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Relationship Pattern of Soil and Water Quality in Sustaining Natural Feed Stocks and the Life of Milkfish, *Chanos chanos* (Forsskal, 1775): The Case study on a Traditional Pond in Kedungpeluk Village, Candi, Sidoarjo, East Java, Indonesia

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Abstract: Traditional aquaculture activities in Kedungpeluk are currently facing the problem of low Survival (SR) and slow growth. The purpose of this study is to examine the development of soil and water quality as well as the behavior of soil and water quality parameters, which act as a limiting factor and determinant of the quality of natural food and fish life during rearing. The study is descriptive and developmental. Data collection is done through longitudinal surveys. The results showed that the pond bottom soil is very acidic and anaerobic, whereas the water is alkaline and aerobic. There are indications of hydrolysis of NH_4 bound to the mud to the water above it, changing NH_4 into $NH_3 + H^+$ and, of decreasing the amount of oxygen as it is absorbed by the base layer of anaerobic soil because of the stirring by the milkfish in search of food. Natural feed quality has decreased because it is dominated by species of phytoplankton of Cyanophyta hated by the milkfish. The condition is considered a causative factor of low survival and slow growth.

Keywords: Soil and Water Quality, Phytoplankton, Natural Feed and Milfish

Introduction

Fish farms in Kedungpeluk are one of the regional development centers for pond arrangement in Sidoarjo, East Java, Indonesia. Fish farms in Kedungpeluk are mostly traditional ponds, major commodity cultivated is milkfish and the pond model is locally known as "Porong type" commonly applied by farmers in Sidoarjo. Milkfish aquaculture is very important in Indonesia, mainly for domestic consumption, the local economy and food security. Smoked milkfish and *presto* milkfish are two very popular local foods in the community. In addition, milkfish can be used as a fishing lure in fishing in the sea.

The milkfish aquaculture has been facing problems of slow growth and low production. A preliminary survey in 2010 in the village which is the center of milkfish aquaculture, Kedungpeluk, Sidoarjo, shows the size of juvenile reared for 4 months has become smaller-as 1 kg used to contain 4 to 5 fish, now it contains 6 to 7 fish. Under normal conditions, juvenile aged 3 to 3.5 months can weigh until 250 grams (Masykur, 2009). Milkfish aquaculture technology applied is traditional and ponds are used continuously. According to Buwono (1993), the quality of the pond bottom usually decreases as time goes by. Carrying capacity of the pond also decreases due to load of organic material input, both from outside and from inside the pond.

In terms of fish farming in traditional ponds (extensive), sediment or soil base and water play an important role in growing natural feed and fish life. The success rate of fish farming in ponds is largely determined by the quality of base soil and water. Sediment is inseparable of the aquatic environment and its role is to determine the quality of aquatic environments. Sediment or base soil plays several roles including (1) demolishing organic material by bacteria, (2) as a regulator of water quality (DO, free CO_2, H_2S) and (3) determining the level of fertility of pond water (Andayani *et al*., 1994).

Water, as the medium of fish life, has a direct effect on the health and growth of fish. The quality of water determines the existence of various types of organisms in the pond ecosystem, both for the cultivated organisms and other biota as a constituent of the pond ecosystem. Water quality far from optimal values may lead to failure of the cultivation. Good water quality is an absolute requirement of cultivation to produce high productivity. In terms of physics, chemistry and biology, water has several functions to support fish life and natural feed, as follows: (1) In terms of physics, water is a place to live and provide space for the fish; (2) in terms of chemistry, water acts as a carrier of nutrients elements, vitamins and other dissolved gases; and (3) in terms of biology, it is a good medium for biological activity as well as the formation and decomposition of organic matter.

This study examines the relationship pattern of soil and water quality in the preparation of natural feed to sustain fish life. The purpose of this study is to determine (1) the development of soil and water quality during cultivation and (2) the behavior of soil and water quality parameters, which acts as a limiting factor and determinant of the quality of natural feed and fish life.

Research Methods

The research took place in Kedungpeluk village, in one of the ponds, with the following considerations: (1) The fishpond is traditionally managed; (2) major commodity cultivated is milkfish; and (3) the pond model is locally known as “Porong type” commonly applied by farmers in Sidoarjo. This study was conducted in one cycle of cultivation, approximately for six months from January to June 2011, as shown in Appendix 1.

The study is descriptive and developmental. Descriptive study systematically describes the facts and characteristic of the following elements: Base soil and water, natural feed and properly cultivated fish. The developmental study is to determine the development of these elements and their interactions over a period of time. Data collection is done through longitudinal surveys i.e., the development of data collection is done through the process and timing of a number of samples observed or measured continuously every 2 weeks following the development period.

The method of measuring soil quality parameters is performed as follows: (1) Soil acidity (pH) and (2) The redox potential (Eh). Prepare air-dried soil samples and distilled water with ratio of volume per volume of 1: 1. Inserting into the film bottle, shake the soil solution by means of swinging by hand, as many as 15 times, measuring pH and Eh with use pH and Eh meter. (3) C organic. Soil samples size <0.5 mm as much as 0.500 g, put into volumetric flask 100 mL. Added 5 mL $K_2Cr_2O_7$ 1 N, shaked, then added 7.5 mL of H_2SO_4 of concentrated, shaked and allowed to stand 30 min. Diluted with water free of ion and allow to cool. The next day the measured of absorbance from clear solution, with spectrophotometer λ 561 nm. As a comparison was made standard 0 and 250 ppm, by pipette 0 and 5 mL from standard solution 5.000 ppm into a flask of 100 mL with the same treatment as workmanship of examples. (4) N total. Move qualitatively, the entire sample extracts into the boiling flask (used of water free of ion and pumpkin of spray). Adding a bit of powder boiling stone and distilled water until half the volume of the flask. Prepare a container for NH_3 that were freed on erlenmeyer containing 10 mL of boric acid 1%, plus 3 drops of indicator Conway and connected by tool of distillation. Added 10 mL NaOH 40% into the boiling flask containing the sample and closed. Distilled to a volume of container: 50-75 mL (green color). Distillate titrated with H_2SO_4 0.050 N until pink and noted titar sample volume (Vc) and blank (Vb). (6) P olsen. Inserted 1000 g of soil samples <2 mm, into the shake bottle, plus 20 mL extraction Olsen and shaken for 30 min. Strain and if the solution is still turbid, filtered again as before. Extract, taken 2 mL with pipette, put in a test tube and further along with the standard solution was added 10 mL phosphate dye reagents, shake until homogeneous and allowed during 30 min. Absorbance of the solution was measured with a spectrophotometer λ 693 nm. (7) Measurement of $NH4^+$ (cation exchange force) can be determined by the method of the Blue indofenol. Pipette 0.5 mL each perkolat NaCl and standard series NH_4^+ (0; 2.5; 5; 10; 15; 20 and 25 me L^{-1}) into a test tube. Each tube plus 9.5 mL of water free of ion (dilution 20 x). Pipette into another test tube each 2 mL of an aqueous extract and standard series. Added consecutive buffering solution Tartrate and Na-phenate respectively: 4 mL, shake and let stand for 10 min. Add 4 mL of NaOCl 5%, shake and measured with a spectrophotometer λ 636 nm after 10 min since the administration of these reagents. (8) NH_4^+. Inserted 20 g soil sample of finely <2 mm into the shake bottle 100 mL, add 1 mL of activated carbon and 40 mL extractors Morgan Wolf. Shake for 5 min with a shaker machine at minimum 180 wobble min^{-1}. Filter with filter paper of Whatman # 1, to obtain a clear extract. Pipette into test tubes each 2 mL of the extract sample and standard series. Adding consecutive: Tartrate buffering solution and Na-phenate each 4 mL, shake and let stand for 10 min. Add 4 mL NaOCl 5%, shake and measured with a spectrophotometer λ 636 nm after 10 min since the administration of these reagents. (9) NO_3. Pipette each 5 mL extract sample and standard series into a test tube.

Added consecutive brucine solution of 0.5 and 5 mL H_2SO_4 of concentrated (p.a.), he whipped until homogeneous and allowed 30 min. The solution is measured with a spectrophotometer λ 432 nm.

The method of measuring water quality parameters is performed as follows: (1) Brightness. Secchi disc is inserted into the water up to the limit visible and note its depth. Slowly, secchi disc pulled back to be seen again and note its depth. The average of the two readings is the brightness value. (2) Temperature. Thermometer Hg dipped in water, allowed during for some time and read the temperature designated by Hg (temperature readings, the thermometer still in the water). (3) pH. Before the pH meter is used, calibrated with buffer solutions pH 4, 7 and 9. The electrode was washed with distilled water, dried with tissue, put in a water sample, allowed during for some time (stable value) and recorded value of pH. (4) DO. Taking water samples with BOD bottles using "Kemmerer water sampler", so there is no contact with air. The sample in the BOD bottles are given 1 mL of KI alkali and 1 mL $MnSO_4$, stirred by flipping through the bottle until homogeneous and allowed to precipitates. Most of the water samples were clear upper discarded, given 2 mL H_2SO_4 of concentrated and stirred until the precipitate dissolved. Titration immediately with $Na_2S_2O_3$ until the color changes from brown to yellow, was given 1-2 drops of starch, titration again until the blue color disappeared. Record the volume of titrant used. (5) free CO2. 50 mL water sample in Erlenmeyer given PP indicator with a pipette, stirring and when no color means it contains free CO_2 gas. Titration with Na_2CO_3 0.0227 N until it turns pink and record the volume of titrant used. (6) TOM. 25 mL water sample was given 0.5 mL of H_2SO_4 6 N and a few drops of $KMnO_4$ 0.01 N until the solution is pink and given again of solution 10 mL $KMnO_4$ 0.01 N until the color becomes red. The solution is heated in the flask erlenmeyer during 10 min. The solution was appointed and allowed of the temperature to drop 80°C, was given 10 mL oxalic acid 0.01 N and the solution became colorless. In a fixed temperature of 70-80°C, the solution was titrated with $KMnO_4$ 0:01 N until color becomes pink usage of $KMnO_4$ 0.01 N as a measure of the amount of organic material that is soluble in water. (7) Ammonia. Water samples were filtered with Whatman paper No. 42. Taken 25 mL samples of filtered water and put in a 50 mL beaker make the standard solution with a concentration of 0.1; 0:25; 0:50; 0.75 and 1.0 ppm N as NH_4^+. Water samples and standard solution were each given Nessler and shake until evenly distributed. The solution is measured with a spectrophotometer λ 430 nm. (8) Nitrate. Taking 5 mL sample of water that has been filtered, put in a 50 mL beaker, added 0.5 mL brucine solution and 5 ml H_2SO_4 of concentrated. Stirred and allowed to stand during 15 min. Make the standard solution Nitrate 0.75; 0.5; 0.25; 0.10; 0.05 and 0.025 ppm N as Nitrate. Benchmarking is done with spectrophotometer λ 410 nm. (9) Orthophosphate. Take 50 mL of water sample and put in a beaker100 mL. Make phosphate standard solution with a concentration of 0.025; 0.05; 0.10; 0.25; 0.50; 0.75 and 1.00 ppm P. The water sample and the standard solution of P each plus 2 ml ammonium molybdate-sulfuric acid and stirred until uniform. Be given 5 drops of $SnCl_2$ and shaked until the resulting blue color of his sharpness comparable to concentrations of phosphorus levels. Comparisons were made with the spectrophotometer λ 690 nm. (10) Salinity, Opening glass handrefractometer and wash with distilled water. Drops water samples in glass of handrefractometer. Handrefraktometer binoculars towards the light source and record the salinity value.

Summary of methods and tools as well as the references used in the determination of the main parameters: Soil quality, water quality and natural food is presented in Appendix 2. Specific phytoplankton sampling is done by filtering as much as 10 liters of water with plankton nets Number 25. Phytoplankton observations and calculations are performed with microscope and Sedgwick Rafter Counting Chamber (SRC). Observation of the predicted condition of fish is done at the time of harvest.

Results and Discussion

Soil Quality

Soil was taken at the beginning and end of cultivation cycle with consideration that changes in soil quality would be much longer than the water quality. Each plot was represented by one composite sample taken at some points considered representatives. The results of observation of soil quality are shown in Table 1.

From the table, it can be seen that during cultivation soil was very acidic, redox potential was negative (anaerobic), while organic C, total N and C/N ratio is categorized as moderate. In acidic soil, nitrate tends to sequestered by the soil colloids, ammonium (NH_4^+) is dominant and phosphate retention occurs due to the reaction of phosphate by Fe, Al and oxide hydrates (Soemarno, 2011). According to Boyd (1982), most of the phosphorus entering the ponds is bound by the soil and small amount becomes water-soluble fraction. Based on the value of CV, there are several parameters of soil quality namely C/N ratio, P Olsen (available), NH_4^+ and varied nitrate. Patel and Shiyan (2001) confirm that a benchmark (critical limit) for CV based on a large number of experiments conducted at different times is 33%. This means that changes in organic matter and soil nutrient are varied and unstable.

Table 1. Quality of soil at the beginning and end of cultivation cycle

No.	Parameter	N	Min.	Max.	Mean	Standard deviation	Coefficient variation
1	pH	6	4.00	4.60	4.333333	0.2338090	5.3955932
2	Redox potential (mV)	6	-124.00	-86.00	-99.000000	14.1279860	-14.2706900
3	C organic (%)	6	2.24	4.07	2.775000	0.6678847	24.0679180
4	N Total (%)	6	0.20	0.34	0.270000	0.0513809	19.0299740
5	C/N ratio	6	7.68	20.35	10.881670	4.7803406	43.9302250
6	P Olsen (mg kg^{-1})	6	9.85	49.84	24.810000	14.7827750	59.5839380
7	KTK (me/100 g)	6	47.50	58.54	52.030000	3.9444645	7.5811349
8	NH_4 (mg kg^{-1})	6	28.50	74.25	47.263330	15.9072090	33.6565540
9	NO_3 (mg kg^{-1})	6	26.73	77.83	49.543330	18.0752280	36.4836740

Table 2. Quality of water at the beginning and end of cultivation cycle

Parameter	N	Min	Max	Average	STDEV	CV
Temperature (°C)	13	29.67	33.67	31.83153846	1.430651636	4.494447032
Light (Cm)	13	16.5	29	24.03846154	4.259604532	17.71995485
pH	13	7.53	10.03	8.855384615	0.91045424	10.28136303
DO (ppm)	13	6.82	11.71	9.543076923	1.373088882	14.38832457
CO_2 (ppm)	13	-	-	-	-	-
TOM (ppm)	13	3.16	36.66	13.07923077	9.995403495	76.42195226
Ammonia (ppm)	13	0	3.32	0.656923077	0.959065384	145.9935596
Salinity ($^0/_{00}$)	13	-	-	-	-	-
Nitrate (ppm)	13	0.97	2.86	1.76	0.541956333	30.79297345
Orthophosphate (ppm)	13	0.04	0.15	0.094615385	0.037330815	39.45533286

Water Quality

Water sample was taken once in 2 weeks as many as 8 times (during cultivation) following the time change of the water. Each plot was represented by one composite sample taken at some point considered representative. The results of observation of water quality are shown in Table 2.

Ammonia amount at some time exceed the maximum limit of quality standards established. Ammonia in the research farm ranged from 0 to 3.32 ppm with an average of 0.66 ppm. According to the Regulation Number 82/2001, the limit of ammonia compounds in water is 0.5 ppm. Nitrate concentrations ranged from 0.66 to 2.86 ppm with an average of 1,689 ppm and orthophosphate ranged from 0.04 to 0.3 ppm with an average of 0.096 ppm. Fertility of water viewed from N (nitrate) is at mesotrophic level and P (orthophosphate) is eutrophic According to Wetzel (1975), nitrates >1-5 ppm is mesotrophic and orthophosphate of 0.031 to 0.100 ppm is eutrophic. According Welch and Lindeel (1980), P nutrient is a key parameter in determining the fertility of waters, as compared to N; therefore, the condition of water in the category eutrophic is also characterized by a high DO and pH and low light and low CO_2. Dissolved Oxygen (DO) ranged from 5.59 to 12.81 ppm with a mean of 9.56 ppm, light ranged from 15 to 29 cm with an average of 23.624 cm and pH ranged from 7.5 to 10.03, with an average of 8.85. Furthermore, the low salinity concentration indicates fresh waters. Based on the CV value, there are several water quality parameters, namely TOM, ammonia and orthophosphate. Patel and Shiyan (2001) confirm that a benchmark (critical limit) for CV based on a large number of experiments conducted at different times is 33%. The ponds have eutrophic conditions, are unstable and contaminated.

The Abundance and Composition of Natural Feed Phytoplankton

Phytoplankton samples were taken following the observation of sampling for water quality. The observation of phytoplankton in the juvenile and semi-adult pond is shown in Fig. 1 and in adult pond is shown in Fig. 2.

Phytoplankton is a biological parameter that can be used as an indicator to evaluate the quality and level of fertility of the water. Changes to water quality and fertility rate are closely related to water potential in terms of abundance and composition of phytoplankton (Ferianita *et al*., 2005). Cyanophyta or Cyanobacteria dominate phytoplankton found in ponds during cultivation. Cyanobacteria are unicellular, live in colony and have the shape of a filament. Each individual cell generally has a thick cell wall and is covered by mucus, flexible and gram negative. When ingested, these cell types are difficult to digest (Segner *et al*., 1987) and can be toxic to fish (Bhaskar and Rao, 1990; Villaluz and Unggui, 1983). The fertility rate based on the abundance of phytoplankton found is eutrophic (in juvenile and semi-adult ponds of 2,204,982.00-30,500,606 individual/ml and in the adult pond of 2,284,796-33,823,049 individual/ml). According to Landner (1976), the abundance of phytoplankton 0-

2000 individual/ml belongs to oligotrophic, 2000-15000 individual/ml belongs to mesotrophic and more than 15000 individual/ml belongs to eutrophic.

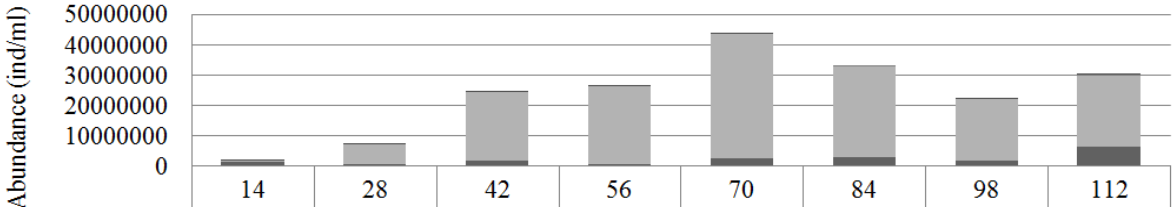
Milkfish Condition

Fish seed reared in pond with a stocking density of 2 *rean* (10,000 head)/20,500 m^2 or about 5000 head/ha, the cultivation period is 3 months and the productivity is 6,056 fish weighing 367 kg. The results of mathematical calculations show that Survival (SR) was only 60.56% and 1 kg contained about 16 to 17 fish. In the business scale, this SR is relatively low and larger size describes slow growth of fish. According to Masykur (2009), under normal conditions on aquaculture, juvenile aged 3-3.5 months, can reach a size of 250 grams of individual fish.

Soil and Water Quality in Sustaining Natural Feed Stocks and the Life of Milkfish

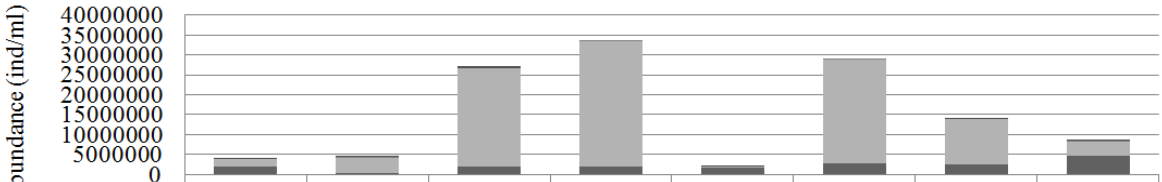
Base soil of the ponds in Kedungpeluk that is very acid and anaerobic is inversely proportional to the water on it that is alkaline and aerobic. This can occur because of the changes of water and the relatively fertile water that irrigates the pond. The presence of a compound of carbon dioxide from the organic material on the pond bottom and nutrients, especially phosphorus in the pond water with high temperature and light, will also trigger the growth of phytoplankton of algae types, Cyanophyta, that can specifically bind nitrogen from the air faster. This will lead to the upper water column bases to be alkaline and aerobic because of the high activity of the photosynthetic process, while the water column below is an interface layer of both conditions. This condition has occurred on the research farm, characterized by the predominance of Cyanophyta and high photosynthetic activity (due to the low CO_2). Such circumstances are not good for the life of the farmed fish as phytoplankton growing rapidly is not a natural food of fish; nutrients depletion may happen as fish relies solely on nutrients from water as the base soil is very acid and anaerobic. According to Hanafi (2008), on acid soils, phosphorus nutrients cannot be utilized as they are bound by the element of Al and Fe. The impact is mass mortality of phytoplankton and threat to the life of fish. This is reinforced by the FAO (2012) stating that fish pond which is always flooded with water or in which water is difficult to flow out throughout the year in the tropics wet (in the tropics with high rainfall) with warm water and a high light provides excellent possibilities for fish farming. However, in the absence of proper environmental management, overall nutrient deficiency in fertile water may occur, although it has a very high production potential. This is because the pond bottom soil has very high levels of organic nutrients in conditions almost locked up and not used because of the nature of the anaerobic pond bottom. It is indicated to have occurred in adult ponds, where the nutrients in the water column on day 70 of cultivation has been reduced or discharged, while the pond bottom cannot provide nutrients because it is very sour and anaerobic. The process of phytoplankton death has occurred, marked by the abundance of phytoplankton were low (Fig. 2).

Furthermore, NH4 accumulation at the base soil that is very acidic and alkaline water (high pH) would be very harmful to fish life. It can be seen that the pond bottom soil stirred by the fish in search of food at the base, NH4 in alkaline water will be hydrolyzed to NH_3 and H+ ions. NH_3 resulted depends on the extent of alkaline water can affect soil pH. Likewise, the anaerobic base soil when mixed with water will lead to dramatic decrease on the availability of oxygen in the water as it is absorbed by the soil. A high level of reduction occurs on farms that have been used for long and haven not gone through reclamation after harvest (Jaya *et al*., 1994). Salinity of the ponds studied was found to be low or it can be said as fresh waters. Subsequent impact of high ammonia concentration will lead to the formation of free NH_3-N that is toxic to fish, especially at low salinity or fresh water with a high temperature and pH (Boyd and Tucker, 1998).



	14	28	42	56	70	84	98	112
Euglenophyta	9977	19954	59864	19955	39909	0	19954	29932
Chrysophyta	239455	319273	399092	299320	419045	219501	59864	508843
Cyanophyta	448978	6345563	22359130	25651639	40996724	29921922	20752782	23396768
Clorophyta	1506572	878002	2005439	927888	2614049	3142850	1785934	6565063

Fig. 1. Phytoplankton in the juvenile and semi-adult pond during cultivation



	1	2	3	4	5	6	7	8
Euglenophyta	9977	29932	49887	0	19954	0	29932	199546
Chrysophyta	249432	429024	488890	329252	229476	159638	169614	289342
Cyanophyta	2055324	3771418	24623977	31647996	319272	26190412	11643509	3472100
Clorophyta	1855778	399091	1915643	1845801	1716094	2703849	2364621	4679354

Fig. 2. Phytoplankton in the adult pond during cultivation

Conclusion

Based on the results of the study, some conclusions can now be drawn. The interface layer on ponds can occur because the base soil is acidic and anaerobic, while the water on top of it is alkaline and aerobic (due to the fertile water). Phytoplankton that grows in such waters is dominated by Cyanophyt, which is not natural feed for the fish and mass death will occur at the time of depleted nutrients because the soil is acidic and anaerobic. Further accumulation of NH_4 on acid and anaerobic soils stirred by the water on top of it, which is alkaline, leads to hydrolysis of NH_4 that changes it into NH_3 and H^+ ions. Likewise, oxygen will drop dramatically as it is absorbed by the anaerobic soil. The condition is considered a causative factor for low SR and slow growing of fish.

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Author's Contributions

Muhammad Musa: A substantial contribution to the concept and design, data collection, interpretation and preparation for writing this article.

Soemarno: Supervisor for experimentation and methodologies, to help provide guidance for data collection and interpretation, preparation of articles and reviews.

UunYanuhari: Helping the analysis and interpretation of data, critical review of research for significant intellectual content and clarity of data, results and discussion are used for this article.

Ethics

This article is the original research and not yet published. The team of authors have read and approved the manuscript and there are no ethical issues involved.

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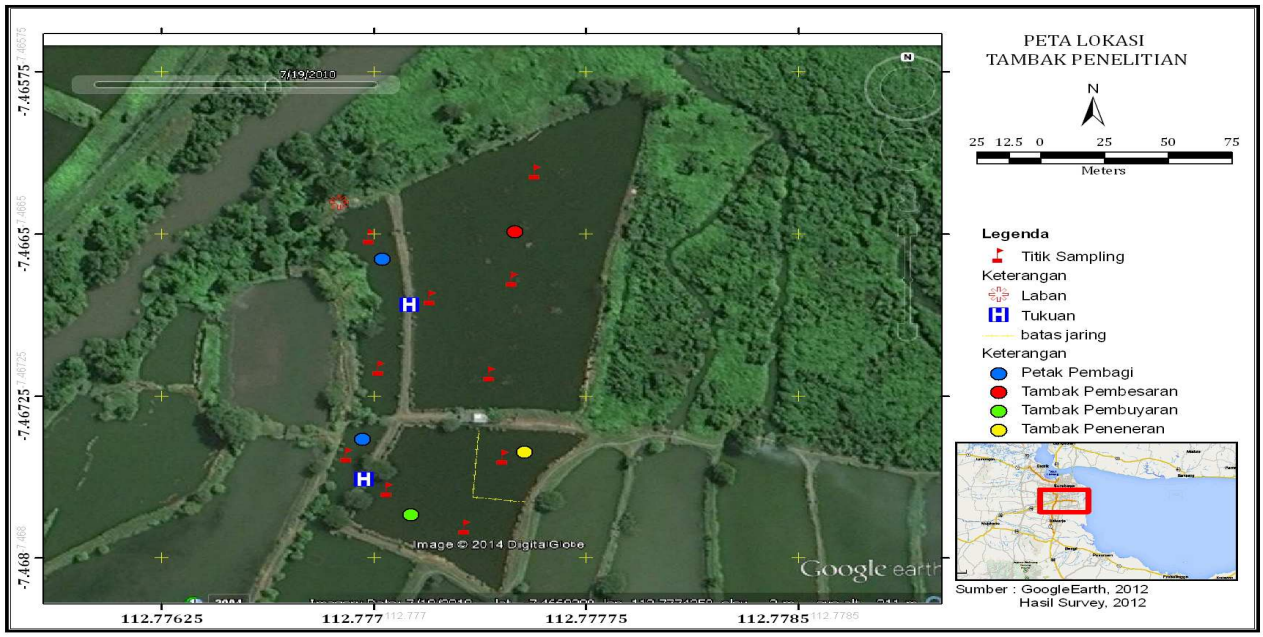
Appendix 1.



Appendix 2.

Water quality

No	Parameter	Methods* and Tools **	Source library
1.	Light (Cm)	Secchi disc**	Jaya *et al*. (1994)
2.	Temperature (0C)	Termometer Hg**	Jaya *et al*. (1994)
3.	pH	pH meter**	Jaya *et al*. (1994)
4.	DO (ppm)	Winkler titimetri*	Wisnuprapto (1987)
5.	CO2 (ppm)	Karbonattitimetri*	Saeni and Darusman (1989)
6.	TOM (ppm)	Permanganometri	Saeni and Darusman (1989)
7.	Ammonia (ppm)	Ammonium Chlorida*	Swingle (1969)
8.	Salinity ($^0/_{00}$)	Refraktometer**	Jaya *et al*. (1994)
9.	Nitrate (ppm)	Brusin*	Saeni and Darusman (1989)
10.	Orthophosphate (ppm)	Ammonium molibdatasamsulfat**	Swingle (1969)
Soil quality			
1.	pH	Soil tester**	Sulaiman *et al*. (2005)
2.	Redox potential (mV)	Milivoltmeter**	Jaya *et al*. (1994)
3.	C organic (%)	C organik**	Sulaiman *et al*. (2005)
4.	N Total (%)	Kjeldahl	-
5.	C/N rasio	-	-
6.	P Olsen (mg kg^{-1})	spektrofotometer**	Sulaiman *et al*. (2005)
7.	KTK (me/100 g)	NH_4OAC pH: 7 1 N*	Sulaiman *et al*. (2005)
8.	NH_4 (mg kg^{-1})	Kolorimetri*	Jaya *et al*. (1994)
9.	NO_3 (mg kg^{-1})	Kjeldahl	-
Natural feed			
1.	Type, composition and abundance of plankton	Microscope **	Davis (1955; Ward and Whipple, 1959; Needham and Needham, 1969; APHA, 1985)