The temporal variation of ammonia and nitrite content in extensive ponds with tilapia

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Abstract. The accumulation of organic material in an extensive pond can cause accumulated ammonia and nitrite compounds, which can be toxic for aquaculture organisms, reducing the rates of fishery production. The purpose of this study was to determine the temporal variations of the levels of ammonia and nitrite in extensive ponds. This study used a survey method on three plots of extensive ponds, conducted every two weeks. Samples were analyzed using the IBM SPSS application with the One-Way ANOVA to determine whether there were differences in ammonia and nitrite levels among the measurement times. In addition to ammonia and nitrite contents, there were also some supporting parameters observed, namely dissolved oxygen concentration, temperature, pH and salinity. The measurement results showed that there were significant changes in ammonia levels ($P<0.01$), with values of 0.112-0.537 mg L^{-1}, and nitrite levels also presented significant differences ($P<0.05$), with values of 0.01-0.12 mg L^{-1}. The measurements of the supporting parameters during the study showed fluctuating results, with pH values between 8.4 and 9.4, temperatures between 31.95 and 35.63°C, dissolved oxygen levels between 6.13 and 9.04 mg L^{-1}, and salinity values from 8.33 to 25.33 mg L^{-1}. There were temporal variations in ammonia and nitrite levels. Fluctuations in ammonia and nitrite levels in extensive ponds did not endanger the cultivation of organisms. The results of hemoglobin from fish cultivation showed that it was in normal levels.

Key Words: cultivation system, natural ecosystems, toxic, water quality.

Introduction. Ammonia and nitrite are toxic compounds found in ponds due to the accumulation of organic material. Organic material in these waters can be found in the form of fish feces, urine, and dead plankton, among others (Effendi et al 2015). The high concentration of ammonia and nitrite compounds is one indicator of a decreased water quality, which can lead to a low production rate of aquaculture.

Ammonia is the final product from the metabolic process of protein, which is excreted by fish. The process of decomposition of organic material is influenced by bacteria (Bhatnagar & Devi 2013). Ammonia compounds that have been ionized in aquaculture ponds will be oxidized by *Nitrosomonas* sp. and become nitrites if the dissolved oxygen level in the water is sufficient. Nitrite is able to kill fish because it can oxidize hemoglobin to methaemoglobin in the blood, blocking the respiration process. It changes the color of the blood, the gills become brown, and it causes damage to the nervous system, liver, spleen, and kidneys of cultivated fish (Bhatnagar & Devi 2013).

Fish farming in Indonesia generally uses extensive systems. Ponds with extensive systems during maintenance activities do not use water changes at all, because of very limited water availability (Dede et al 2014). This causes the water quality to decrease because of a higher accumulation of organic matter, resulting in ammonia and nitrite compounds. One such farm is located in Gresik, Indonesia, with an area of 15.6 ha with a total production of 53.8 tons in 2017 (Statistic-Jawa Timur 2017a; 2017b). Most of the ponds in this area use an extensive system with rainwater without water changes, the availibility of water being limited during the cultivation process. Due to this, the water gets saturated with different compounds.

Therefore, during the aquaculture process, the ammonia and nitrite levels have fluctuations. This study aimed to determine the temporal variations in the levels of ammonia and nitrite in extensive ponds, so that fish farmers can anticipate the negative effects caused by these two compounds and improve fish production.

Material and Method

Location. This research was conducted from September to December 2017. The overview of the sampling locations was conducted in three extensive ponds located in Tanggulrejo Village, Manyar Sub-district, Gresik District, East Java, Indonesia. The coordinates are 7°05'40.1"S 112°32'21.8"E. 3 ponds were observed (Figure 1).

The research was conducted in several stages, and it included sampling, measurements of ammonia, measurements of nitrites, measurements of other water quality parameters. Also, blood was collected from fish and hemoglobin concentrations were determined as supporting parameters for nitrite levels.

Figure 1. Sampling locations. X - points of sampling.

Research media preparation. The water sample collection was conducted at 4 PM, because at that time the interaction of photosynthesis and respiration would have a high influence on the water pH. Ponds were in peak condition before sunset, so the highest ammonia concentration occurred in the afternoon (Wurts 1992). Sample collection was conducted in four months, from September to December. Sampling was conducted seven times, every two weeks. The measurement are marked as follows: P1 - September 10, 2017; P2 - September 24, 2017; P3 - October 8, 2017; P4 - October 22, 2017; P5 - November 5, 2017; P6 - November 19, 2017; P7 - December 3, 2017.
This study used three replications in the form of pond plots where the sampling locations were in three points (the end, edge, and center).

The equipment used in the study included spectrophotometers, analytical scales, dark containers, Erlenmeyer glass (100 mL), measuring cups (50 mL), measuring pipettes (10 mL), fillers/bulbs, DO meters, pH, refractometers, syringes, microtubes, sample containers, and cool boxes. The substances used were phenol solution (C_6H_5OH), 0.5% sodium nitroprusside ($C_5FeN_6Na_2O$) solution X, alkaline citrate solution ($C_6H_5Na_3O_7$) 200 g L^{-1}, 5% sodium hypochlorite solution (NaClO), sulfanilamide solution ($H_2NC_6H_4SO_2NH_2$) 10 g L^{-1} , NED dihydrochloride solution 1 g L^{-1}, EDTA solution, 0.1 N HCl solution, distilled water, and filter paper.

Measurement of ammonia, nitrite, and other water quality paramaters. Ammonia and nitrite levels were measured in the laboratory of the Faculty of Fisheries and Marine, Airlangga University, Surabaya city, Indonesia. The measurements of temperature, pH, salinity, and dissolved oxygen (DO) were conducted at the sampling location. The ammonia level was measured using a spectrophotometric method in accordance with SNI 06-6989.30-2005 and the measurement of nitrite levels was conducted by spectrophotometric methods in accordance with SNI 06-6989.9-2004. In addition to ammonia and nitrite, other parameters were also observed, namely DO concentration, temperature, pH and salinity. Measurements of DO oxygen concentration and water temperature were conducted using an AZ 8402 Dissolved Oxygen Meter. Whereas for pH and salinity measurements, a pH pen and refractometer were used.

Calculation of hemoglobin. Tilapia (*Oreochromis niloticus*) hemoglobin (Hb) levels were observed in this study. 6 fish from each pond were collected, totaling 18 fish with a mean weight of 300 g. The procedure for calculating Hb levels was carried out with the methods used by Hartika et al (2014). 0.2 mL of blood were collected using a Sahli pipette. The blood in the pipette is transferred into the Hb-meter tube, which was filled with 10 mL of 0.1 N HCl and stirred for 5 minutes. Distilled water was added until the color of the mixture became like the color of the standard solution available in Hb-meters.

Statistical analysis. The data were analyzed using the IBM SPSS software with the One-Way ANOVA test to determine the differences in ammonia and nitrite levels of the different times of measurement.

Results and Discussion

Ammonia levels. The level of ammonia ranged from 0.112 to 0.537 mg L^{-1}. The mean values of ammonia content at each measurement are presented in Table 1. ANOVA test results shows that there were significant differences ($P<0.01$). Duncan's Multiple Range Test results show that values differed significantly, except in P4, P6, and P7. The highest value of ammonia content was at P5 and the lowest value at P1.

Table 1

The mean value of ammonia content in extensive tilapia (*Oreochromis niloticus*) ponds

Measurement	*Ammonia value (mg L^{-1}) ± SD*
P1	0.1231883^{e}±0.01370723
P2	0.1899153^{d}±0.01047693
P3	0.2914657^{c}±0.02204169
P4	0.3538647^{b}±0.02895914
P5	0.4789653^{a}±0.05085083
P6	0.3832530^{b}±0.03852962
P7	0.4167673^{b}±0.05141435

Note: different superscripts in the second column show significant differences ($P<0.05$). P1 - September 10, 2017; P2 - September 24, 2017; P3 - October 8, 2017; P4 - October 22, 2017; P5 - November 5, 2017; P6 - November 19, 2017; P7 - December 3, 2017.

Nitrite levels. The level of nitrite during the study ranged from 0.01 to 0.12 mg L^{-1}. The mean value of nitrite content at each measurement are presented in Table 2. ANOVA test results show that there were significant differences ($P<0.05$). Duncan's Multiple Range Test results show that nitrite levels from P1, P2, P3, and P4 did not differ significantly. The highest level of nitrite content was at P5 and the lowest value was found at P4.

Table 2

The mean value of nitrite content in extensive tilapia (*Oreochromis niloticus*) ponds

Measurement	*Nitrite value (mg L^{-1}) ± SD*
P1	0.0759644^{ab}±0.01406217
P2	0.0696340^{abc}±0.04613624
P3	0.0347511^{bcd}±0.00468277
P4	0.0214969^{d}±0.01057451
P5	0.0866469^{a}±0.01149425
P6	0.0294758^{cd}±0.02498539
P7	0.0677877^{abc}±0.02671894

Note: Different superscripts show significant differences ($P<0.05$). P1 - September 10, 2017; P2 - September 24, 2017; P3 - October 8, 2017; P4 - October 22, 2017; P5 - November 5, 2017; P6 - November 19, 2017; P7 - December 3, 2017.

Water pH. The pH value of the water ranged from 8.4 to 9.4. The pH mean value at P1 was 8.83 and it gradually increased until it reached 9.32 in P5. The pH value decreased in P6 and increased in P7 with the mean values of 8.72 and 8.87, respectively. The mean value of pH in each measurement can be seen in Figure 1.

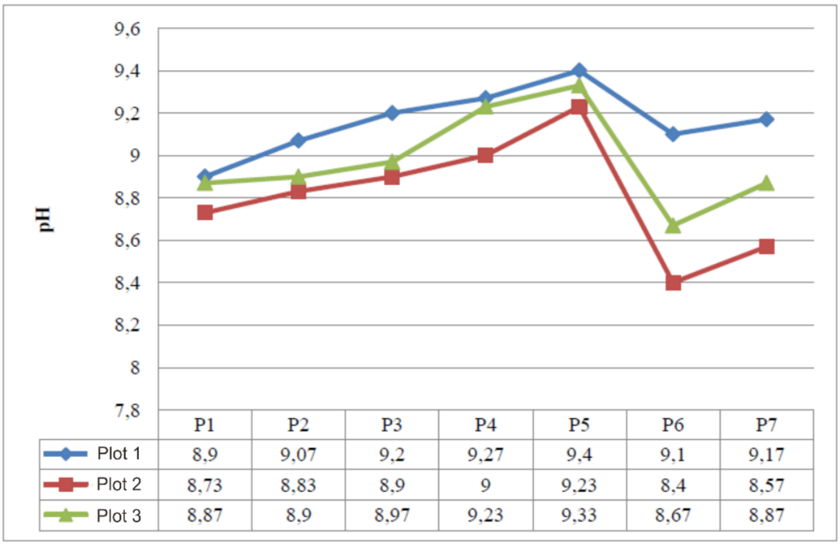

Figure 1. The mean value of pH in extensive tilapia (*Oreochromis niloticus*) ponds. P1 - September 10, 2017; P2 - September 24, 2017; P3 - October 8, 2017; P4 - October 22, 2017; P5 - November 5, 2017; P6 - November 19, 2017; P7 - December 3, 2017.

Temperature. The temperature at P1 showed the mean value of 33.03°C and decreased with 0.29°C at P2. The temperature gradually increased until P4, with the mean value of 35.24°C, and decreased again until the last measurement, with the mean value of 32.26°C. The mean value of temperature in each measurement is presented in Figure 2.

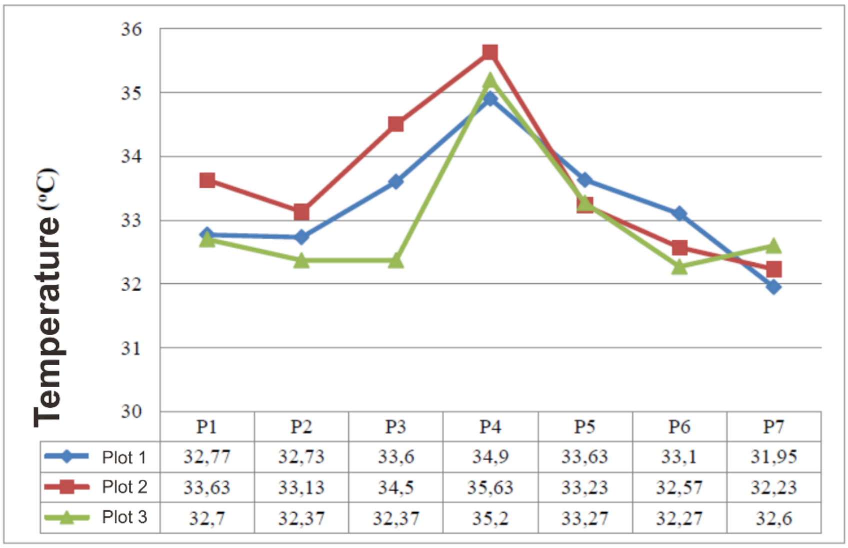

Figure 2. The mean value of temperature in extensive tilapia (*Oreochromis niloticus*) ponds. P1 - September 10, 2017; P2 - September 24, 2017; P3 - October 8, 2017; P4 - October 22, 2017; P5 - November 5, 2017; P6 - November 19, 2017; P7 - December 3, 2017.

Dissolved oxygen (DO). The DO values ranged from 6.13 to 9.04 mg L^{-1}. The center of the ponds had the highest mean value, while the end of ponds had the lowest mean value. The mean value of DO at each measurement is presented in Figure 3.

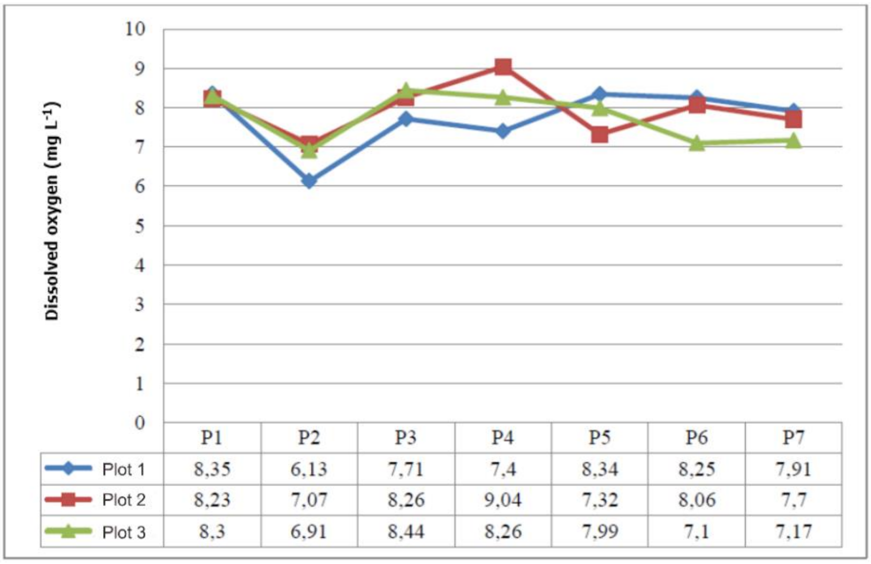

Figure 3. The mean value of dissolved oxygen (mg L^{-1}) in extensive tilapia (*Oreochromis niloticus*) ponds. P1 - September 10, 2017; P2 - September 24, 2017; P3 - October 8, 2017; P4 - October 22, 2017; P5 - November 5, 2017; P6 - November 19, 2017; P7 - December 3, 2017.

Salinity. The mean values of salinity in extensive ponds during the measurements tend to increase. The salinity mean value at P1 was 8.89 g L^{-1} and gradually increased to 24.55 g L^{-1} at P5. The salinity mean value slightly decreased to 2.22 g L^{-1} at P6 and increased at P7. The mean salinity values are presented in Figure 4.

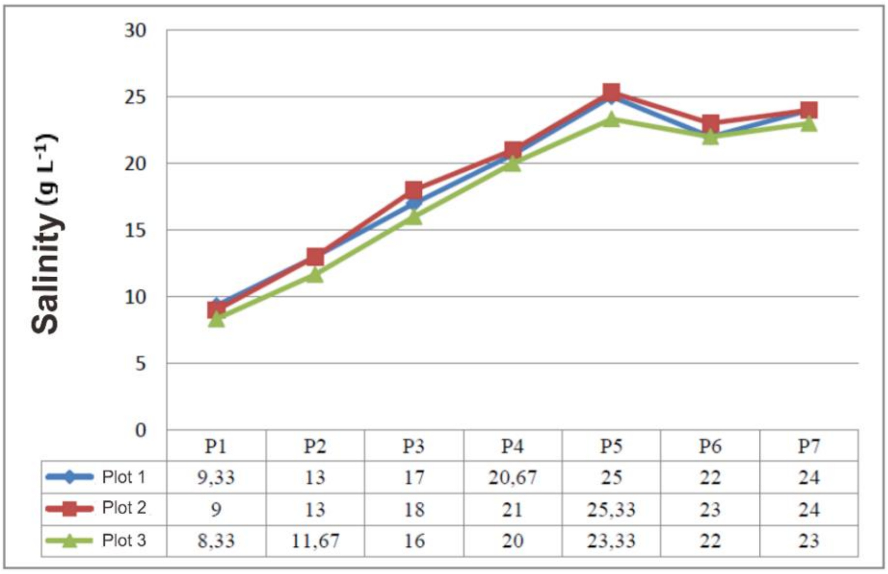

Figure 4. The mean value of salinity (g L^{-1}) in extensive tilapia (*Oreochromis niloticus*) ponds. P1 - September 10, 2017; P2 - September 24, 2017; P3 - October 8, 2017; P4 - October 22, 2017; P5 - November 5, 2017; P6 - November 19, 2017; P7 - December 3, 2017.

Hemoglobin levels of tilapia. The mean value of hemoglobin levels of tilapia at the end of the observations are 7.53 g dL^{-1} in the first pond, 6.53 g dL^{-1} in the second pond, and 6.03 g dL^{-1} in the third pond. The results of the overall measurements of the hemoglobin levels of tilapia are presented in Table 3.

Table 3

Hemoglobin levels (g dL^{-1}) of tilapia (*Oreochromis niloticus*) from extensive ponds

Plot I		*Plot II*		*Plot III*	
Fish 1	9.8	Fish 1	7.2	Fish 1	6.2
Fish 2	7.4	Fish 2	6.4	Fish 2	5.4
Fish 3	7.8	Fish 3	5.6	Fish 3	6.8
Fish 4	8.2	Fish 4	6.6	Fish 4	5.2
Fish 5	5.8	Fish 5	7.2	Fish 5	6.2
Fish 6	6.2	Fish 6	6.8	Fish 6	6.4
Mean	7.53	Mean	6.63	Mean	6.03

Hb levels in each plot had different levels, which ranged from 5.4 to 9.8. The measurements showed that the highest mean level of Hb was in plot I, while the lowest mean value was in plot 3.

The average increase of ammonia content from P1 to P5 was 0.089 mg L^{-1}. This is because during the study period, the extensive ponds did not have any water replacement, so fish feces and plankton accumulated, acting a source of organic material (Effendi et al 2015). The organic material in the ponds will be decomposed by bacteria in nitrogen compounds.

The concentration of ammonia decreased in P6 with a mean value of 0.383 mg L^{-1}, due to the rain that occurred previously. Rain can cause dilution in the ponds, so that the level of ammonia will decrease (Whitehead et al 2009).

The level of ammonia in extensive ponds from Tanggulrejo Village was tolerated by cultured organisms, with the highest values of 0.537 mg L^{-1}. The concentration of ammonia compounds that can kill aquatic organisms is 0.6 mg L^{-1} (Joel & Chinomso 2010). This is because ammonia compounds in high concentrations can inhibit the process of transferring oxygen from gills to blood and causing damage to the gills. Ammonia compounds in the waters in aerobic conditions will undergo the process of nitrification into nitrate compounds through nitrite intermediate compounds.

The nitrite concentration in the ponds is usually unstable or tends to always change (Samsundari & Wirawan 2013). Nitrite in aerobic conditions will be oxidized easily in nitrate, with the help of *Nitrobacter* spp.

The levels of nitrite compounds in this study still could be tolerated by culture organisms, with the highest mean value reaching 0.12 mg L^{-1}. The nitrite concentration that could support the life of aquatic organisms is 0.5 mg L^{-1} (Santhosh & Singh 2007). On the other hand, nitrite concentrations should be no more than 0.2 mg L^{-1} in freshwater and 0.125 mg L^{-1} in seawater (OATA Water Quality Criteria 2008). This is because nitrite can bind hemoglobin in the blood and inhibit the oxygen transfer process.

Supporting water quality parameters were also observed in this study, including pH, temperature, DO, and salinity. This is because the supporting parameters can affect the concentration of ammonia and nitrite directly or indirectly. The pH values ranged from 8.3 to 9.5. The presence of ammonia concentration will increase directly proportional to pH values (Wurts 1992). This can be seen from the ammonia mean values and the pH values that have the same tendency. The increase in ammonia levels from the first measurement to the 5^{th} measurement was followed by an increase in the pH value, and both parameter values decrease in the 6^{th} measurement.

The temperature ranged from 31.2 to 35.9°C. Besides pH, temperature also plays a role in influencing ammonia concentration. Ammonia concentration in the ponds will increase directly proportional with the activity of organic matter reform process and an increase in water temperature (Suwoyo 2011). However, the effect of temperature was lower than the water pH water (Boyd 1998). Therefore, it can be seen that the pattern of temperature fluctuations did not resemble the pattern of fluctuations in ammonia and pH.

The DO plays an important role in determining the levels of ammonia and nitrite in the waters. This is because DO is needed by *Nitrosomonas* spp. and *Nitrobacter* spp. during the nitrification process (Lestari 2014). The level of nitrite in pond waters is closely related to organic material (Afriansyah 2016). Decomposition of organic material by microorganisms in waters requires oxygen. But if the DO is insufficient, oxygen is taken from nitrate, which results in dangerous nitrite. The DO content of the water during the study ranged from 5.88 to 9.58 mg L^{-1}.

Pond salinity also fluctuated between measurements. In extensive aquaculture, the water is not replaced in its maintenance period, so water salinity should increase over time due to the evaporation of water. However, at the 6^{th} measurement, there was a decrease in salinity. This was probably caused by the rain that occurred before the measurement, which caused the water volume to increase and decreased the total ion concentration.

Tilapia Hb levels observed at plots 1, 2 and 3 were 7.53 g dL^{-1}, 6.63 g dL^{-1}, and 6.03 g dL^{-1}, respectively. Hb levels were used as supporting parameters of nitrite levels in the waters. Nitrite can oxidize ferrous ions in Hb to ferric ions, which can reduce the ability of Hb to bind and transport oxygen throughout the body of the fish (Tilak et al 2007). Fish with high levels of methemoglobin can cause anoxic conditions. Therefore, the Hb level obtained from the measurements was proportional to the level of nitrite at the end of sampling, which was still below the threshold for the life of organisms. The Hb levels of tilapia ranged from 5.05 to 8.33 g dL^{-1} (Safitri et al 2013).

It can be considered that the concentration of nitrite was directly proportional to the content of Hb in tilapia. It can be seen that the highest nitrite level was found in plot I, 0.097 mg L^{-1}, where the Hb content in the samples showed the highest value as well, 7.53 g dL^{-1}. The mean value of Hb levels in plots II and III were 6.63, and 6.03 g dL^{-1}, with nitrite levels of 0.043 mg L^{-1} and 0.063 mg L^{-1}, respectively. Ammonia and nitrite levels that are dangerous to tilapia can be observed through the levels of fish Hb. The Hb levels of tilapia in extensive system cultivation ponds were within the normal range. Some measures can reduce toxic levels of nitrogen compounds in extensive ponds, like the application of probiotics.

Conclusions. Based on the results of the study, it can be concluded there were temporal variations in ammonia and nitrite levels every two weeks. Fluctuations in ammonia and nitrite in extensive ponds did not endanger the cultivation organisms, because they had not exceeded the threshold for mortalities in fish. This can be observed from the content of the hemoglobin of tilapia in extensive ponds, which was in the normal range.

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