Faculty of Resource Science and Technology

COMPARISON OF WATER QUALITY BETWEEN SITES WITH AND WITHOUT CAGE CULTURE IN BATANG AI RESERVOIR, SARAWAK

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Comparison of Water Quality between Sites with and without Cage Culture at Batang Ai Reservoir, Sarawak

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DECLARATION

I, Khoo Yen Nee, final year student of Aquatic Resource Science and Management hereby declare that this thesis is my own work and effort with the guidance of my supervisor, Professor Dr. Lee Nyanti. No part of the thesis has previously been submitted for any other degree, university or institution of higher learning.

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LIST OF ABBREVIATIONS

m	Metre
hr	Hour
µg/L	Microgram per litre
mg/L	Milligram per litre
°C	Degree Celsius
DO	Dissolved oxygen
L	Litre
NH_3-N	Ammonia nitrogen
NO_3^-	Nitrate
NO_2^-	Nitrite
PO_4^{3-}	Orthophosphate
SiO_4	Silica
pH	Potential of hydrogen
TDS	Total dissolved solids
TSS	Total suspended solids
CO_2	Carbon dioxide
km^2	Kilometre square
m^3	Cubic metre
ml	Millilitre
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
GPP	Gross primary productivity
NPP	Net primary productivity
H_2S	Hydrogen sulphide
rpm	Round per minute
nm	Nanometre
µS	MicroSiemen
NTU	Nephelometric Turbidity Unit
mg/L/hr	Milligram per litre per hour
GPS	Global positioning system

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ABSTRACT

This study was carried out in the months of October and November 2011, and April 2012 to assess the impact of cage culture activities on the water quality at Batang Ai Reservoir, Sarawak. Water quality parameters were recorded at 3 sampling stations which are S1 (cage culture area), S2 (area without cage culture), and S3 (unpolluted area of the reservoir) at five different depths (subsurface, 5 m, 10 m, 20 m, and 25 m). For nutrients, the range for ammonia was 0 to 0.83 mg/L, for nitrate 0.4 to 9.6 mg/L, for nitrite 0.004 to 0.115 mg/L, for orthophosphate 0 to 0.43 mg/L, and silica 0.030 to 9.583 mg/L. The concentrations of nutrients in April 2012 were significantly different compared to in October and November 2011 because it was raining season, fishes were harvested, and water level of the reservoir rose in April 2011. The range for BOD_5 was from 1.73 to 19.75 mg/L, COD from 0 to 21 mg/L, TSS from 0.500 to 11.019 mg/L, and chlorophyll-*a* from 0.192 to 11.706 (µg/L). TSS was highest at station 3 for all three samplings but is still under acceptable level for freshwater aquaculture. Chlorophyll-*a* was higher in station 1 and station 3 compared to the concentration of chlorophyll-*a* in station 2. Primary productivity and exposure tests of juvenile fishes were conducted during November 2011 and April 2012. The net primary production was higher at cage culture area compared to area without cage culture. The dissolved nutrients from urea and waste from feed fertilized the water and promote primary productivity. All juvenile fishes survived at depth of 5 m, 6 m, 7 m, and 8 m, but 100 % morlity at the depth of 10 m. Dissolved oxygen was found to be the main cause of juveniles' mortality. These results indicated that cage culture activities and water input from upstream affects water quality at the reservoir.

Keywords: Water Quality, primary productivity, fish cage culture, reservoir

ABSTRAK

Kajian ini dijalankan pada bulan Oktober dan November 2011, dan April 2012 untuk menilai kesan aktiviti ternakan sangkar terhadap kualiti air di Takungan Batang Ai Reservoir, Sarawak. Parameter kualiti air telah direkod di 3 stesen kajian iaitu S1 (kawasan bersangkar ikan), S2 (kawasan yang tiada sangkar ikan), dan S3 (kawasan takungan air yang tidak tercemar) pada lima kedalaman yang berbeza (permukaan, 5 m, 10 m, 20 m, dan 25 m). Untuk nutrien, kandungan ammonia berjulat diantara 0 sehingga 0.83 mg/L, nitrat diantara 0.4 sehingga 9.6 mg/L, nitrit diantara 0.004 sehingga 0.115 mg/L, ortofosfat diantara 0 sehingga 0.43 mg/L, dan silica diantara 0.030 sehingga 9.583 mg/L. Kandungan nutrien pada bulan April 2012 adalah berbeza secara signifikan berbanding dengan bulan Oktober dan November 2011 kerana pada ketika itu adalah musim hujan, ikan telah dikeluarkan dari sangkar dan paras air juga meningkat. BOD_5 pula berjulat diantara 1.73 sehingga 19.75, mg/L, COD diantara 0 sehingga 21 mg/L, TSS diantara 0.500 sehingga 11.019 mg/L, dan klorofil-a diantara 0.192 sehingga 11.706 (µg/L). TSS paling tinggi di stesen 3 untuk ketiga- tiga bulan tetapi masih lagi berada dalam tahap yang sesuai untuk akuakultur air tawar. Klorofil-a adalah lebih tinggi pada stesen 1 dan stesen 3 berbanding dengan stesen 2. Produktiviti primer dan pendedahan ikan pada kedalaman yang berbeza dijalankan pada bulan November 2011 dan April 2012. Pengeluaran utama bersih adalah lebih tinggi di kawasan yang mempunyai sangkar ikan berbanding dengan kawasan tiada sangkar ikan. Nutrien daripada urea dan makanan ikan telah meningkatkan produktiviti primer. Semua ikan juvenil masih hidup pada kedalaman 5 m, 6 m, 7 m, dan 8 m, manakala semua ikan juvenil mati pada kedalaman 10 m. DO merupakan parameter utama yangmenyebabkan kematian ikan tersebut. Berdasarkan keputusan daripada kajian ini, aktiviti penternakan ikan di sangkar dan kemasukan air dari hulu sungai ke empangan akan menpengaruhi kualiti air di empangan ini.

Kata kunci: kualiti air, productiviti primer, ternakan sangkar ikan, takungan

1.0 Introduction

Aquaculture started in China around 2000 years BC and has been practiced for millennia (Stickney, 2009). According to statistic published by FAO (2010), world fisheries and aquaculture production in 2009 was 145.1 million tonnes. Of that, 55.1 million tonnes was from inland aquaculture and marine sources excluding plants. In Malaysia, aquaculture began only in the early 1900's. In 2009, fisheries and aquaculture production in Malaysia was 1.87 million tonnes with a value of RM8683.80 million (DOF, 2009). In Sarawak, aquaculture activities started in Batang Ai Reservoir since 1993 and has provide protein source for human consumption.

Batang Ai Reservoir is located at the district of Lubok Antu, Sri Aman Division in the state of Sarawak. The surface area of the reservoir is 84 km^2 and the catchment area is 1200 km^2 (Asian Development Bank, 1999). Construction of Batang Ai Hydroelectric Dam started 1982 with the river diversion work and the last turbine was completed in 1985. Batang Ai Reservoir was formed by inundation after the completion of Batang Ai Hydroelectric dam construction. The reservoir is utilized for generating electrical power and for floating fish cage cultures.

Other than cage culture, eco-tourism activities also occur surrounding the reservoir. Eco-tourism is prominent in Batang Ai Reservoir with the existence of Hilton Batang Ai Resort. The Batang Ai National Park and human settlement are located upstream of the lake area. Water quality of the reservoir could be affected by those activities due to nutrient input from urea and waste discharge. It is important to document the water quality of the reservoir as many cage cultures are being set up since 1993.

Waters can be described in terms of the physical, chemical, and biological conditions present (Abdullah & Yasin, 2000). Water quality parameters such as the amount of ammonia, pH, and dissolved oxygen are important to describe the water quality of reservoir (Maleri, 2011). Monitoring water quality of the reservoir also allows future development and expansion of aquaculture activities. Other than that, parameters of water quality are also important to be analyzed to ensure that fish survival and growth are optimum. During dry season, minimum flow releases also can cause poor water quality for aquatic life to live in the Batang Ai Reservoir. In dry season, very deep ponds are likely to experience low dissolved oxygen and ammonia or nitrite build up problems.

The success in introducing floating cage aquaculture brings production of 2,696 fish cages and the main cultured species is red tilapia (DOA, 2007). In Batang Ai Reservoir cage culture, the main species cultured is *Oreochromis* sp. (Red tilapia). Red tilapia is known as a very hardy species. It is able to tolerate high range of temperature and salinity compared to other species. Fishes are prone to get diseases when water temperatures dropped below 22°C and death occur when temperature drop below 16°C (Watanabe et al., 1997). The ideal temperature to ensure optimal growth of red tilapia is 27°C to 32°C, at salinity of 0 PSU and 32°C at salinity of 18 to 30 PSU (Watanabe et al., 2006). The overall growth and health of red tilapia will be affected when exposed to long period of low DO although the species is known to be able to withstand low DO (Shelton & Popma, 2006). Low DO concentrations for long period can markedly affect fish growth, feeding and assimilation efficiencies. Ammonia toxicity of tilapia is dependent on the content of DO, CO_2 and pH in the water bodies (Shelton & Popma, 2006).

All life form depend on primary production, a biochemical synthesis of organic substances from inorganic substances in certain space per unit time by autotrophs which form the base of the trophic chain (Kumar & Dubey, 2006). Primary productivity through photosynthesis assimilates carbon in the biosphere and made oxygen available. The production of oxygen through photosynthesis can be used to monitor the primary productivity of an aquatic ecosystem. In reservoir system, the primary productivity is heterogeneous and not well understood. The resultant primary productivity is influenced by abiotic and biotic characteristics of the reservoir system, often due to diel or seasonal characteristic (Prabhakar et al., n.d.). The formation of organic substances and oxygen is as follow:

$$6\ H_2O + 6\ CO_2(g) + \text{energy} \longrightarrow C_6H_{12}O_6 + 6O_2(g)$$

The changes in water quality of Batang Ai reservoir provides valuable information on the quality of water, the sources of variations and their impacts on cage culture activities. The government allocated RM 252 million in recent budget for aquaculture industry to develop large-scale integrated Aquaculture Zones in Pitas, Sungai Telaga, and Sungai Padas in Sabah as well as Batang Ai and Tanjung Manis in Sarawak that meet the standard as well as produce high quality products (Yusof, 2010). It is important for this study to be conducted as aquaculture is the main protein source for local population and state agency. The aims of this study were i) to assess water quality at five depths at cage culture site and outside the cage culture site located at Batang Ai Reservoir, ii) to determine the gross and net primary productivity of the Batang Ai Reservoir and iii) to determine the survival rate of juvenile tilapia fishes at six different depths

2.0 Literature review

2.1 Reservoir

Over 70% of the surface of the planet is covered by vast majority of saltwater and leaving 2.5% of it is freshwater that can be used in production of the largest proportion of aquaculture products (Stickney, 2009). Reservoirs are managed much like fish ponds in many countries including China and Israel. In Malaysia, there are 63 large reservoirs with a total storage of 25 billion m^3 (Makhlough, 2008). According to Beveridge (1987), reservoirs have characteristics that make them less than ideal for aquaculture.

Reservoir's environmental conditions are intermediate between those in rivers and lakes. Differences between reservoirs compared to rivers and lakes are reflected in the morphology, hydrology, physicochemical and biological conditions and greatly affect fish culture in reservoirs (Li & Xu, 1995). Reservoir can be affected by precipitation, evaporation and ground movement. Runoff waters from rainfall to reservoir are larger than to natural lakes. Water bodies of reservoirs are exposed to extensive loads of dissolved and particulate matters.

Stratification normally happens in reservoir. If reservoir stratification happened, substrate exchange between surface and deep water reduces and eventually leading to anoxic conditions (Friedl & Wuest, 2002). According to Baharim (2011), during the stratification, the development of the thermocline layer prevents the circulation of oxygen from the epilimnion to the hypolimnion layer. Thermocline layer act as a physicochemical barrier to diffusion where organic material can sink through but salts or gases cannot diffuse across it (Meon & Busuiocescu, 1993). Accumulation of organic material in hypolimnion by dying phytoplankton and sinking of detritus through the thermocline can results in oxygen utilization which cannot be replaced from the epilimnion. Besides that, drawdown also occurs in reservoirs. In hypolimnion, drawdown may cause upwelling of deoxygenated water that is rich in hydrogen sulphide, H_2S (Beveridge, 1987).

2.2 Cage culture

Cage culture is commonly developed in reservoirs and lakes as an independent process parallel to the enhancement of capture fisheries. Cages can be in various shapes and sizes such as square, rectangular, or cylindrical in shape. Cages used in freshwater are usually small with volume of less than one to a few cubic meter. Floatation materials used such as styrofoam and sealed metal cans such as oil or grease drums can be used to float cages that are at the surface. Installation of cages in a water body will enhance harvests from the wild stock as nutrients and excess food become available for the fish. In other reservoirs, nutrient loading and sedimentation are caused by cage culture operations due to large inputs of nutrients (Abery et al., 2005). Nutrients become sediment and accumulated at the bottom of the reservoir under cages and can create considerable oxygen demand in the already low oxygen conditions.

2.3 Primary productivity

The method of measuring the production of oxygen is the light and dark bottle method. This method is use to determine the major components of primary production that can be estimated from measurements in closed systems. The major components to be measured are gross primary productivity (GPP), net primary productivity (NPP) and respiration (Cullen, 2001). GPP is the rate of photosynthesis which losses through excretion or respiration are not deducted. NPP refers to gross primary production after deducted losses to respiration by phytoplankton.

2.4 Water quality

Water quality is the basic chemical and physical characteristics of water that determine its suitability for aquaculture. During rainy season, the floodplain reservoirs store more water to protect the downstream area from flood damage and the water level increased during this period (Rast & Ryding, 1989). Water level fluctuates much greater in reservoir compared to natural lakes. In reservoirs, water quality deterioration usually comes from excessive nutrient inputs, eutrophication, acidification, heavy metal contamination, organic pollution, and obnoxious fishing practices.

2.4.1 Temperature

When upper and lower layers of reservoir's water body have great variation in temperature, thermocline usually develops. Each aquatic species has a temperature range within which helps enhance growth to optimal as long as other condition are appropriate and sufficient food of the proper quality is available. Thermocline which normally develops in deep reservoirs occurs at a depth of 8-23 m (Li & Xu, 1995). Thermal stratification occurred in reservoir is an important natural process that can have significant effect on the water resources quality. The potential changes in chemical contents in water resulting from stratifications are the production of ammonia, sulphide and algal nutrients which are important in reservoir cage culture (Baharim et al., 2011). Besides that, vertical distribution and change in water temperature has a great effect on the productivity and habitat of natural organisms in the reservoirs. However, effect varies from reservoir to reservoir.

2.4.2 Dissolved oxygen (DO)

DO is also one of the limiting environmental factors affecting fish feeding, growth and its metabolism. Dissolved oxygen is an important indicator of water quality, ecological status, productivity and health of the reservoir due to its importance as a respiratory gas and its use in biological and chemical reactions (Mustapha, 2008). For deep reservoirs, DO level at surface is usually higher than that in bottom, whereas, shallow reservoirs have similar DO content at the surface and bottom layers of shallow reservoirs (Li & Xu, 1995).

Fluctuation in DO is affected by photosynthesis, respiration and diel fluctuation. Thermal stratification affects DO in deep water bodies as the amount of oxygen that the water can hold at saturation is reduced if temperature, salinity and altitude increase. Reservoirs are unsuitable for aquatic life if stratification happens and lake's upper zone (epilimnion) is thermally divided from deeper zone (hypolimnion). The latter becomes stagnant and lacking in dissolved oxygen (anaerobic). According to Baharim (2011), dissolved oxygen reduction in the hypolimnion happened during insufficient wind action which decreases the extent of mixing between hypolimnion and epilimnion.

2.4.3 pH

Changes in pH directly affect utilization and transfer of nutrients and affect the physical condition and survival of fish in water (Li & Xu, 1995). Presence of carbon dioxide at surface water caused pH of the water to range between 6.0 to 6.8. Intense photosynthesis by phytoplankton, periphyton or submerged macrophytes can lead to increase in pH due to consumption of CO_2, perhaps to pH 9 to 10 in shallow reservoirs (Straskraba, 1999). Therefore, lakes that support large algal populations could be expected to show a pH that exceeds the pH of the supply waters prior to the growth of algae, at least near the lake surface and during seasons when the algae are photosynthesizing most rapidly.

2.4.4 Chlorophyll-*a*

Chlorophyll-*a* is an important indicator for the presence of algae, and large fluctuations in dissolved oxygen and pH are characteristics of eutrophication (Lee et al., 2006). In freshwater, phosphorus is often use as indicator of eutrophication. However to assist interpretation of the actual phosphorus content, chlorophyll-*a* levels or biomass of phytoplankton can be used (Wetzel, 2001).

2.4.5 Nutrients

Reservoirs are subject to higher element loading compared to natural lakes because of the greater catchment area and higher inflow rates (Straskraba, 1999). The concentration of nutrients varies from reservoir to reservoir due to differences in soil and vegetation in the catchment area and also hydrological condition of the reservoir (Li & Xu, 1995). According to Li & Xu (1995), nitrates, phosphates, silicate, and iron are important nutrients required by fish and bait organisms in reservoirs. Eutrophication and water quality problems can be cause by nutrient input from watershed. Eutrophication occur in reservoir due to its shallowness and it could affect the water quality of the reservoir by giving rise to unpleasant taste and odour, and affects dissolution of other gases, especially dissolved oxygen (Mustapha, 2008).

Reservoirs also have a higher capacity of retaining phosphorus (Straskraba, 1999). Naturally, phosphorus originates from the weathering of rocks. Phosphorus and nitrogen promote algal growth, which in turn promotes the growth of zooplankton (Stickney, 2005). Fry fish will also benefit from having plankton available upon which they can feed. For example, species such as tilapia, which accept prepared foods when they begin to feed, will also forage on plankton (Stickney, 2005).

3.0 Materials and Methods

3.1 Sampling locations

Batang Ai Hydroelectric Reservoir is a man-made reservoir which located 250 km south-east of Kuching, close to the border between Sarawak and Kalimantan (Figure 1). The reservoir contained average water volume of 750 million m^3. The water resources for the reservoir are from Batang Ai, Engkari River and precipitation. Water samples were collected at an area with cage culture and outside the cage culture in the month of October and November 2011 and April 2012. Exposure tests of fish in water condition of six different depths were conducted at the same time. Primary productivity was conducted at two area which were area with cage culture activities and area without cage culture activities.

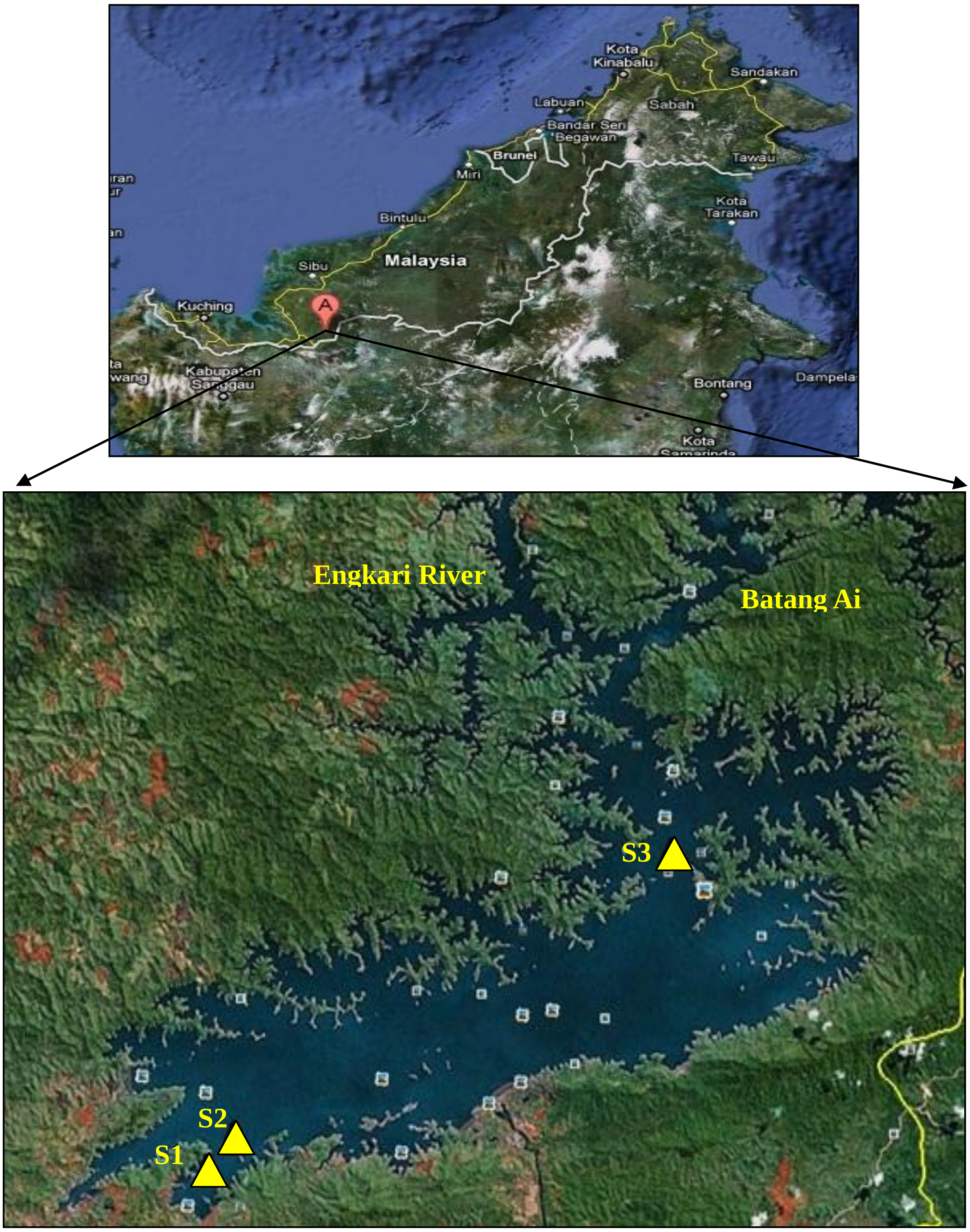

Figure 1: Map showing the three sampling stations at Batang Ai Reservoir (Source: Google Earth)

3.2 Experiment procedures

3.2.1 Water samples

The water samples were taken using Van Dorn water sampler at three stations (Table 1) and at five different depths which are subsurface, 5 m, 10 m, 20 m and 25 m (Figure 2). At each station, three replicates of *in-situ* parameters and water samples were taken at each depth for laboratory analysis. Water samples were kept in 2 L polyethylene water bottles that had undergo acid wash and were stored in cooler box filled with ice. All samples were taken to laboratory for further analysis.

Table 1: The coordinates of the three sampling stations

Stations	GPS	Description
1	N 01° 09’ 43.1” E 111° 50’ 09.7”	At the center to the Tiang Laju cage culture
2	N 01° 09’ 45.8” E 111° 50’ 16.2”	100 m away from Tiang Laju cage culture
3	N 01° 10’ 43.1” E 111° 55’ 20.0”	8.05 km away from the Tiang Laju cage culture

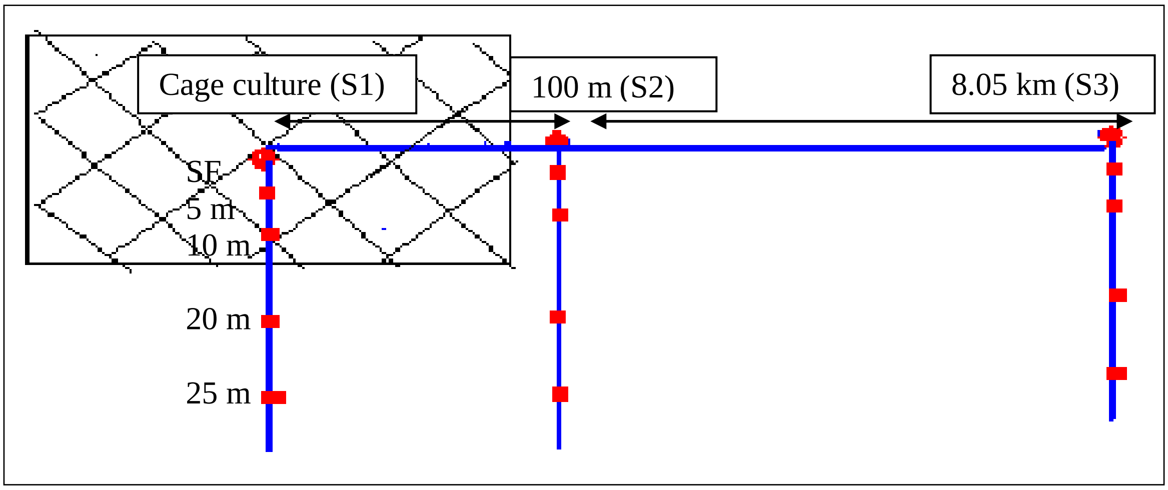

Figure 2: Horizontal and vertical view of sampling stations at Batang Ai Reservoir

3.2.2 *In-situ* water quality parameters

Temperature, dissolved oxygen (DO), pH, electrical conductivity, and total dissolved solids (TDS) were taken using Eutech Multiparameter Series 600. Turbidity was measured using Eutech Turbidity Instrument TN-100. The depth of each station was measured using depth finder. Water transparency was measured using secchi disc at each station. All the parameters were recorded in triplicates during each time water samples were collected. TDS was recorded in triplicates for October and November 2011.

3.2.3 Laboratory analysis

Water quality parameters such as biochemical oxygen demand in five days (BOD_5), total suspended solids (TSS), chlorophyll-*a*, ammonia-nitrogen, nitrate, nitrite, orthophosphate, silica and chemical oxygen demand (COD) were carried out in the laboratory.

3.2.3.1 Biochemical oxygen demand in five days (BOD_5)

BOD_5 was determined for November 2011 and April 2012. To determine the BOD_5 values, water samples was filled into 300 ml BOD bottles. Dilution was done on water samples using distilled water with dilution factor of 1:2. Then the water was shaken to aerate it. DO readings of the water samples were measured from the bottles. All BOD bottles were wrapped with aluminium foil to prevent light penetration and were kept in a cooler box for 5 days. The initial DO value is the value for D_1. On the 5^{th} day, DO readings were recorded as the value for D_5. The formula that was used for measuring BOD_5, followed the protocol outlined by APHA (1998):

$$BOD_5 \text{ (mg/L)} = \frac{D_1 - D_5}{V}$$

Where, D_1 = Initial DO of sample immediately after preparation (mg/L)
D_5 = DO value after 5 days incubation at 25°C (mg/L)
V = Proportion of dilution used

3.2.3.2 Total suspended solids (TSS)

Total suspended solids were analyzed using standard method APHA (1998). For TSS analysis, there were pre-fieldtrip sampling method and post-fieldtrip method. For pre-fieldtrip method, glass fibre filter paper was soaked in distilled water. Each filter paper was placed on a piece of aluminium foil and was dried in the oven at 103°C-105°C overnight. Filter paper was allowed to cool for at least 10 minutes before weighing using analytical balanced. The initial reading of filter paper was recorded. For post-fieldtrip method, the glass fibre filter paper was placed on the inter-plate of the filter funnel using forcep. A known volume of water samples were filtered using the vacuum pump. After that, filter paper was removed from the filtration funnel and it will be placed back to aluminium foil. Filter paper was dried in the oven at 103°C-105°C overnight. Filter paper was taken out of the oven and allowed to cool until reading room temperature before weighing. Final reading of the filtered glass fibre filter paper was recorded and TSS was calculated using formula:

$$TSS \text{ (mg/L)} = \frac{W_F - W_I}{V}$$

Where, W_I = Initial weight of filter paper
W_F = Final weight of filter paper
V = Volume of water samples filtered (L)

3.2.3.3 Chlorophyll-*a*

The concentration of chlorophyll-*a* in water samples was analyzed using standard method APHA (1998). For chlorophyll-*a* analysis, water samples were filtered using vacuum pump. Filter paper containing chlorophyll-*a* was taken for analysis. The samples were grinded by using a grinder and 5-6 mL of 90% acetone were added into the mortar. Samples were grinded for about 5 minutes and all materials in the mortar were placed into a capped test tube. Ninety percent acetone was added into test tube to make up the volume to 10 mL. Test tube was folded with aluminium foil and was placed in the refrigerator for 4-18 hours to facilitate complete extraction of the pigments. Then the liquid extracted was transferred into centrifuge tube. The samples was placed into a centrifuge for about 10 minutes under 3000 rpm. Optical density was determined using spectrophotometer at wavelength of 750 nm, 664 nm, 647 nm, and 630 nm. Each extinction for small turbidity blank was corrected by subtracting 750 nm from 664 nm, 647 nm, and 630 nm absorptions.

The concentration of chlorophyll-*a* in the extract of the pigment after correction:

$$C_a = 11.85(E_{664}–E_{750}) – 1.54(E_{647}–E_{750}) – 0.08(E_{630}–E_{750})$$

Where E = the absorption in the respective wavelength

After determining the concentration chlorophyll-*a* in the extract, the amount of chlorophyll-*a* in the pigment per unit volume of water filtered was calculated as follows:

$$\text{Chlorophyll-}a\ (\mu g/L) = \frac{C_a\,(v)}{VL}$$

Where C_a= Chlorophyll-*a* pigment concentration in µg/mL
v = Volume of acetone in mL
V= Volume of samples in L

3.2.3.4 Ammonia-nitrogen (NH_3–N)

For ammonia-nitrogen (NH_3–N), the concentration was determined using standard method 8038, Nessler Method. Polyvinyl Alcohol Dispersing Agent was added. Ammonia concentration was measured using DR 2010 Spectrophotometer (HACH, 2000).

3.2.3.5 Nitrate (NO_3^-)

For nitrate analysis, the concentration was determined using standard method 8192, Cadmium Reduction Method. Nitrate in the sample was reduced to nitrite by cadmium metal where nitrite ions reacted with sulfanilic acid in an acidic medium. Nitrate concentration was measured using DR 2010 Spectrophotometer (HACH, 2000).

3.2.3.6 Nitrite (NO_2^-)

For nitrite analysis, the concentration was determined using standard method 8507, Diazotization Method. Nitrite in the sample reacted with sulfanilic acid. The amount of nitrite was measured using DR 2010 Spectrophotometer (HACH, 2000).

3.2.3.7 Orthophosphate (PO_4^{3-})

For orthophosphate analysis, the concentration was determined using standard method 8048, Phos Ver3 Method. Concentration of orthophosphate was measured using DR 2010 Spectrophotometer (HACH, 2000).

3.2.3.8 Silica (SiO_4)

For silica analysis, the concentration was determined using standard method 8186, Heteropoly Blue Method. Silica reacted with molybate ion under acidic condition. The concentration of silica was measured using DR 2010 Spectrophotometer (HACH, 2000).