tactics are necessary. Live feed is necessary as an initial feeding for many farmed fish, and it aids in rapid development and survival for others. Fish larvae are unable to consume artificially supplied food. For nutrition, they require small-sized living meals. Live feeds are a high-protein, readily digested meal for fish and shellfish (Das *et al.* 2012).

2.3 Live feed

Live feed is basically the first meal for newly – hatched fish and shrimp larvae, because their digestive system is still incomplete and lack of enzyme. Live feed is an important type of feed in the aquaculture sector. In aquaculture, phytoplanktons are used to culture zooplankton for the fry. Phytoplankton can also be used to feed fry, bivalves, crustacean and zooplanktons in the hatcheries. In the food web, phytoplankton plays a major role in the marine water column as a producer, it is because they have the nutrient needed by fish larvae (Das *et al.* 2012). Many hatcheries achieve success in their production of fish fingerlings for grow-out production because they have availability of suitable live feed to feed their fish larvae, fry and fingerlings (Lim *et al.* 2003). Nowadays, a commonly used procedure while culturing fish and prawn larvae is to add in microalgae or "green water" together with zooplanktons like marine rotifer (*Brachionus plicatilis* and *Brachionus rotundiformis*) and artemia nauplii into intensive culturing system. Phytoplankton can provide many type of phytonutrient and some biologically active ingredients, for examples fatty acids, amino acids, sterols, organic minerals, enzymes, carotenoids, chlorophyll, vitamin and trace metal, which is needed for the first larval stage of the fish or other aquatic animal in the hatchery (Napiórkowska-Krzebietke 2017). These nutrients are significant in the aquaculture business.

2.4 Phytoplankton Culturing

Phytoplankton culturing is a method to produce a large volume of phytoplankton mainly for live feed application in aquaculture. To obtain phytoplankton usually they were collected from the nature environment like rivers, ocean, estuaries, wastewater and lake through sampling. During sampling there might be some difficulty like the microalgae attached on rock or stones. Then we need to use a toothbrush to brush down the samples or swab it. But the most commonly used method is by using plankton net with a mesh size of 20 µm (Hassan and Alkam 2006). There are two kinds of cultivation system, open cultivation and closed photobioreactor. Open cultivation is commonly used in open pond and raceways, which there are large in scale, simple, low cost and high production capacity. But there are still some cons about this cultivation system, the most obvious is not axenic which means it is easily mixed with bacteria, parasites, zooplankton and maybe other species of phytoplankton. Besides that, the open ponds will be low in carbon dioxide, sunlight penetration, high evaporation and easily affected by the rain and temperature. Another way of cultivation is closed photobioreactors, they are design to culture a more pure culture, with controlled light intensity, gas exchange, and evaporation. But to set up and maintain a photobioreactor, it needed a high cost for both maintenance and installation. To maintain the culture it should be place under a controllable environment and subculture it once a week or two weeks. In order to achieve success in culturing, it is important for the farmers to provide an optimum culture environment (filtered water, nutrient, light, temperature, pH, aeration) for the microalgae allowing them to grow at its optimum rate.

The culturing medium should be free of planktons, bacteria, heavy metal and pesticides. To remove all these unwanted substances it could be a costly and complicated process. For example, there are mechanical filtration, chemical filtration, heat sterilization, chemical sterilization, and ultraviolet irradiation. For open pond culture mostly chemical sterilization method is used to sterilize the pond with chlorine (Creswell 2010). The nutrient is also an important part while culturing phytoplankton. Nutrients helps them to grow at a higher rate in a shorter period of time. Domestic sewage are used in some open ponds as culture medium according to a research (Assemany *et al*. 2015).

The next major component in culturing phytoplankton is the light source, it is important because phytoplankton need light energy to convert nutrients into algal biomass. During outdoor culturing, the sun provides solar energy allowing the culture to grow. With this method of culture is can be said is very cost effective to the hatchery and can be produce in a large volume. The low cost high value practice can benefit the hatchery, by supplying live feed to the larvae, fry and zooplanktons.

There are several types of light been used during an indoor culture, the most common is fluorescent bulbs which can produce irradiation of 2500 lux, but it is not the best choice for intensive culture. For intensive culture metal, halide lamps are used and need to be placed at least 30 cm away from the culture as it generates some heat that may affect the culture if placed too near. For the temperature, it is best to be controlled at 24 °C.

Table 2.4: Temperature, light, and salinity ranges for culturing selected microalgae species (Hoff and Snell 2008)

Species	Temperature (°C)	Light (Lux)	Salinity (ppt)
Chaetoceros muelleri	25 – 30	8000 - 10000	20 – 35
Phaeodactylum tricornutum	18 – 22	3000 – 5000	25 – 32
Dicrateria sp.	25 – 32	3000 – 10000	15 – 30
Isochrysis galbana	25 – 30	2500 – 10000	10 – 30
Skeletonema costatum	10 – 27	2500 – 5000	15 – 30
Nannochloropsis oculate	20 – 30	2500 – 8000	0 – 36
Pavlova viridis	15 – 30	4000 – 8000	10 – 40
Tetraselmis tetrathele	5 – 33	5000 – 10000	6 – 53

Tetraselmis subcordiformis	20 – 28	5000 – 10000	20 – 40
Chlorella ellipsoidea	10 - 28	25000 – 5000	26 - 30

Different species have different optimum temperatures for growing, we need to consider the temperature by including the size of the room, heat energy from the light, volume and temperature of the air pumped into the culture. Aeration is very important when having a larger culture because aeration provides the carbon dioxide needed by the phytoplanktons for photosynthesis, stabilizing the pH, and mixing the culture so that the nutrient is evenly distributed. In large culture aeration without air stone is preferable because it does not create a fine bubble that can promote bacterial growth. Jet pumps, paddle wheels, and continuous recirculation are also commonly used in microalgae culture. When the culture density increases the carbon dioxide supply is low the water pH will slowly increase. For most marine phytoplanktons the optimum pH is 7.5 to 8.2, so a pH monitor is advised to be used in order to supply carbon dioxide to the culture on time. When the pH reached 10 the algal growth will stop and the culture will collapse (Chilmawati & Suminto 2016).

3.0 MATERIALS AND METHODS

3.1 Study Area

The sampling was carried out at river mouth of Bintulu Flood Mitigation Plan (Rancangan Tebatan Banjir Bintulu) (3°11'59.5"N 113°02'40.4"E), Tanjung Batu Vista Point 1 (3°12'29.1"N 113°02'38.7"E) and ABF Kidurong Beach Fish Market (Pasar Nelayan Pantai ABF Kidurong) (3°13'38.8"N 113°03'45.4"E) from Bintulu, Sarawak. Meanwhile, sampling site at Mukah area was carried out at Mukah Beach (2°55'21.2"N 112°08'32.9"E).

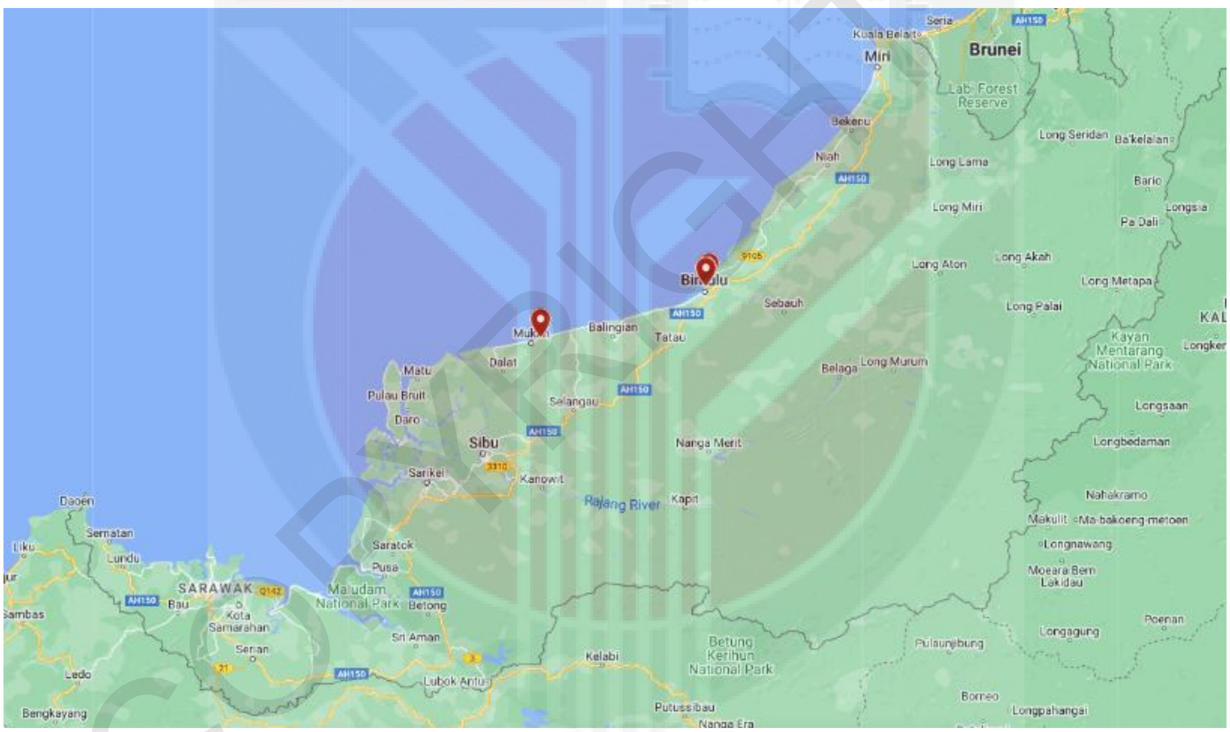


Figure 3.1: An overview of Sarawak and sampling location at Bintulu and Mukah, Sarawak.

3.2 Sampling Method

Water sample were collected twice a month from April 2022 to December 2022 at the selected location. Test and collect the water salinity if it reaches the standard with a mutliparameter. Plankton net with a mesh size of 20 µm were used to collect the plankton samples following method by Hassan and Alkam (2006). Plankton net were tossed into the water for around 5 meters and approximately 250ml of water sample were collected at each location. The sample that was collected were poured into a 50ml falcon tube and were preserved with 50 µl of acidic Lugol's solution (Sano *et al.* 2020).

3.3 Single-cell Isolation Technique

During single-cell isolation set up a invertered microscope equipped with a micropipettes. 1 ml of water sample were placed on the Sedgewick rafter and viewed under the microscope, micropipette was used to individually pick up the cell. The isolated single cell were observed and photographed under the inverted microscope to perform unbiased isolation (Genomics 2021). Single cell isolated were placed into a 96 – well plate, and the outermost well were filled to slow down evaporation effecting the well with target cell. The cell isolated were placed starting from B2 well, with some filtered seawater and culture medium. Filtered seawater used were filtered through a biofilter with 0.22 µm filter paper to filter away any microorganisms or debris to prevent contamination (Marie *et al.* 2017).

3.3 Identification

1 ml of preserved sample and live sample were observed under a microscope (Leica DFC7000T, Germany). The phytoplankton were compared by using identification books like

"Identify Marine Phytoplankton", " Handbook of Marine Microalgae" and "Microalgae: Biotechnology and Microbiology" to get know their genus or species. Besides that, online data base like "Algae Base" was used to search what is the species of the sample obtained (Tomas 1997) (Kim *et al.* 2015) (Becker, 1994) (Guiry, M.D. & Guiry, G.M. 2022).

4.0 RESULT

4.1 Identification of marine phytoplankton at Bintulu and Mukah, Sarawak

In total, 21 genera was identified at both Bintulu and Mukah (Table 4.1). There are several genera that can be found at both sites, like *Bacteriastrum* sp., *Ceratium* sp., *Coscinodiscus* sp., *Guinardia* sp., *Lauderia* sp., *Odontella* sp., *Pleurosigma* sp., *Rhizosolenia* sp., *and Striatella* sp. Meanwhile, during sampling, the following genus were discovered only at Bintulu Beach: *Bellerochea* sp., *Cyclotella* sp., *Dactyliosolen* sp., *Ditylum* sp., *Navicula* sp., *and Nitzschia* sp. Genus like *Asteronellopsis* sp., *Hemiaulus* sp., *Leptocylindrus* sp., and *Thalassiosira* sp. are indigenous at Mukah. Micrographs of these phytoplanktons were taken from preserved and live samples. The samples were photographed and categorized into three categories namely, live feed, harmful phytoplankton, and potential phytoplankton as live feed as shown in Figure 4.1, 4.2, and 4.3.

Table 4.1 Phytoplanton found in Bintulu (Bintulu Flood Mitigation Plan, Tanjung Batu Vista Point 1, ABF Kidurong Beach Fish Market) and Mukah (Mukah Beach)

No	Taxon Name	Bintulu	Mukah
1	*Asterionellopsis glacialis*	-	+
2	*Asterionellopsis* sp.	-	+
3	*Bacteriastrum delicatulum*	+	-
4	*Bacteriastrum* sp.	+	+
5	*Bellerochea horologicales*	+	-
6	*Ceratium furca*	+	-
7	*Ceratium fusus*	+	+
8	*Ceratium trichocerus*	-	+
9	*Ceratium tripod*	+	-
10	*Chaetoceros affinis*	-	+
11	*Chaetoceros costatus*	+	-
12	*Chaetoceros curvisetus*	+	-
13	*Chaetoceros lorenzianus*	+	-
14	*Chaetoceros mitra*	+	-
15	*Coscinodiscus* sp.	+	+
16	*Cyclotella* sp.	+	-
17	*Dactyliosolen* sp.	+	-
18	*Ditylum brightwellii*	+	-

Continue

Table 4.1 Continued

No	Taxon Name	Bintulu	Mukah
19	*Guinardia* sp.	+	+
20	*Helicotheca tamesis*	+	+
21	*Hemiaulus indicus*	-	+
22	*Hemiaulus* sp.	-	+
23	*Lauderia* sp.	+	+
24	*Leptocylindrus* sp.	-	+
25	*Navicula* sp.	+	-
26	*Nitzschia longissima*	+	-
27	*Odontella mobiliensis*	+	+
28	*Odontella sinensis*	+	+
29	*Pleurosigma fasciola*	+	-
30	*Pleurosigma* sp.	+	+
31	*Rhizosolenia bergonii*	-	+
32	*Rhizosolenia hebetata*	-	+
33	*Rhizosolenia imbricata*	+	-
34	*Rhizosolenia setigera*	-	+
35	*Rhizosolenia styliformis*	-	+
36	*Rhizosolenia* sp.	+	+
37	*Thalassionema frauenfeldii*	+	-
38	*Thalassionema javanecum*	+	-

Continue

Table 4.1 Continued

No	Taxon Name	Bintulu	Mukah
39	*Thalassionema* sp.	+	+
40	*Thalassiosira pseudonana*	-	+

* (+) Present, (-) Absent

Table 4.2 Diatom and dinoflagellate found and their potential to be used as live feed.

No	Diatom / Dinoflagellate	Taxon Name	Live Feed / Potential / Harmful	Notes
1	Diatom	*Asterionellopsis glacialis*	Potential Live Feed	Population of barnacles and bivalves increased together with *Asterionellopsis* population (Sahu *et al.* 2022)
2	Diatom	*Asterionellopsis* sp.	Potential Live Feed	Population of barnacles and bivalves increased together with *Asterionellopsis* population (Sahu *et al.* 2022)
3	Diatom	*Bacteriastrum delicatulum*	Potential Live Feed	Stated as Non-HAB diatom (Ravelo *et al*. 2022)
4	Diatom	*Bacteriastrum* sp.	Potential Live Feed	Stated as Non-HAB diatom (Ravelo *et al*. 2022)
5	Diatom	*Bellerochea horologicales*	Potential Live Feed	
6	Dinoflagellate	*Ceratium furca*	Harmful Phytoplankton	Produce ichthyotoxin (Tan *et al*. 2016)
7	Dinoflagellate	*Ceratium fusus*	Harmful Phytoplankton	Produce ichthyotoxin (Tan *et al*. 2016)
8	Dinoflagellate	*Ceratium trichocerus*	Harmful Phytoplankton	Produce ichthyotoxin (Tan *et al*. 2016)
9	Dinoflagellate	*Ceratium tripod*	Harmful Phytoplankton	Produce ichthyotoxin (Tan *et al*. 2016)
10	Diatom	*Chaetoceros affinis*	Potential Live Feed	Contain high concentration of lipid (Patten *et al.* 2012)
11	Diatom	*Chaetoceros costatus*	Potential Live Feed	Contain high concentration of lipid (Patten *et al.* 2012)
12	Diatom	*Chaetoceros curvisetus*	Potential Live Feed	Contain high concentration of lipid (Patten *et al.* 2012)
13	Diatom	*Chaetoceros lorenzianus*	Potential Live Feed	Contain high concentration of lipid (Patten *et al.* 2012)

Continue

Table 4.2 Continued

No	Diatom / Dinoflagellate	Taxon Name	Live Feed / Potential / Harmful	Notes
14	Diatom	*Chaetoceros mitra*	Potential Live Feed	Contain high concentration of lipid (Patten *et al*. 2012)
15	Diatom	*Coscinodiscus* sp.	Potential Live Feed	Protein and carbohydrates contribute 20-40% of the dry weight (Aitken *et al.* 2016)
16	Diatom	*Cyclotella* sp.	Potential Live Feed	High lipid content (Li *et al.* 2017)
17	Diatom	*Dactyliosolen* sp.	Potential Live Feed	
18	Diatom	*Ditylum brightwellii*	Potential Live Feed	Contains acyl lipid which can catalyses the production of complex lipid (Watkins 2013)
19	Diatom	*Guinardia* sp.	Potential Live Feed	
20	Diatom	*Helicotheca tamesis*	Potential Live Feed	
21	Diatom	*Hemiaulus indicus*	Potential Live Feed	Contain glycolipid compositions from cyanobacteria and the endosymbiont *Richelia intracellularis* (Schouten 2013)
22	Diatom	*Hemiaulus* sp.	Potential Live Feed	Contain glycolipid compositions from cyanobacteria and the endosymbiont *Richelia intracellularis* (Schouten 2013)

Continue

Table 4.2 Continued

No	Diatom / Dinoflagellate	Taxon Name	Live Feed / Potential / Harmful	Notes
23	Diatom	*Lauderia* sp.	Potential Live Feed	Contain wax ester which can help in improving feed conversion on Atlantic salmon (Bogevik 2011).
24	Diatom	*Leptocylindrus* sp.	Live Feed	Important oyster food (Middleton *et al*. 2021)
25	Diatom	*Navicula* sp.	Potential Live Feed	High lipid content (Li *et al.* 2017)
26	Diatom	*Nitzschia longissima*	Potential Live Feed	*Nitzschia navis-varingica* is the only *Nitzschia* sp. producing amnesic shellfish poisoning toxin named domoic acid (Tan *et al*. 2016)
27	Diatom	*Odontella mobiliensis*	Potential Live Feed	Contains Omega-3 fatty acids (Patten *et al*. 2012)
28	Diatom	*Odontella sinensis*	Potential Live Feed	Contains Omega-3 fatty acids (Patten *et al*. 2012)
29	Diatom	*Pleurosigma fasciola*	Potential Live Feed	Lipid content found in *Pleurosigma* sp. (Jayakumar 2021)
30	Diatom	*Pleurosigma* sp.	Potential Live Feed	Lipid content found in *Pleurosigma* sp. (Jayakumar 2021)
31	Diatom	*Rhizosolenia bergonii*	Potential Live Feed	Non-HAB diatom (Ravelo *et al*. 2022)
32	Diatom	*Rhizosolenia hebetata*	Potential Live Feed	Non-HAB diatom (Ravelo *et al*. 2022)
33	Diatom	*Rhizosolenia imbricata*	Potential Live Feed	Non-HAB diatom (Ravelo *et al*. 2022)
34	Diatom	*Rhizosolenia setigera*	Potential Live Feed	Non-HAB diatom (Ravelo *et al*. 2022)

Continue

Table 4.2 Continued

No	Diatom / Dinoflagellate	Taxon Name	Live Feed / Potential / Harmful	Notes
35	Diatom	*Rhizosolenia styliformis*	Potential Live Feed	Non-HAB diatom (Ravelo *et al*. 2022)
36	Diatom	*Rhizosolenia* sp.	Potential Live Feed	Non-HAB diatom (Ravelo *et al*. 2022)
37	Diatom	*Thalassionema frauenfeldii*	Potential Live Feed	
38	Diatom	*Thalassionema javanecum*	Potential Live Feed	
39	Diatom	*Thalassionema* sp.	Potential Live Feed	
40	Diatom	*Thalassiosira pseudonana*	Potential Live Feed	Lipid extracted from this genus (Borges *et al*. 2011)

Figure 4.2 shows that both sites have a total of 21 genera, 20 of which are diatoms (95%) and only one is dinoflagellate (5%). Besides that, 19 genera (90%) found at both sites have the potential to be used as live feed. While both live feed and harmful phytoplankton have only 1 genus (5%) found at these sites which is *Leptocylindrus* sp. for live feed and *Ceratium* sp. for harmful phytoplankton.

4.1.1 Phytoplankton used as Live Feed

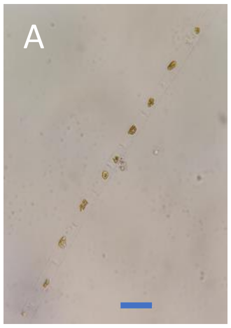


Figure 4.1 Diatom from Mukah (Mukah Beach). (A) *Leptocylindrus* sp. Scale bar = 20 µm.

4.1.2 Harmful Phytoplankton

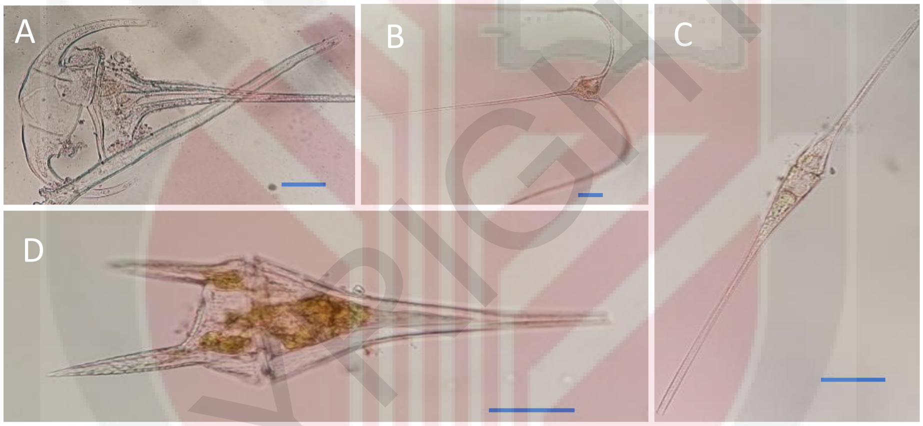
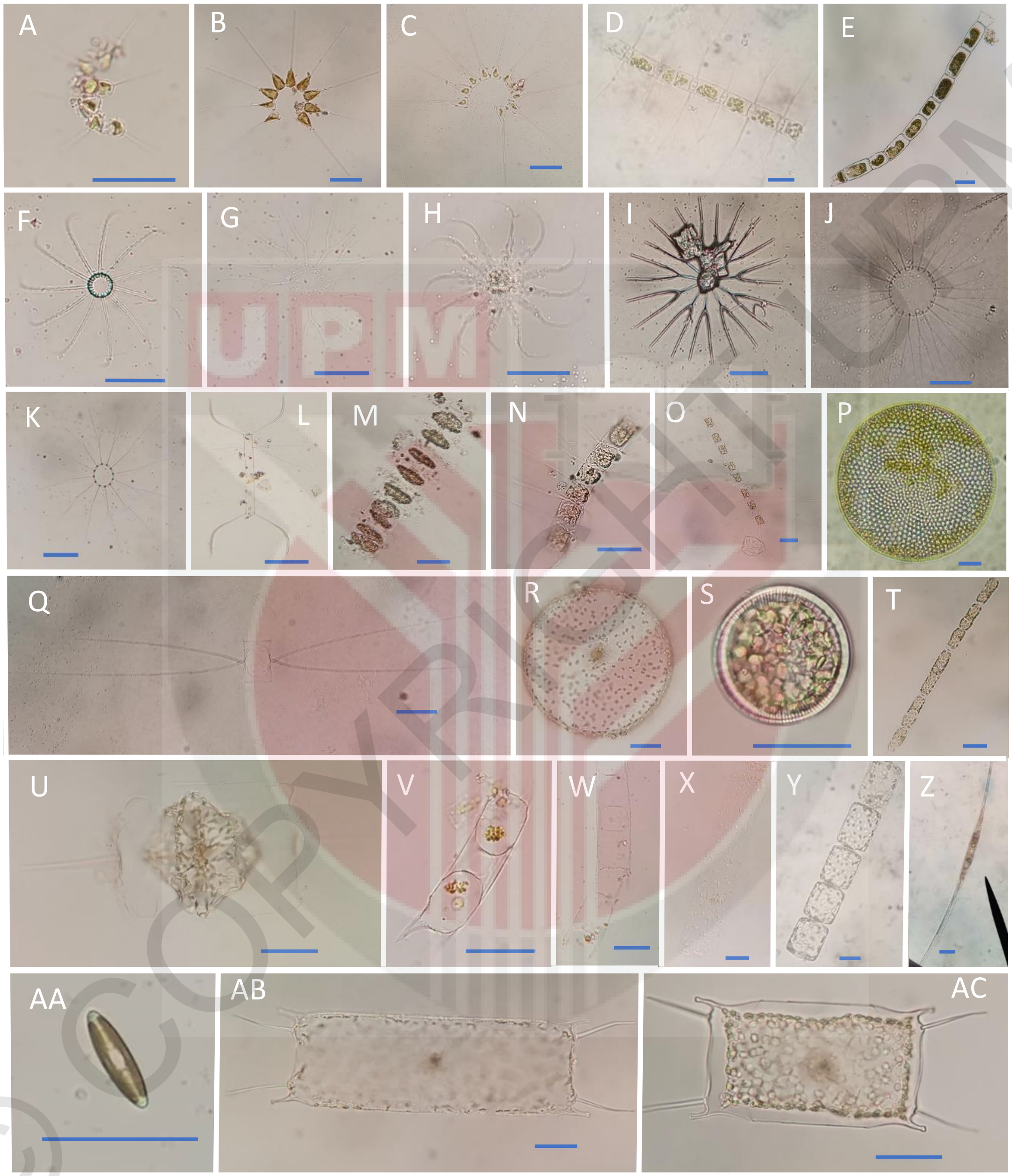


Figure 4.2 Dinoflagellates from Bintulu and Mukah. (A) *Ceratium tripod* (B) *Ceratium trichocerus* (C) *Ceratium fusus* (D) *Ceratium furca* . Scale bar = 20 µm.

4.1.3 Potential Phytoplankton as Live Feed



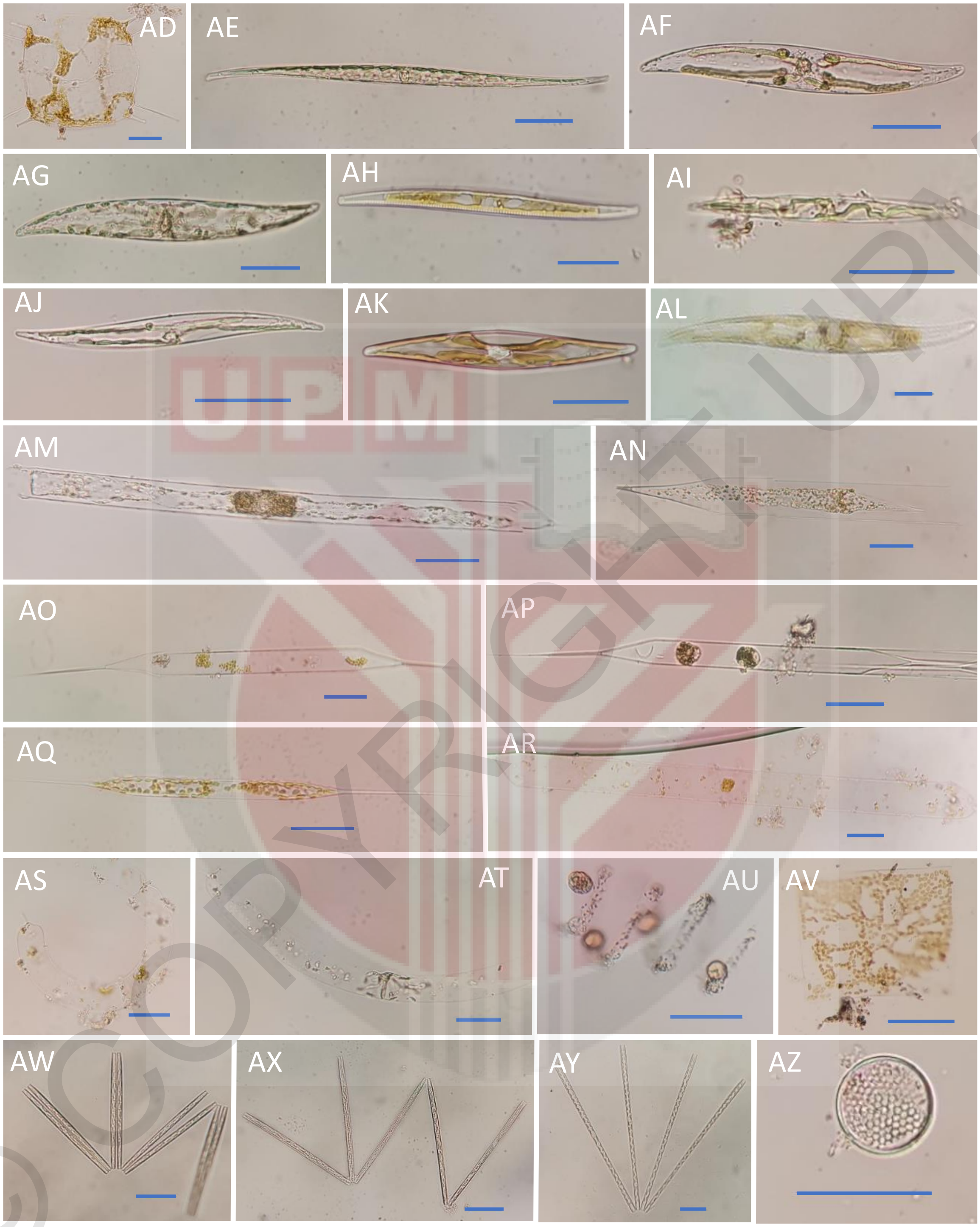

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Figure 4.3 Diatoms and Dinoflagellates from Bintulu (Bintulu Flood Mitigation Plan, Tanjung Batu Vista Point 1, ABF Kidurong Beach Fish Market) and Mukah (Mukah Beach). (A) *Asterionellopsis* sp. (B) *Asterionellopsis glacialis* (C) *Asterionellopsis* sp. (D) *Bacteriastrum delicatulum* (E) *Bellerochea horologicales* (F-K) *Bacteriastrum* sp. (L) *Chaetoceros affinis* (M) *Chaetoceros costatus* (N) *Chaetoceros curvisetus* (O) *Chaetoceros mitra* (P) *Coscinodiscus* sp. (Q) *Chaetoceros lorenzianus* (R) *Coscinodiscus wailesii* (S) *Cyclotella* sp. (T) *Dactyliosolen* sp. (U) *Ditylum brightwellii* (V) *Hemiaulus indicus* (W) *Hemiaulus* sp. (X) *Guinardia* sp. (Y) *Lauderia* sp. (Z) *Nitzschia longissima* (AA) *Navicula* sp. (AB) *Odontella sinensis* (AC) *Odontella* sp. (AD) *Odontella mobiliensis* (AE) *Pleurosigma fasciola* (AF-AL) *Pleurosigma* sp. (AM) *Rhizosolenia imbricata* (AN) *Rhizosolenia bergonii* (AO) *Rhizosolenia hebetata* (AP-AQ) *Rhizosolenia setigera* (AR) *Rhizosolenia styliformis* (AS-AU) *Rhizosolenia* sp. (AV) *Helicotheca tamesis.* (AW) *Thalassionema frauenfeldii* (AX) *Thalassionema javanecum* (AY) *Thalassionema* sp. (AZ) *Thalassiosira pseudonana* . Scale bar = 20 µm.

5.0 DISCUSSION

5.1 Potential of the marine phytoplankton to be used as live feed

Ninety percent of the genera found at both sites have the potential to be used as live feeds. While both live feed and harmful phytoplankton have only 5% of their genus at these sites (shown in Table 4.2), The potential of the phytoplankton to be used as live feed can be measured in many ways, but their nutrient content is the most important. Napiórkowska-Krzebietke (2017) mentioned that many valuable phytonutrients and biologically active ingredients are found in phytoplankton, including fatty acids, amino acids, sterols, organic minerals, enzymes, carotenoids, chlorophyll, trace elements, and vitamins, which are available directly to the first larval or juvenile stages of fish or indirectly (via trophic chains) to the more mature forms. Lipids are the most nutritious phytoplankton biochemical composition, influencing the development, health, and reproduction of aquatic animals, particularly fish species (Napiórkowska-Krzebietke 2017). Not every genus discovered through the study has been studied; of the 19 genera with the potential to be used as live feeds, 5 of them have almost no research on their nutrient content.

5.1.1 Live feed genus

a. *Leptocylindrus* (Cleve, 1889)

Patten *et al.* (2012) stated that the genus *Leptocylindrus* is a diatom that is cylindrical, has golden chloroplasts, and has clear silica covering it. This genus has a high tolerance for temperature, and they reproduce both asexually and sexually (Patten *et al.* 2012). In this study, there is only one place that *Leptocylindrus* is found, which is at Mukah Beach, as shown in Figure 4.1 (A). Past studies written in the article "Marine Phytoplankton of South Central Alaska" stated that *Leptocylindrus* sp. are an important oyster food source (Middleton *et al*. 2021).

5.1.2 Harmful phytoplankton genus

a. *Ceratium* (F. Schrank, 1793)

Ceratium has 53 species discovered to date; it is the genus that primarily causes HAB (Harmful Algal Bloom) all over the world, including China, Malaysia, Vietnam, and Singapore (Tan *et al.* 2016). *C. tripod, C. trichocerus, C. fusus,* and *C. furca* were the only dinoflagellates found in my study at both sites, Bintulu and Mukah, as shown in Figure 4.2. Tan *et al.* (2016) mentioned that these genera produce ichthyotoxins, which can cause hypoxia and anoxia, and that when they form HAB, large amounts of fish are killed.

5.1.3 Potential live feed genus

a. *Asterionellopsis* (F.E. Round, 1990)

This is a genus under the class Bacillariophyceae. Genus *Asterionellopsis* is a diatom that has triangular-shaped cells with a long spine protruding from one end, which are frequently united at the broad end, creating star-shaped colonies. (Patten *et al.* 2012). Through study, there are a total of three species of *Asterionellopsis* found in the Mukah coastal area, as shown in Figure 4.3 (A–C). Sahu (2022) mentioned that the intense bloom of *Asterionellopsis* was caused by suitable temperature, salinity, and nutrient levels during the post-northeast monsoon. Bioenvironmental (BIOENV) analysis and Principle Component Analysis (PCA) confirmed that salinity and nitrate are the primary contributors to their abundance. The larvae of the relatively small population of barnacles and bivalves proliferated because of the blooming of *A. glacialis* and became their primary food supply (Sahu *et al.* 2022). Because of their spiny morphology, *Asterionellopsis* may not be suitable as live feed for fish because it may clog and cause bleeding to fish gills; however, it may be a superior alternative in bivalve culture (Sahu *et al.* 2022).

b. *Bacteriastrum*

The genus *Bacteriastrum* is a diatom that has so far been reported in more than 30 taxa. Except for a recently identified solitary species, the majority of these genera form filament-like colonies (Guiry, M.D. & Guiry, G.M. 2022). Filamentous colonies are formed by connecting cells with numerous setae at a distance equal to 2–3 cell diameters, generally more than two per valve, which places *Bacteriastrum* in the family *Chaetocerotaceae* Ralfs (Godrijan *et al.* 2012). The general shape of the genus *Bacteriastrum* is quite similar to several *Chaetoceros* species (Sarno et al., 1996). Figure 4.3 (D and F–K) shows there are a total of 7 different species of *Bacteriastrum* found in both Bintulu and Mukah coastal areas throughout the study. However, because the *Bacteriastrum* and *Chaetoceros* are so similar, it is possible that the *Bacteriastrum* also has a high lipid content like *Chaetoceros* sp. (Patten *et al.* 2012). Besides that, the genus *Bacteriastrum* is also described as a non-HAB diatom, which increases the possibility of the genus being used as live feed in the future (Ravelo *et al.* 2022).

c. *Chaetoceros* (Ehrenberg, 1844)

More than 400 *Chaetoceros* species have been described (Li *et al.* 2013). When the conditions are favourable, *Chaetoceros* can proliferate. According to past studies, the genus *Chaetoceros* has a high concentration of lipid content (Patten *et al.* 2012). Which gives it the value and potential to be cultured as live feed in the aquaculture sector. Although *Chaetoceros* sp. has some risk of damaging or blocking the gills of fish when the population blooms, this genus still has the potential to be used as live feed for bivalve species like oysters, which will not be affected (Perry *et al.* 2010). In this study, a total of five species were found. *C. affinis* is found in Bintulu coastal water, while *C. costatus*, *C. curvisetus*, *C. lorenzianus*, and *C. mitra* were found in Mukah coastal water, as shown in Figure 4.3 (L-O and Q).

d. *Coscinodiscus* (Ehrenberg, 1839)

According to "Algae Base" the genus *Coscinodiscus* is a type of diatom in the class Bacillariophyceae, which has hundreds of species with different numbers and arrangements of pores and chloroplasts (Guiry, M.D. & Guiry, G.M. 2022). The form of the *Coscinodiscus* is centric; it looks like a wheel. As a result, it seems circular from the top and there are several openings in the "glass house" that allow water and nutrients to flow through, as well as numerous round or oval chloroplasts (Patten *et at.* 2012). There are no exterior spines, and it is found alone rather than in chains or groups (Patten *et at.* 2012). Through this study there are 2 species of *Coscinodiscus* found at both Bintulu and Mukah coastal areas as shown in Figure 4.3 (P,R), which shows their abundance throughout Sarawak's coastal waters. Aitken (2016) mentioned that, protein and carbohydrates contribute 20-40% of the dry weight inside the diatom silica valves.

e. *Cyclotella* (Brébisson, 1838)

In "Algae Base" it is stated that there are 126 species of *Cyclotella* sp., but they are mainly found in freshwater. It has been discovered that there are two species found in coastal water, implying that it is most likely an evolutionary invasion from brackish waters. *Cyclotella* is characterised by drum-shaped cells that are short, filamentous, and rarely cluster (Guiry, M.D. & Guiry, G.M. 2022). There is only one species found in Bintulu coastal area throughout this study, which is shown in Figure 4.3 (S). Therefore, according to "Algae Base" there are only 2 species found in the coastal area which makes current result likely accurate. Roessler, (1988) mentioned that in diatom *Cyclotella* sp. the effects of silicon deficiency on the activity of numerous enzymes involved in lipid and storage carbohydrate synthesis were explored (Roessler 1988).

f. *Ditylum* (Grunow, 1885)

According to Patten *et al.* (2012) says the genus *Ditylum* looks like a capsule, with a ring of tiny ridges on the two short, rounded ends and a long, thick, hollow cylindrical spine emerging from each. A cluster of golden brown chloroplasts gathered at the cell's core. According to "Algae Base" there are slightly more than ten species of *Ditylum*. Meanwhile, in the current study, there is one species found in the Bintulu coastal area, which is identified as *Ditylum brightwellii* (Figure 4.3) (U). Döhler and Biermann (1994) stated that *Ditylum brightwellii* contains acyl lipids that can catalyse the creation of a thioester link between a fatty acid and coenzyme A in the presence of adenosine triphosphate. This basic reaction enables the fatty acid to be degraded for energy production, integrated into complex lipids, or involved in other metabolic processes (Watkins 2013).

g. *Hemiaulus* (Heiberg, 1863)

The genus *Hemiaules* is described by Harwood and Nikolaev (1995) as a chain-forming colony of bipolar cells. There is a total of two species of *Hemiaulus* found in the Mukah coastal area, as shown in Figure 4.3 (V, W). Schouten (2013) stated that *Hemiaules* sp. has been found to contain glycolipid compositions from cyanobacteria and the endosymbiont *Richelia intracellularis*. *G*lycolipids can aid in the activation of host immune systems. Besides that, glycolipids have the potential to be utilised as a practical, cost-effective, and safe adjuvant for farmed fish (Matsumoto *et al.* 2018).

h. ***Lauderia* (Cleve, 1873)**

Middleson (2021) described the genus *Lauderia* as a centric diatom that formed straight chains of cells; every cell has a lot of small, lobed chloroplasts around it. Both coastal areas of Bintulu and Mukah *Lauderia* sp. are found in my study as shown in Figure 4.3 (Y). In an experiment, the genus *Lauderia* was tested and result in it providing a sufficient amount of wax ester (Lee *et al.* 1971). According to Bogevik (2011), salmon have some adaptation to a diet high in wax ester. This includes increased feed conversion, increased bile synthesis, and increased midgut lipolytic enzyme activity (Bogevik 2011). Atlantic salmon have been demonstrated to eat and thrive on diets containing a moderate level of wax esters (30% of the lipid), with outcomes comparable to fish kept on fish oil diets (Bogevik 2011).

i.*Navicula* (Bory, 1822)

Being in the class Bacillariophyceae, this diatom has the most species (approximately thousands of species) (Patten *et al.* 2012). The genus *Navicula* is described as a boat-shaped diatom that is bilaterally symmetrical and has two chloroplasts. Besides that, when observed in a live sample, they can be actively moving (Middleton *et al.* 2021). Patten (2012) says that it is so common worldwide that we can find it almost whenever we sample. (Figure 4.3)(AA) shows the *Navicula* sp. caught during sampling. In a study, it is stated that the total lipid content in *Navicula gregaria* is 19.17 pg cell^{-1} (Popovich *et al.* 2012).

j. *Nitzschia* (Hassall, 1845)

Middleton (2021) described the genus *Nitzschia* as an elongated cell with long, pointed ends that might be slightly curved. *Nitzschia* sp. also has two big chloroplasts located at the center, and its cytoplasm extends to the point of the cell (Middleton *et al.* 2021). In this study, there is only one species found in the Bintulu coastal area as shown in Figure 4.3 (Z). There are around 400 species in this genus, but only one species (*Nitzschia navis-varingica*) was responsible for a toxic bloom in 2000 (Tan *et al.* 2016). Tan *et al.* (2016) mentioned that another 15 species of *Nitzschia* found in the study were identified as non-toxic species. According to Alzahrani (2019), compared to *Chlorella* sp., *Nitzschia* sp. *has a* higher protein content, and besides that, it also produces bioactive lipids.

k. *Odontella* (C. Agardh, 1832)

The genus *Odontella* were described as centric diatoms that has lanceolate horns and tiny chloroplasts that can be observed (Middleton *et al.* 2021). *Odontella mobiliensis* and *Odontella sinensis* as shown in Figure 4.3 (AB-AD) were found at both Bintulu and Mukah coastal area throughtout my study. According to Patten *et al.* (2012) *Odontella* sp. contain Omega-3 fatty acids which is healthy to animals. *Odontella aurita* has been approved to be human food in France.

l. *Pleurosigma* (W.Smith, 1852)

Pleurosigma is a pennate diatom that is always blunt at the ends, they were never pointed. When observed via side view it may not look bending (Middleton *et al.* 2021). There are hundreds of species name accepted and documented in “Algae Base” (Guiry, M.D. & Guiry, G.M. 2022). According to this study carried out at Bintulu and Mukah coastal areas, there is a total of eight species discovered as shown in Figure 4.3 (AE-AL) . Jayakumar (2021) stated that the lipid content found in *Pleurosigma* sp. is high at 27%.

m. *Rhizosolenia* (Brightwell, 1958)

Patten *et al.* (2012) described the genus *Rhizosolenia* as a very long, straight, and narrow cylinder or tube tapering at both ends to a point, resembling a pencil sharpened at both ends. The coloration is clear, with numerous little golden chloroplasts. Girdle band sections are frequently stacked. The length to width ratio can reach 20:1. According to my study at both Bintulu and Mukah coastal areas, there are nine species found including *Rhizosolenia bergonii*, *Rhizosolenia hebetata*, *Rhizosolenia imbricata*, *Rhizosolenia setigera*, and *Rhizosolenia styliformis* which is shown at Figure 4.3 (AM-AU) . In *Rhizosolenia setigera* polyunsaturated monocyclic sester- and triterpenes were found and derived from it (Massé *et al.* 2004).

n. *Thalassiosira* (Cleve, 1873)

Thalassiosira is a genus that can be observed as a circle from "top" and "bottom" , Thalassiosira's general characteristics include chains linked together by chitin threads or cells buried in mucilage, tiny spines, radial symmetry, and numerous small holes in the valves (Patten *et al.* 2012). In this study, *Thalassiosira* was obtained at the Mukah coastal area as shown in Figure 4.3 (AZ). Borges (2011) stated that solar energy is efficiently converted by microalgae into metabolites such as lipids, proteins, carbs, pigments, and vitamins. Meanwhile in a lipid extraction experiment carried out by Borges in 2011, it shown that a minimum mass fraction of 2.77% was obtained for the CPF (Flopam®) treatment, with a maximum mass fraction of 4.12% obtained for the control from the species *Thalassiosira weissflogii*.

6.0 CONCLUSION

To sum up, 40 marine phytoplankton individuals were isolated using a single-cell isolation technique, and their genera and species were identified using books, an online data base, and a poster. It ends up having 35 out of 40 individuals of marine phytoplankton found in both Bintulu and Mukah coastal areas that have the potential to be used as live feed in the future aquaculture sector. They have the characteristics to be a great choice for live feed due to their nutrient content (lipid, protein, vitamin, carbohydrate, and pigment). Therefore this study, it can help increase the number of choices of live feed to be used in the future aquaculture sector. In addtion, there are some "potential ones" that are found in this study that have little research on them, especially regarding the nutrient content. An increase in site for investigation is needed in order to identify more species that can be used as live feed in the aquaculture sector.

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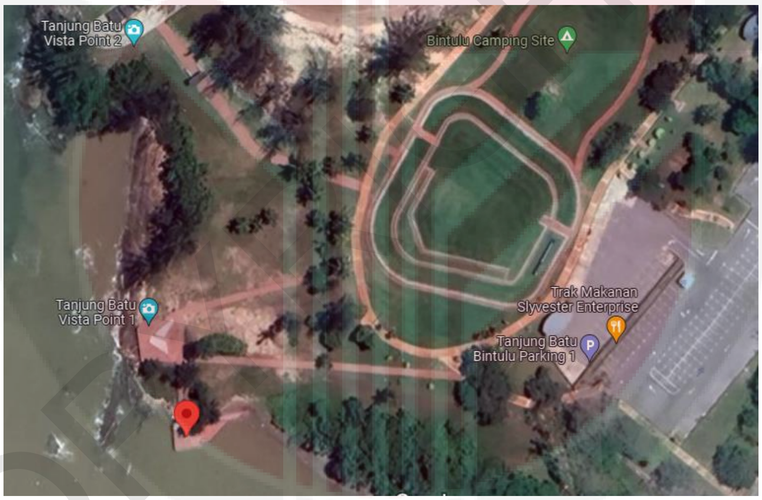
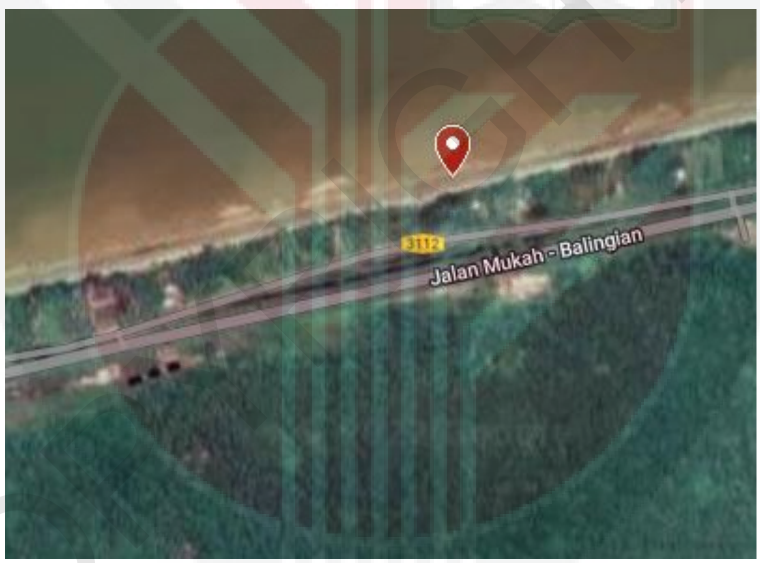
8.0 APPENDICES

Bintulu Flood Mitigation Plan



Tanjung Batu Vista Point 1

ABF Kidurong Beach Fish Market



Mukah Beach