Analysis of nitrogen and phosphate in hybrid grouper media for better understanding of water management practices

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Abstract. Indonesia is globally known as the third biggest grouper producer, but its production lately raises some environmental concerns. Aims of this study were identification of nitrogen and phosphorus concentrations derived from feed and metabolism of hybrid grouper. Simple vessels were built and arranged into five main components in culture system: culturing tank, sedimentation, oxygenation, reservoir and filtration. Two sets of systems were used as study replications. Twenty juveniles of the hybrid were stocked in 100 L culture tanks and fed to satiation. Four testing times were taken: at initial (0), 1, 3 and 6 hours after feeding. Results showed that nitrogen contents (ammonia, nitrite, nitrate and total nitrogen was performing nitrogen cycle starting from 0 and peaked at 3 to 6 hour after feeding time. The highest values were majority found in culture tank at 0.08; 0.0015; and 1.372 ppm for ammonia, nitrite and nitrate, respectively. The highest values of total nitrogen and total phosphates were 1.9 and 0.33 ppm that slightly fluctuated over the period even though still in fair values. This study also documented that feeding intake was relatively high (14.78% of feed/BW) that may indicate greater degree in generating solid waste materials to water media.

1. Introduction

Groupers species (Epinephelus spp.) are one of the high-value marine products of Indonesia, beside shrimps, barramundi and seaweeds [1]. Even at seeds production, Indonesia noted as the biggest exporter that supplied seed for many Asia Pacific region [2]. In an attempt to meet the high demand for grouper product, there are so many trials in its culture practices, including the hybridization process. One of the most successfully cultured and well-known product is hybrid of Tiger grouper (Epinephelus fuscoguttatus, ♀) × giant grouper (Epinephelus lanceolatus, ♂), known as TGGG or kerapu cantan. This species is quite popular to be cultured as it has a high resistance to disease, fast-growing and high tolerance to the extreme environment [3].

Grouper fingerling is commonly cultured in net cages along sheltered coastal regions, employs local water and freely discharges excess feed and metabolite residues to the surrounding environment. In line as documented in many marine culture areas, the development grouper industries in Indonesia also leads in some natural and ecological issues. Some studies reported that marine aquaculture gives direct consequences in organic nutrient enrichment, solids accumulation at sediment, degradation in diversity and structure of plankton community, also water tropic-level disturbance [4] [5] [6] [7].

Two main factors were reported as reasons for these problems, low-efficiency feed and massive stocking fish densities [8] [9]. Solids and dissolved material from feed and faeces will partially degrade in the water column and give direct impact in deteriorating water quality and disturbing local
sediment condition [10]. Waste from the feed, in the form of uneaten fees and faeces, may carry of solids particle that contains total nitrogen (7-32%) and total phosphorus (30-84%) [11]. Protein in feed is leached and binds into the organic form of ammonium that in certain values may toxic to the aquatic organism [12]. In particular, for grouper culture, data showed that 69% nitrogen and 85% phosphorus were released from each productive farm to its surrounding area [13]. However, details on how these materials diffuse in the marine water environment, in regards to feeding time activities, are still not yet investigated.

The efforts on performing fish culture at suitable environment condition and with least ecological concerns are unavoidable important in recent years. Using small scale laboratorial testing, this study aims to identify and measure the organic nutrient contents (mainly nitrogen and phosphate), which generate from feeding activity and establish the changing patterns of water quality during and after feeding time of the grouper culture.

2. Methodology
2.1. Tested Fish and Experimental Tanks
Laboratory scale testing was designed to investigate the concentration of nitrogen and phosphate material loading in water media of hybrid grouper. This study was conducted in DIFITA Hatchery, Institute for Mariculture Research and Fisheries Extension (IMRAFE), Bali. A simple project of culture vessels was built and arranged into five main components that performed several key factors in the culturing system, i.e. culture tank (A), sedimentation (B), oxygenation (C), reservoir (D) and filtration (E), as illustrated as in Figure 1. Two sets of systems were used as study replication.

![Figure 1. The experimental culture vessels designed for water quality maintenance](image)

A. Cultured tank. The tanks of cultured, sedimentation, oxygenation and sump tanks are rectangular-plastic containers volume 100 L. The cultured tank is outfitted with an outlet located in the base corner of the tank. One air stone was placed in the centre of the tank for providing oxygen in the media for fish respiration. Debit water of 3 L/m was set to let the water naturally flowing between each tank. Twenty individual’s fish were placed in both culture tanks. Hybrid grouper of Tiger grouper (Epinephelus fuscoguttatus, ♀) × Giant grouper (Epinephelus lanceolatus, ♂) juveniles (TL 12.08±1.42 cm, BW 28.38±3.82 g) or known as kerapu cantang were used as tested species.

B. Sedimentation tank. Solids particles were passively removed in this component. Oyster mesh screen diameter 120 μm were placed diagonally for trapping the solid materials and let the clarified water moving out of the tank.

C. Oxygenation tank. One air stone was placed in the centre of this tank to infuse additional oxygen. Dissolved oxygen about 6 mg/L was aimed in this media to provide a sample amount of oxygen in the water for fish respiration and bacteria to perform nitrification process in this system.

D. Reservoir tank. The outflowing water from oxygenation tank will enter in this tank, and One pump is settled and used to transferred water media to filtration tanks.
E. Filtration tank. The filtration tank is circular container diameter 70 cm volume 1000 L and made of polyethylene (HDPE) material. Sets of rocks, pebbles and sands were layered (±20 cm each) and separated by a baffle screen to performs as mechanical filtration for purifying water. Filtrated water was transferred back to the cultured tank.

All tested fish were fasted up to 24 hours prior to this study. Four-time of testings were taken from each component to investigate the nutrient waste loading during the reduction process, which are initial (just after feeding), 1 hour, 2 hours and 6 hours after feeding. The feed used in this study was commercial extruded pellets size 2.3 mm, and has high nutrition contents remarkable for grouper.

2.2 Data collection and analysis
Nitrogen and phosphate were examined as the primary parameter in this experiment. Nitrogen content was measured using standard methods proposed by American Public Health Association in an analysis of ammonia (phenate method), nitrite (Colorimetric method), nitrate (cadmium reduction method and total nitrogen analysis (Kjeldahl method) [14]. Total phosphate was analyzed using ascorbic acid spectrophotometry method [14]. Secondary parameters such as temperature, Dissolved Oxygen (DO) and pH were observed on-site during the experiment. Total suspended solids (TSS) also measured by the gravimetric method [14]. All data collected were analyzed descriptively by plotting and drawing into graphs.

3. Result and Discussion
In general, amounts of nitrogen parameters displayed similar trends in increasing values from initial to 6 hours after feeding. Data showed that cultured tanks (A) have the highest ammonium levels, and its numbers were decreasing to the other following tanks (Figure 2a). The ammonium contents were started low at 0.035 ppm until 0.794 ppm at 6 hours after feeding in tank A. Similarly, nitrite levels started to increased in tanks A and B from 3 to 6 hour after feeding (< 0.001 to 0.0015 ppm), then stable at tanks C and D (0.0015) ppm and back to <0.0010 ppm at tank E (Figure 2b). The concentrations were started from about 0.5 ppm after feeding time, then doubled in the first hour (0.96-1.32 ppm) then stagnant until remaining sampling times. Tanks A, B, C and D have similar amounts of nitrate, while tank E seemed to have lower amount throughout sampling times (Figure 2c).

There are two major elements in aquaculture wastewater, nitrogen and phosphorus. These results are linear with the natural process of the nitrogen cycle. Ammonium and nitrite levels in water can be expected to increase since the beginnings of fish were reared. Ammonium is commonly known as the major type of excreted nitrogen than any other catabolize protein materials in teleost fish [15]. Ammonium may occur in non-toxic form ammonia (NH4) and toxic form un-ionized ammonium (NH3). Ammonium plays an important role in the nitrification cycle, which is an oxidative process, and first converted into nitrite by naturally occurring Nitrosospira and Nitrosomonas bacteria in the aquatic environment. The next process will be converting nitrite (NO2) into nitrate (NO3) by Nitrospira and Nitrobacter bacteria.

Based on sampling times, data showed that ammonium values were continuously increased from just after feeding to 6 hours, indicating that NH4+ degradation process was still continued throughout experimental tanks. The range of ammonia amounts from 1 to 6 hour after feedings (0.258-0.794 ppm) were noted higher than its maximum tolerable level of ammonia for marine culture (< 0.1 ppm) [16]. Even its concentrations were noted quite high, and it was mentioned that NH4 (ammonia) is generally harmless and can be easily removed from water to the atmosphere [17]. The toxicity of ammonia likely occurs in the form of NH3 (un-ionized ammonium), and dependent on direct expose into greater temperature and pH values. At the same time, nitrite and nitrate in this study were considerably lower than its ambient criteria (<4 ppm and <200 ppm, respectively) [16]. Nitrite form still has a toxic effect on fish species compared to nitrate. Nitrate-nitrogen is usually non-toxic, and the amounts in culture water mostly can be managed by the comparatively low level of water exchange [18].
Figure 2. Trends of Ammonium (2a), Nitrite (2b) and Nitrate contents (2c) in media observed in different tanks started from after feeding, to 1, 3 and 6 hours after feeding.

Total Nitrogen (TN) and Total Phosphate (TP) data are significant to investigate the total loads of various forms of nitrogen and phosphate in the water column. Total nitrogen concentration in Kjeldahl method refers to the total amount of ammonia and organic nitrogen. TN data displayed downward trends in Tank A, B and upward figure in Tank C, D and E (Figure 3). It seems that the TN values increase just after feeding as the impact of food leaching during feeding (started from 1 hour), and decrease by the time of digestion (2 to 3 hour period), and rise again when fish were releasing faeces to culture media (started from 4 hours). This condition has similarly occurred from Tank A simultaneously to the next following tanks. While in Tank E, TN performed in stable and less fluctuated values.

Total nitrogen values have relatively similar trends as total phosphate (Fig. 3a). Both values were recorded high in the beginning hour after feeding, and then significantly decreased during 3 hours period before having another increasing trend. It can be predicted that the first peak values after feeding were generated mostly by leaching process of uneaten feed in the culture media, while the second peak was the result of both leaching and residue of fish metabolism. Nutrient transfers from aquaculture system will give a direct impact on the variation of physical and chemical water parameters [19] [20], including total phosphorous, total nitrogen and dissolved oxygen [21] [22] [23].
Figure 3. Trends of Total Nitrogen (3a) and Total Phosphate contents (3b) in media observed in different tanks started from after feeding, to 1, 3 and 6 hours after feeding.

In this experiment, the feed intake of cantang hybrid grouper at satiation stage was high at 4.19 g or equal to 14.78% of feed per total fish body weight. Comparative studies found that cansir hybrid grouper (hybrid of tiger grouper (Epinephelus fuscoguttatus) x spotted grouper (Epinephelus corallicola)) consumed about 11.68% of feed [24], while spotted grouper only 2.71% [25]. Hybrid grouper are known to have high feed consumption so that it grows faster than other grouper species. The ratio of faeces/feeding was 0.41 g or 0.097% of its total consumed feed. This high amount of metabolite waste is not ideal for environmental standpoints. The high amount of feed and feeding frequency (2 to 3 times/day) of grouper in the net cage will consequently lead to a greater quantity of solid material and soluble nutrients on that leach into neighbouring water environment [26] [27]. Solids waste may accumulate on the substrate and give negative impacts on sea minerals, benthic organism and sediment structure [19] [28] [29].

Temperature and DO during the experiment are displayed as in Figure 4. Temperatures were low initially (about 28 °C), then slightly increased by 0.5 °C at the end of the study. It is general facts that digestive and metabolism process needs oxygen and release heat. In grow-out net cages situation, at sheltered coastal area, temperatures will be more stable in the range between 25 to 32 °C with daily fluctuation less than 1 °C [30]. An increase in temperature will affect on lower solubility of oxygen in the water. Even though DO concentrations were noted decrease at just 1 hour after feeding, its majority values were still in the range of feasible for grouper culture (4.65 to 5.93 ppm). The lowest DO levels were recorded at during sedimentation process (Tank B at 3 to 6 hour after feeding), indicating that many processes were occurring at the same time in these periods, such as fish metabolism, excretion through gills, and leach-feed degradation process.
Figure 4. Trends of Temperature (4a) and Dissolved Oxygen (4b) in media observed in different tanks started from pre-feeding, to 1, 3 and 6 hours after feeding.

pH and total suspended solids (TSS) tend to have downward trends at culture tanks (A) to the next following tanks and through the experiment periods (Figure 5). Seawater has relatively stable pH values and commonly varied just between 7.5 and 8.4 [31]. This study documented pH values from 7.54 to 8.21, which can be considered within its standard optimum range. Similarly, TSS values were generally noted between 4 and 5 mg/L, with a remarked peak at 6.81 mg/L. TSS is the weight of filterable solid material suspended in the water column [32]. These amounts are considered quite low based on its study condition which performed in controlled, filtered water. This finding is linear with reports by Turcios and Papenbrock that in a flow-through system, solid concentration is detected between 5 to 50 mg/L [33].

Figure 5. Trends of pH (5a) and TSS (5b) in media observed in different tanks started from pre-feeding, to 1, 3 and 6 hours after feeding.

4. Summary
In summary, this study documented that feeding activities gave direct impact in enriching organic materials (ammonia, nitrite and nitrate) in the local water environment. Nitrogen and phosphate levels have upward trends over first to six hours after feeding. Temperature, dissolved oxygen, and pH slightly fluctuated just after feedings, but overall still in optimum and safe range values. In bigger culture scale, such as net cages culture, great attention on feedings activities (total feed, frequency, feeding techniques) are important to balance the efficiency of feed needed by fish and the least amount of uneaten feed in the system. Thus, failure in balancing these two main factors may impact in either fish growth or surrounding water environment.
5. References

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