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Effect of fertilizer addition on water quality and fingerling production of loach (*Misgurnus anguillicaudatus*) in natural outdoor ponds

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Abstract

The present study examined the effect of fertilizer addition on water quality and loach (*Misgurnus anguillicaudatus*) fry growth in outdoor ponds for the promotion of loach culture in rice paddy fields. Three ponds received the following treatments: fertilizer with no loach (6-1), fertilizer with loach (6-4), and no fertilizer and no loach (6-2). Concentration of NH_4^+-N and PO_4^{3-}-P were highest in ponds that received fertilizer. Chl. *a* concentration was two times higher in ponds 6-1 and -4 than in pond 6-2. Furthermore, Chl. *a* concentration in pond 6-4 was 78% lower than that in pond 6-1. The density of diatoms and green algae was two times higher in ponds 6-1 and -4 than in pond 6-2, and the average density of diatoms was lower in pond 6-4 than in pond 6-1. Loach cultured with fertilizer in this study were slightly longer than the Chinese loach cultured in an indoor fiber-reinforced plastic tank.

Key words

Fingerling production, Loach, *Misgurnus anguillicaudatus*, Natural outdoor pond, Water quality

Introduction

Loach (*Misgurnus anguillicaudatus*) is a small freshwater fish and an indigenous species in Korea that is rich in calcium, protein, essential amino acids, vitamin A and various minerals. Thus, it has been used as an essential ingredient in foods such as loach soup, Eojuk and Sook Hee, which are popular in Korea, and for a long time has been used in tonics to improve health (Kim, 1997; Kang *et al.,* 2002). Recently, due to increasing interest in health in Korea, the demand for loach soup has also been increasing, and more than 90% of the loach consumed in Korea (10,000 tons) is imported from China.

In the early 1990s, loach culture in paddy fields was a rural sideline. Live food and artificial seed production technology had not yet been developed, so wild seed was widely used during that time (Oh *et al.,* 1991; Song *et al.,* 1991a, b). Recently, the amount of the captured wild fry in Korea has decreased therefore prices have risen as compared to the Chinese loach (Kang *et al.,* 2002). According to FAO, paddy field culture is performed in 28 countries in six continents. It is effective in removing malarial mosquito larvae, and is on rise because pesticide usage is then reduced and pests can be controlled (Halwart and Gupta, 2004). Recently, culture of loach in paddy fields without the use of pesticides and chemical fertilizer has attracted a great deal of attention in Korea, where rice is mostly grown in paddy fields, as in Southeast Asia. By utilizing idle farmland that can be obtained through FTA negotiations, the culture of loach in paddy fields can provide a new source of income for farmers; some farmers who have cultured loach in paddy fields in 2012 are earning more than four times the income produced by rice cultivation alone. Additionally, by culturing them in paddy fields, farmers can produce loach without using pesticides or antibiotics and obtain rice, which is produced as by-product, without the use of pesticides as well. Furthermore, it may be possible to protect ecosystems from pesticides and control red tides or blooms of green algae by reducing nitrogen and phosphorus loads.

Research on various aspects of loach culture technology (germplasm, gonadal sex differentiation, multiple spawning) was conducted in 1990s (Kim *et al.,* 1995; Kim and Nam, 1994; Lim *et al.,* 1996; Nam *et al.,* 1999) and loach spawning can now be easily induced. While few farmers are currently culturing loach in paddy fields with rice, little is known about culture methods and a systematic approach has not been established; the most important objective is to produce low-cost, healthy loach fry.

To culture loach in paddy fields in Korea, the fry should be released into paddy fields in June at the latest, during rice-planting season, to grow as large as possible (total length: 120 mm; weight: 10 g) by October, the rice harvesting season, for sale in Korean markets. Therefore, ideally, large loach fry should be released into the paddy fields. However, breeding loach fry of 0.7 g in weight is difficult; conventional methods of indoor breeding in PVC tanks with artificial feed require a great deal of time and money. As part of loach culture research in rice paddy fields, the present study evaluated the effect of addition of fertilizer on water quality and loach fry growth in natural outdoor ponds.

Materials and Methods

Three outdoor ponds (6-1, -2 and -4) located at the Inland Aquaculture Research Center were selected as study area. The ponds were 30 m long, 15 m wide, 1 m high and 50 cm deep. Ten kilograms of urea fertilizer (Super Alalyi, Namhae Chemical) and 40 kg of manure (Hannong Green, Hannong Industry) were added to ponds 6-1 and -4, and 500,000 loach fry (*Misgurnus anguillicaudatus*), which were cultured with rotifers for 10 d, were added to pond 6-4 on 22nd May, 2012 (Wang *et al.,* 2010). The manure consisted of 10% cow dung, 10% pig droppings, 30% fowl droppings and 45% sawdust by weight. The size of the loach fry (mean ± SD) was 7.57 ± 2.54 mm in total length 6.59 ± 1.86 mm in body length and 0.02 g in weight. Fertilizer and loach fry were not added to control pond, 6-2. For ponds 6-1 and -4, 20, 40, and 40 kg of manure were added on May 29, June 11 and June 20, using an onion bag with 1 mm pore size for slow nutrient release.

In the present study, manure was used to produce prey organisms for the loach fry because it is cheaper and more eco-friendly than artificial fertilizer. *M. anguillicaudatus* was also choosen because it, more fleshy and has more desirable taste than *Misgurnus mizolepis* (Chinese loach). Surface water was collected at 2-3 day interval to measure water quality and cell density of phytoplankton. pH and dissolved oxygen (DO) were measured, using a portable water-quality meter (model 6920; YSI, Yellow Springs, OH, USA) in the field. Concentration of ammonium-nitrogen (NH_4^+-N), nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N) and phosphate-phosphorus (PO_4^{3-}-P) in water samples were analyzed by Spectrophotometer (Cary 300 Conc; Varian, Mountain View, CA, USA), using colorimetric methods after filtration with GF/F filter paper (APHA-AWWA-WEF, 1995). To determine chlorophyll *a* (Chl. *a*) concentration water samples were filtered through a membrane filter (0.45 µm; Whatman, Maidstone, Kent, UK); pigments were extracted from the filter with 90% acetone in dark place for 1 day (APHA-AWWA-WEF, 1995), and then of Chl. *a* concentration was estimated by Spectrophotometer (Cary 300 Conc; Varian). Phytoplankton was observed under an optical microscope (Leica, Wetzlar, Germany; Chung, 1993). Loach was harvested from 26th June to 20th July 2012, using a creel containing paste bait.

Results and Discussion

The spatial variation in pH, DO and Chl. *a* concentration at three outdoor ponds (6-1, -2, and -4) are shown in Fig. 1. pH values of ranged from 7.16 to 9.62 (average: 8.28), with no difference between the fertilized and non-fertilized ponds. DO concentration in ponds 6-1, -2 and -4 ranged from 2.56 to 16.67 mg l^{-1} (average: 9.33 mg l^{-1}), 3.59 to 16.30 mg l^{-1} (average: 9.62 mg l^{-1}) and 1.53 to 16.73 mg l^{-1} (average: 8.23 mg l^{-1}), respectively; and no clear difference was detected between the fertilized and non-fertilized ponds, similar to pH. The highest DO concentration (>13 mg l^{-1}) was observed around May 21 and lowest concentration (<5 mg l^{-1}) was observed between 29th May and 8th June after DO concentration increased. Chl. *a* concentration in pond 6-1, -2 and -4 ranged from 14.02 to 164.70 µg l^{-1} (average: 73.74 µg l^{-1}), 4.90 to 96.45 µg l^{-1} (average: 25.24 µg l^{-1}) and 4.04 to 119.26 µg l^{-1} (average: 41.17 µg l^{-1}), respectively. Chl. *a* Concentration in pond 6-1 and -4, which were fertilized, were two times higher than in pond 6-2, which was not fertilized, and the highest concentration was observed in pond 6-1, which did not contain loach fry.

Han *et al*. (2010) found that the average pH and Chl. *a* concentration in nine lakes in Korea ranged from 7.31 to 7.63 and 3.59 to 8.65 µg l^{-1}, respectively. In comparison, the average pH value of the ponds in the present study was higher (8.28), as were the average Chl. *a* concentration in ponds 6-1, -2 and -4 (73.74, 25.24, and 41.17 µg l^{-1}, respectively). Therefore, high pH value in the present study could have been the result of higher phytoplankton biomass. As shown in Fig. 2, a positive correlation was observed between pH and DO (r^2=0.632, p value=0.000). In general, both pH and DO concentration decrease when organic matter decomposes and increase when phytoplankton grow. Therefore, very low DO concentration in this study was likely due to increased organic matter decomposition.

Chl. *a* Concentration ranged from 4.2 to 9.9 µg l^{-1} in Lake Juam, 4.8 to 17.6 µg l^{-1} in the surface waters of Lake Andong during the summer season, and averaged 5.9 µg l^{-1} at Unmun Dam from 1996 to 2010 (Heo *et al.,* 2000; Lee and Lee, 2003; Kim, 2012). In the present study, average Chl. *a* concentration in ponds 6-1, 2 and 4 was 73.74, 25.24 and 41.17 µg l^{-1}, respectively. These concentrations, including that of pond 6-2 which was not fertilized, were significantly higher than those reported for Juam and Andong lakes, as well as the Unmun Dam. Pond 6-2 is a stagnant pond and small as compared to a lake. It was previously used for fish farming, which may have led to higher nutrient concentration in the pond. These characteristics may explain high

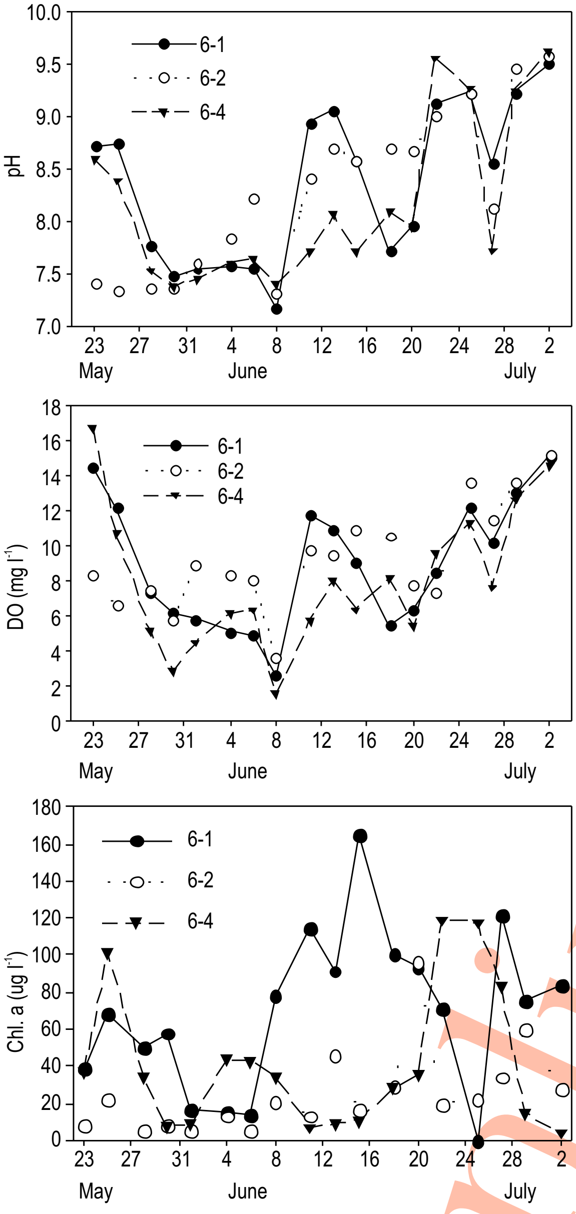

Fig. 1 : Special variations of pH, DO and Chl. *a* concentrations at pond 6-1, 6-2 and 6-4 during May 23-July 2, 2012.

Chl. *a* concentration in pond 6-2, despite the fact that no fertilizer was added. High Chl. *a* concentration in pond 6-1 and 4 were likely due to addition of fertilizer that promoted phytoplankton growth. In comparison, the average Chl. *a* concentration in pond 6-4 was 78% lower than that in pond 6-1. Loach fry were added to pond 6-4 and not to pond 6-1. In pond 6-4, the loach fry grew from 0.02 to 0.72 g in weight within 36~60 days (Table 1). According to Wang *et al*. (2008), larval loach feed on microparticles and *chlorella*. Therefore, low Chl. *a* concentration in pond 6-4, compared to pond that in 6-1, may have been due to the consumption of phytoplankton by loach fry.

The spatial variation in NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-}-P concentration are shown in Fig. 3. NH_4^+-N concentration in pond 6-1, -2 and -4 ranged from 0.082 to 1.070 mg l^{-1} (average: 0.456 mg l^{-1}), 0.061 to 0.284 mg l^{-1} (average: 0.141 mg l^{-1}) and 0.071 to 1.080 mg l^{-1} (average: 0.658 mg l^{-1}), respectively. The concentrations of NH_4^+-N in pond 6-1 and 4, which were fertilized, were three times higher than in pond 6-2, which was not fertilized. High concentration of NH_4^+-N in pond 6-1 and 4 were observed from 23rd May to 4th July and 16th July, respectively; then dramatically decreased to concentration comparable to those in pond 6-2. NO_2^--N concentration in pond 6-1, -2 and -4 ranged from 0.009 to 0.209 mg l^{-1} (average: 0.049 mg l^{-1}), 0.008 to 0.342 mg l^{-1} (average: 0.034 mg l^{-1}) and 0.011 to 0.354 mg l^{-1} (average: 0.097 mg l^{-1}), respectively; the highest value was detected in pond 6-4, which received additions of both fertilizer and loach fry. NO_3^--N concentration in ponds 6-1, -2 and -4 ranged from 0.680 to 1.952 mg l^{-1} (average: 1.280 mg l^{-1}), 0.676 to 1.924 mg l^{-1} (average: 1.195 mg l^{-1}) and 0.071 to 2.059 mg l^{-1} (average: 0.855 mg l^{-1}), respectively, and almost no difference were detected between the fertilized and non-fertilized ponds. However, the concentration in pond 6-4 was very low on 20th June. PO_4^{3-}-P concentration in pond 6-1, -2 and -4 ranged from 0.044 to 0.214 mg l^{-1} (average: 0.077 mg l^{-1}), 0.036 to 0.102 mg l^{-1} (average: 0.063 mg l^{-1}) and 0.043 to 0.193 mg l^{-1} (average: 0.088 mg l^{-1}), respectively. The highest PO_4^{3-}-P concentration was observed on 23rd May in pond 6-1 and -4.

An (2011) reported peak NH_4^+-N concentration of 0.10–0.15 mg l^{-1} in Yongdam Lake. Average concentration of NH_4^+-N in this study were significantly higher in fertilized ponds (6-1: 0.456 mg l^{-1}; 6-4: 0.658 mg l^{-1}) and similar in non-fertilized ponds (6-2: 0.141 mg l^{-1}) as compared to Yongdam Lake. The average

Table 1 : Total length and weight of young loach by raising term (n=30)

Rearing term	Total length (mm)	Weight (g)	Yield or survival rate (%)	Loach type	Reference
30-60 days (Pond)	51.61±6.21	0.72±0.26	5.07	Muddy loach *(M. anguillicaudatus)*	This study
50 days (Indoor in FRP)	19.42±3.27	0.074	95	Muddy loach *(M. anguillicaudatus)*	
60 days (Indoor in FRP)	47.1	1.03	34	Chinese muddy loach *(M. mizolepis)*	Kang *et al.*, 2002

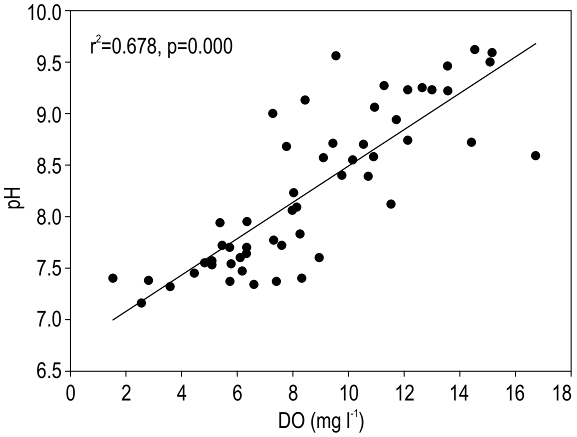

Fig. 2 : Relationship between DO concentration and pH at stations 6-1, 6-2, and 6-4

NO_2^--N concentration was <0.02 mg l^{-1} in Andong, Imha and Naju lakes, and 0.04 mg l^{-1} in Yongdam Lake. The average NO_2^--N concentration was 0.049 and 0.097 mg l^{-1} in pond 6-1 and -4, respectively. These values were higher than those reported for Andong, Imha and Naju lakes, where water quality was relatively good and even Yongdam Lake, which has been contaminated for a long time. The average NO_2^--N concentration in pond 6-2, where fertilizer was not added, was 0.034 mg l^{-1}, which was lower than the concentration in Yongdam Lake. Average NO_3^--N concentrations of in three ponds, in study area, were 1.280, 1.195 and 0.855 mg l^{-1}, and these values were lower than those reported for Lake Doam during dry (3.573 mg l^{-1}) and rainy (2.550 mg l^{-1}) seasons (Kwak *et al.,* 2010). However, the monthly average concentration of NO_3^--N in Lake Daecheong ranged from 0.96 to 1.34 mg l^{-1} (Park, 2005), which was similar to the concentrations observed in the present study. Average PO_4^{3-}-P concentration of in three ponds were 0.077, 0.063 and 0.088 mg l^{-1}. The average PO_4^{3-}-P concentration in Lake Doam and Lake Shihwa was 0.102 and 0.081 mg l^{-1}, respectively (Baek *et al.,* 2010; Kwak *et al.,* 2010), which is comparable to the concentration observed in the present study. However, PO_4^{3-}-P concentration increased sharply in pond 6-1 and -4 on 23rd May (Fig. 3), with addition of urea fertilizer as NH_4^+-N.

Average concentration of NO_2^--N and NO_3^--N were similar among ponds (fertilized or not), and no considerable differences

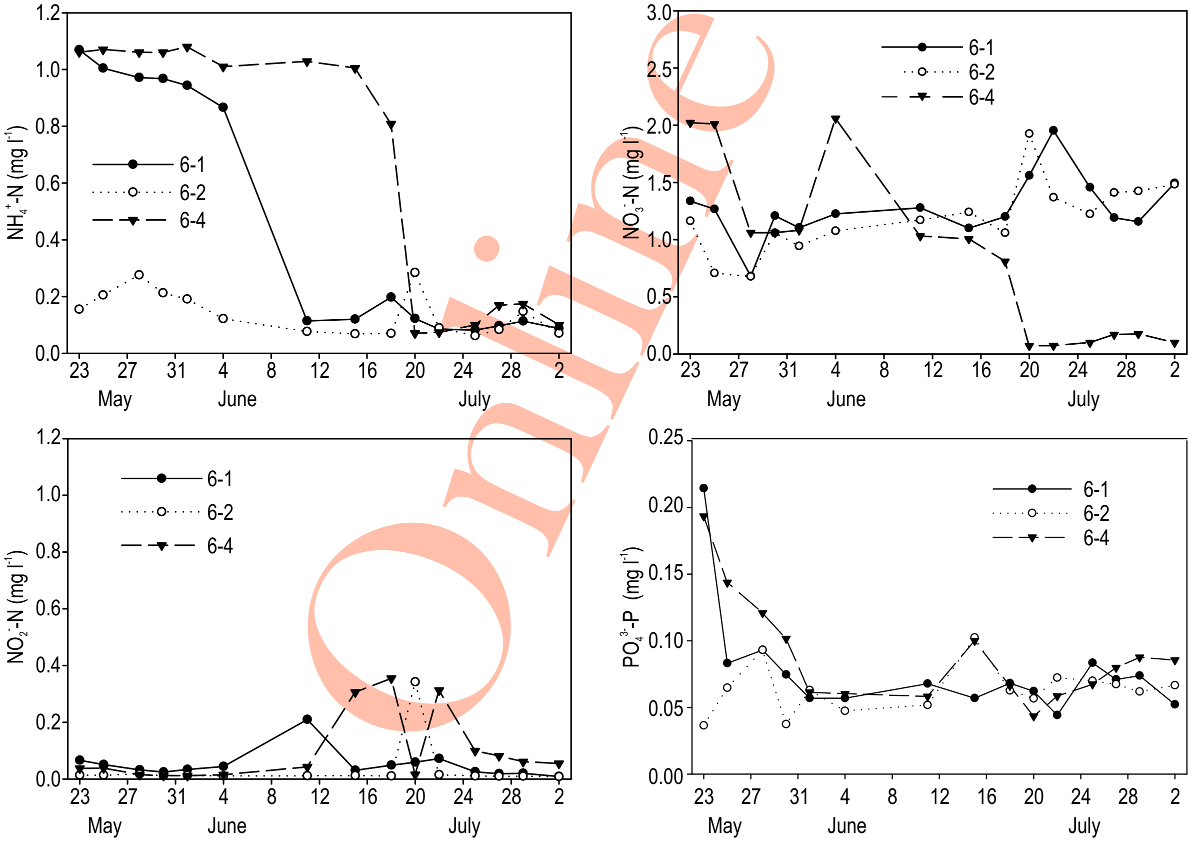

Fig. 3 : Special variations of NH_4^+-N; NO_2^--N; NO_3^--N and PO_4^{3-}-P concentration at pond 6-1, 6-2 and 6-4 during May 23-July 2, 2012

were noted between the concentrations observed in this study and other Korean lakes previously mentioned. However, NH_4^+-N and PO_4^{3-}-P concentration was much higher in pond 6-1 and -4, to which urea fertilizer was added. Therefore, addition of urea and manure to produce live food for loach in this study may have led to increases in NH_4^+-N and PO_4^{3-}-P concentration, but not other nutrients.

The spatial variation in diatom, green algae, blue-green algae and euglena densities are shown in Fig. 4. Phytoplankton cell densities in ponds 6-1, -2 and -4 ranged from 44 to 1,914 cells ml^{-1} (average: 570.5 cells ml^{-1}), 16 to 590 cells ml^{-1} (average: 177 cells ml^{-1}) and 30 to 9,306 cells ml^{-1} (average: 1,032 cells ml^{-1}), respectively, and the highest density was observed in pond 6-4, to which fertilizer and loach fry were added. The standing crops of phytoplankton in the East River and Unmun Dam were 104–1,467 and 1,399–6,472 cells ml^{-1}, respectively, during summer (Cho and Shin, 2012; Kim, 2012). In the present study, the standing crop of phytoplankton in pond 6-2, to which fertilizer was not added, was lower (16–590 cells ml^{-1}) than that in the East River or Unmun Dam, whereas the standing crop of phytoplankton in pond 6-1, to which fertilizer was added, was similar (44–1,914 cells ml^{-1}) to that in the East river but lower than that at Unmun Dam. The standing crop of phytoplankton in pond 6-4 (30–9,306 cells ml^{-1}), to which both fertilizer and loach fry were added was similar to that at the Unmun Dam. As previously mentioned, Chl. *a* concentration in this study was much higher than those reported at the Unmun Dam, which suggests that the ponds in this study contained phytoplankton species that were either larger and/or had higher Chl. *a* content as compared to phytoplankton at the dam.

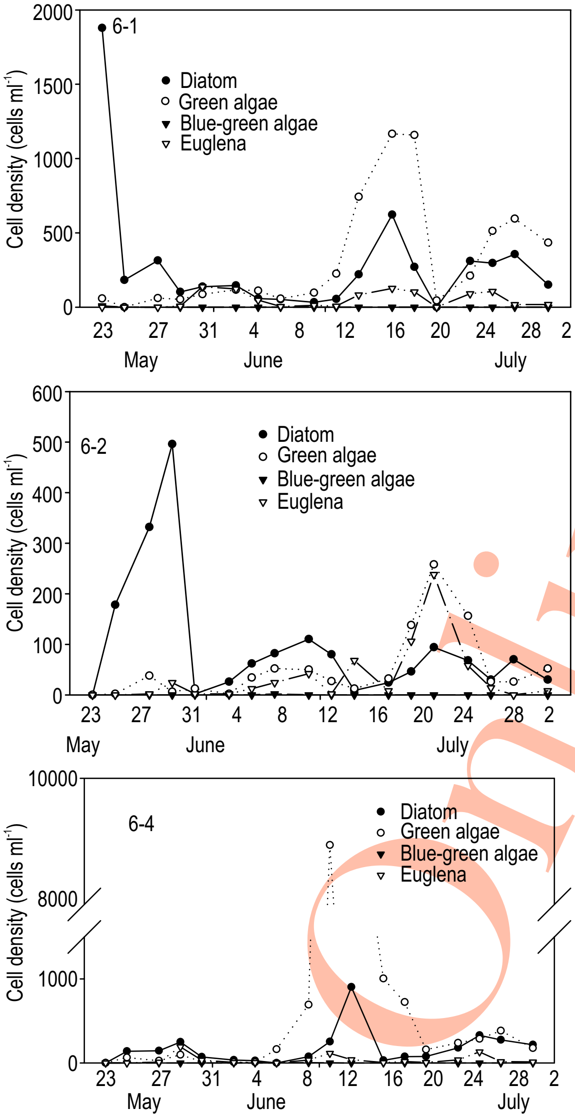

Fig. 4 : Special variations of phytoplankton species at pond 6-1, 6-2 and 6-4 during May 23-July 2, 2012

The average densities of diatoms, green algae, blue-green algae and *Euglena* in pond 6-1 were 273, 319, 1 and 43 cells ml^{-1}, respectively. The highest densities of diatoms and green algae were observed on 23rd May and 14th June, respectively. The average densities of diatoms, green algae, blue-green algae, and Euglena in pond 6-2 were 89, 50, 1, and 30 cells ml^{-1}, respectively, and the highest diatom density (496 cells ml^{-1}) was observed on 27th May. The average densities of diatoms, green algae, blue-green algae and *Euglena* in pond 6-4 were 151, 798, 1 and 33 cells ml^{-1}, respectively, and the density of green algae was highest (8,940 cells ml^{-1}) on 12th June. The average density of diatoms in pond 6-4, to which loach fry were added, was lower than in pond 6-1, which did not contain loach fry, and the density of green algae was higher in pond 6-4 than in 6-1. Diatoms and green algae were dominant phytoplankton species in ponds 6-1 and -4, despite the presence or absence of loach. Diatoms and green algae are also dominant phytoplankton species in the East River and Unmun Dam (Cho and Shin, 2012; Kim, 2012). Knowing what loach fry eat is critical to successfully culture loach in paddy fields. Larval loach (*M. anguillicaudatus*) feed on microparticles, *Daphnia, Chlorella*, and freshwater rotifers (Kang *et al.,* 2002; Wang *et al.,* 2008). In general, zooplankton such as *Daphnia* are commonly found in paddy fields and ponds. Therefore, results suggest that loach fry consumed more diatoms and/or zooplankton feed diatom than green algae.

The loach fry (mean ± SD), which were raised indoors for 10 days and outdoors for 36~60 days, were 51.61 ± 6.21 mm in total length, 44.01 ± 5.25 mm in body length and 0.72 ± 0.26 g in weight. The harvest yield was 5.07%, for a total of 25,370 loach (Table 1). According to Kang *et al*. (2002), Chinese loach (*M. mizolepis*) are grown in fiber-reinforced plastic (FRP) tanks indoors for 60 days until they are 47.1 mm in total length and 1.03 g in weight (survival rate: 34%; Table 1). Loach (*M. anguillicaudatus*) are grown in an indoor FRP tank for 60 days until they are 19.42 mm in total length and 0.07 g in weight (survival rate: 95%), as shown in Table 1. The size of loach *M. anguillicaudatus*, in the present study cultured with manure in outdoor ponds, was 2.7 times longer and 9.7 times heavier than the *M. anguillicaudatus* grown in an indoor FRP tank, and slightly longer than the Chinese loach cultured indoors.

Typically, Chinese loach (*M. mizolepis*) inhabit the downstream regions of rivers and loach (*M. anguillicaudatus*) inhabit the headwater regions of rivers, mud in paddy fields and morass. Higher survival and growth rates of loach (*M. anguillicaudatus*) were observed with a mix of live *Daphnia* plus live *Chlorella*, and live *Daphnia* plus microparticles, compared to microparticles or live *Daphnia* only (Wang *et al.,* 2008). Many types of microparticles and several types of plankton were observed in the ponds of study area (Fig. 4). Therefore, high growth rate of loach in outdoor ponds as compared with indoor tanks, may have been due to greater diversity of microparticles available for consumption, including phytoplankton and zooplankton.

Survival rates and harvest yields are very important in the fry production industry. In this study, the harvest yield was 5.07% for a culture period of 36–60 days. This value is too low compared with the results of the laboratory experiments discussed previously. A comparison of the survival rate and harvest yield results from this study with those for the loach (*M. anguillicaudatus*) cultured in indoor FRP tanks is difficult due to the small volume of prey that was added to the tank to control disease (Table 1). One reason for this is that loach fry were not captured in the field, and a second reason is increased mortality due to the presence of multiple predators such as diving beetles, water spiders, and dragonfly larvae. Research on the removal of predators and effective harvest methods are needed to develop stable seed production of loach.

Concentration of NH_4^+-N, PO_4^{3-}-P, and Chl. *a* were highest in ponds that received fertilizer. The density of diatoms and green algae was two times higher in fertilized ponds than in non fertilized pond. Loach cultured with fertilizer in the current study was slightly longer than the Chinese loach cultured in an indoor fiber-reinforced plastic tank.

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