# Growth and Feeding Behaviour of *Barbonymus gonionotus* (Bleeker, 1850) and *Hypsibarbus wetmorei* (Smith, 1931) in Added-Substrate and No-Added-Substrate Cages

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## ABSTRACT

This research was conducted to study the growth and feeding behaviour of two indigenous herbivorous fish species, *Barbonymus gonionotus* (Bleeker, 1850) and *Hypsibarbus wetmorei* (Smith, 1931) under different culture systems. Experimental units were composed of net cages with and without substrates, suspended in a nutrient-enriched pond. Bamboo poles with an approximately submerged surface area equal to the total cage surface area were used as substrates for periphyton growth, and were installed vertically inside the net cages. Survival and growth rates of the fish were evaluated after a 56-day rearing period. The results indicated that the provision of substrates to enhance periphyton production in net cages during the early nursing of *B. gonionotus* and *H. wetmorei* did not improve growth and survival of the fish stocked at 300 fish $m^{-3}$. *B. gonionotus* showed a higher growth rate than *H. wetmorei* ($p < 0.05$). Average yields of *B. gonionotus* and *H. wetmorei* obtained in cages were 0.32 and 0.22 kg $m^{-3}$ $month^{-1}$, respectively.

**Keywords:** Thai silver barb, Periphyton, Fish nursing, Cage culture

## INTRODUCTION

Periphyton-based aquaculture systems have been developed and found to be efficient for herbivorous fish culture. Adding artificial substrates for periphyton growth in pond-based culture systems could increase production of some cultured species (Hem and Avit, 1994; Wahab *et al*., 1999; Ramesh *et al*., 1999; Azim *et al*., 2001a; Keshavanath *et al*., 2001; 2004). The provision of substrates could also reduce the need for supplemental feeding, and have a positive effect on fish survival (Keshavanath *et al*., 2002). Periphyton may be an alternative or a complement for supplemental feed in fingerling production (Keshavanath *et al*., 2004). Adding substrates in the ponds up to 100% of the pond surface area will increase production (Azim *et al*., 2004). It sequesters excess nutrients (van Dam *et al*., 2002) and improves health of

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cultured organisms (Shankar and Mohan, 2001). The use of substrates for periphyton growth is a low-cost culture method which results in a more favorable environment for the cultured organisms (organic loading avoided) and at the same time provides an extra source of food (Uddin *et al*., 2008).

Aquaculture species such as tilapias (Huchette *et al*., 2000; Dempster *et al*., 1993) and Indian major carps (Azim *et al*., 2001a, b; Azim *et al*., 2002; Azim *et al*., 2004) were observed to consume periphyton. Rohu (*Labeo rohita*) and masher (*Tor khudree*) are very suitable for exploiting periphyton, while others (e.g., *Catla catla*) do not utilize periphyton directly but may be used in a polyculture system with periphyton (Azim *et al*., 2001b). Other carp species may have the potential to utilize periphyton, however, this has not been verified, for e.g. Java carps, *Barbonymus gonionotus* (synonyms *Barbodes gonionotus* or *Puntius gonionotus*), and yellow-belly barb, *Hypsibarbus wetmorei*. Among these two species, *Barbonymus gonionotus* was recorded as a common fish species in rice-fish culture in South (Bangladesh and India) and Southeast (Indonesia, Malaysia, Thailand and Vietnam) Asia. In the Mekong Delta, Java carp is often raised in polyculture with *Oreochromis niloticus* (L.) and *Cyprinus carpio* L. (Vromant *et al*., 2002). Together with *B. gonionotus*, *H. wetmorei* is also an indigenous species which is very similar to *B. gonionotus*, not common in aquaculture, but are found with *B. gonionotus* in aquaculture ponds. The most distinguishing characteristic that differentiates between the two species is the anal fin, which reaches until the caudal base for the latter species (Vidthayanon, 2008).

Most suggestions, comments and recommendations concerning aquaculture of herbivorous fish species indicate that the feeding behaviour of fish is considered to be more complex in periphyton-based systems. Thus it is necessary to study the feeding behaviour of these species under two conditions: added and no-added substrates. The purpose of this study was to compare the growth and survival of two indigenous herbivorous fish species, and discuss whether periphyton grazing in early nursing is a significant means of increasing growth and survival of the fish. Feeding behaviour of fish is also discussed.

## MATERIALS AND METHODS

The experiment was conducted at Khon Kean University's Nong Khai Campus, Thailand, from September to November 2010. Blue nylon net cages, measuring 1 x 1 x 1.2 m, with 1-mm square mesh netting were used as experimental units. The submerged volume of each cage was 1 $m^3$. Twelve cages were suspended from a bamboo structure set in a 400-$m^2$ fertilized fish pond. Six cages were provided with bamboo poles (mean diameter 5 cm, length 50 cm), which were suspended vertically into the cages, at a total density of 13 poles per cage (added-substrate). The poles gave an additional submerged surface area approximately equal to that of the total cage surface area. The remaining six cages were substrate free (no-added-substrate). Hatchery-produced, mixed sex populations of two herbivorous fish species, *Barbonymus gonionotus* (Bleeker, 1850) and *Hypsibarbus wetmorei* (Smith, 1931) were used as experimental fish.

Prior to the beginning of the experiment, all fish were fed a daily diet of local rice bran and fish meal (1:1) at a rate of 15% body weight.day$^{-1}$.

Prior to the trial, the pond was renovated, and all larger aquatic animals were eradicated by frequent netting. It was then treated with lime ($CaCO_3$) at the rate of 250 kg ha$^{-1}$. After 1 week of liming, it was fertilized with cow manure, urea and triple super phosphate (TSP) at the rate of 2000, 100, and 100 kg ha$^{-1}$ (80, 4, and 4 kg per 400 m$^2$-pond), respectively. Fertilization continued thereafter at fortnightly intervals at levels which were half of the initial rates (40, 2, and 2 kg per pond, respectively). The cow manure was applied at the four corners of the pond, while the urea and TSP were soaked together with water for 6 hours and then spread over the water surface. On the same day of the first fertilization, bamboo poles were suspended vertically in the cages from a bamboo rack. After the first fertilization on September 17$^{th}$ and before fish stocking, the cages were left for 10 days to allow periphyton growth on the bamboo poles. On September 28$^{th}$, fry of two barbs species, *B. gonionotus* (individual weight 0.17 g) and *H. wetmorei* (individual weight 0.17g), were stocked in the cages at 300 fish cage$^{-1}$ (1 m$^3$). Each treatment combination was tested in triplicate. All fish in each cage (300 fish) were bulked weighed at the beginning of the experiment for growth evaluation. Fish from all treatments were harvested after 56 days.

Water quality in each cage was measured throughout the experimental period. Temperature (C thermometer), dissolved oxygen (Winkler method; APHA, 1989), Secchi disc depth (Secchi disc) and pH (IQ Scientific Instruments) were monitored weekly between 0900 and 1000 h on each sampling day. Total alkalinity, total hardness and total ammonium nitrogen were measured fortnightly (APHA, 1989). To determine chlorophyll *a* concentrations (Boyd, 1979), 100 ml water samples were collected fortnightly and filtered through glass microfiber filters (Whatman GF/C) which were then analyzed.

After 10 days of substrate installation, periphyton samples were collected and there after continued to be taken at fortnightly intervals. At each sampling time, one pole from each cage was selected randomly and from each pole, a 10 x 10 cm-band sample of periphyton was taken at the middle depth level and analyzed for dry matter and ash-free dry matter content. After periphyton sample collection, the poles were returned to their original positions and marked so that previously sampled poles were always excluded from subsequent samples. Dry matter (DM) was determined by drying the samples overnight at 105°C to a constant weight, upon which ash content was determined using a muffle furnace (4 h at 540°C). The final weight of the crucible along with the remains was weighed for the calculation of ash and ash-free dry matter (AFDM).

At the end of the experiment, all fish were removed from the cages, then bulk weighed and counted. Survival rate (%) was calculated as (no. of fish harvested/no. of fish stocked) x 100. The final mean weight of the individual fish in each cage

was estimated by dividing the total final biomass in the cage by the number of survivors. The specific growth rate (% $day^{-1}$) was calculated as $(\ln W_2 - \ln W_1)/t \times 100$, where $W_1$ is the initial mean weight (g), $W_2$ is the final mean weight (g) and t is the time in days. The weight gain (g $fish^{-1}$) of the individual fish in each cage was estimated as final mean weight (g) – initial mean weight (g). The daily weight gain (g $fish^{-1}$ $day^{-1}$) of the individual fish in each cage was estimated as (final mean weight (g) – initial mean weight (g))/time in days. The gross yield in each cage was estimated by dividing the total final biomass (kg) by the cage volume ($1m^3$).

Among the survivors in each cage, thirty fish were randomly selected. The fish were weighed individually with a digital balance to an accuracy of 0.01 g. The total length of each weighed fish was also measured to the nearest 0.1 cm total length. Then the condition factor was calculated as weight in g/(total length in cm)$^3$.

The data on final mean weight, weight gain, daily weight gain, specific growth rate, survival rate, condition factor and gross yield were compared using analysis of variances (ANOVA) and Tukey-HSD test. The assumptions of normal distribution and homogeneity of the variances were checked before analyses. The differences were considered statistically significant at an alpha level of 0.05.

## RESULTS

The water quality parameters between treatments were not significantly different. Means of water quality parameters are given in Table 1. Adding substrates did not show significant effects on growth and survival of *Barbonymus gonionotus* and *Hypsibarbus wetmorei* ($p > 0.05$; Table 2). The final weight, mean weight gain, daily weight gain and specific growth rate of the fish show that *B. gonionotus* had a higher growth rate than *H. wetmorei* ($p < 0.05$) in both added-substrate and no-added-substrate cages. Average yields of *B. gonionotus* and *H. wetmorei* obtained in cages were 0.32 and 0.22 kg $m^{-3}$ $month^{-1}$, respectively. The results of periphyton biomass analysis are given in Table 3. However one pole sample from each cage at each sampling time was not representative enough to allow for acceptable statistical treatment of data. The fluctuations of periphyton biomass in cages stocked with different species are shown in Fig. 1. There was no significant difference in AFDM between the cages of the two species except in week 8. Chlorophyll *a* concentrations tended to increase and were highest on week 4 of the experiment. Unfortunately, there was no data for chlorophyll *a* concentrations for week 6; however, lower chlorophyll *a* concentrations were recorded on week 8 of the experiment (Fig. 2).

Table 1. The culture environment of *Barbonymus gonionotus* and *Hypsibarbus wetmorei* in added -substrate and no-added-substrate cages during a 56-day period. All are means ± SD.

| Parameters | Mean ± SD | n |
|---|---|---|
| Dissolved oxygen (mg $l^{-1}$) | 4.24 ± 1.00 | 96 |
| pH | 7.87 ± 0.41 | 96 |
| Water temperature ( $^{o}C$ ) | 28.49 ± 2.32 | 96 |
| Secchi disc depth (cm) | 41.31 ± 7.75 | 96 |
| Chlorophyll *a* (µg $l^{-1}$) | 156.82 ± 103.74 | 48 |
| Total ammonia nitrogen (mg $l^{-1}$) | 0.30 ± 0.13 | 48 |
| Total alkalinity (mg $l^{-1}$ of $CaCO_3$) | 165.64 ± 0.79 | 48 |
| Total hardness (mg $l^{-1}$) | 106.77 ± 0.12 | 48 |

Table 2. Average growth parameters of *Barbonymus gonionotus* and *Hypsibarbus wetmorei* in added-substrate and no-added-substrate cages during a 56-day period. All values are means ± SD (range), n = 3, except condition factor data where n, number of examined fish per treatment = 90. In each row, different letter superscripts indicate significant difference (ANOVA and Tukey's test).

| Parameters | Treatments | | | |
|---|---|---|---|---|
| | *H. wetmorei* with substrates | *H. wetmorei* without substrates | *B. gonionotus* with substrates | *B. gonionotus* without substrates |
| Initial mean weight (g) | 0.17 ± 0.00 | 0.17 ± 0.00 | 0.17 ± 0.00 | 0.17 ± 0.00 |
| Harvest (no. fish) | 268.5 ± 10.6 | 251.3 ± 59.3 | 261.0 ± 9.5 | 276.0 ± 17.0 |
| | (261 – 276) | (183 – 289) | (255 – 272) | (264 – 288) |
| Final mean weight (g) | 1.60 ± 0.16 $^{a}$ | 1.83 ± 0.18 $^{ab}$ | 2.44 ± 0.19 $^{c}$ | 2.21 ± 0.10 $^{bc}$ |
| | (1.49 – 1.72) | (1.62 – 1.94) | (2.33 – 2.66) | (2.14 – 2.28) |
| Weight Gain (g fish-1) | 1.43 ± 0.16 $^{a}$ | 1.66 ± 0.18 $^{ab}$ | 2.28 ± 0.19 $^{c}$ | 2.04 ± 0.10 $^{bc}$ |
| | (1.32 – 1.55) | (1.45 – 1.77) | (2.16 – 2.49) | (1.97 – 2.11) |
| Daily Weight Gain | 0.026 ± 0.003 $^{a}$ | 0.030 ± 0.003 $^{ab}$ | 0.041 ± 0.003 $^{c}$ | 0.037 ± 0.002 $^{bc}$ |
| (g $fish^{-1}$ $day^{-1}$) | (0.02 – 0.028) | (0.026 – 0.032) | (0.039 – 0.044) | (0.035 – 0.038) |
| Specific Growth Rate | 4.01 ± 0.18 $^{a}$ | 4.23 ± 0.19 $^{ab}$ | 4.76 ± 0.13 $^{c}$ | 4.58 ± 0.08 $^{bc}$ |
| (% $day^{-1}$) | (3.88 – 4.13) | (4.02 – 4.35) | (4.67 – 4.91) | (4.52 – 4.64) |
| Survival rate (%) | 89.50 ± 3.54 | 83.78 ± 19.76 | 87.00 ± 3.18 | 92.00 ± 5.66 |
| | (87.0 – 92.0) | (61.0 – 96.3) | (85.0 – 90.7) | (88.0 – 96.0) |
| Condition factor | 1.29 ± 0.13 $^{ab}$ | 1.47 ± 0.15 $^{b}$ | 1.08 ± 0.08 $^{a}$ | 0.97 ± 0.05 $^{a}$ |
| | (1.20 – 1.38) | (1.30 – 1.56) | (1.03 – 1.17) | (0.94 – 1.01) |
| Gross yield (kg $m^{-3}$) | 0.43 ± 0.03 $^{a}$ | 0.45 ± 0.09 $^{ab}$ | 0.64 ± 0.04 $^{b}$ | 0.61 ± 0.01 $^{ab}$ |
| | (0.41 – 0.45) | (0.36 – 0.54) | (0.59 – 0.68) | (0.60 – 0.62) |

Table 3. Average periphyton biomass in added-substrate cages of *Barbonymus gonionotus* and *Hypsibarbus wetmorei* during a 56-day period. All values are means ± SD (range), n = 15

| Periphyton | Fish species | |
|---|---|---|
| | ***B. gonionotus*** | ***H. wetmorei*** |
| Dry matter (mg $cm^{-2}$) | 0.789 ± 0.313 (0.394 – 1.550) | 0.847 ± 0.513 (0.402 – 2.214) |
| Ash (mg $cm^{-2}$) | 0.519 ± 0.205 (0.263 – 1.080) | 0.457 ± 0.142 (0.243 – 0.732) |
| Ash free dry matter (mg $cm^{-2}$) | 0.270 ± 0.127 (0.115 – 0.503) | 0.390 ± 0.519 (0.141 – 1.860) |

% ash free dry weight of periphyton biomass is approximately 40%

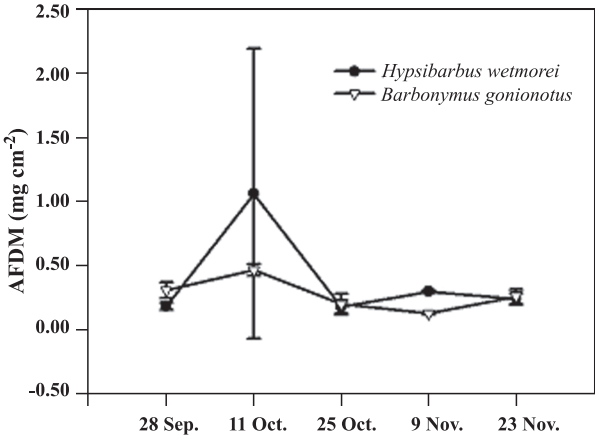


Figure 1. Average periphyton biomass in ash free dry matter (AFDM) per unit surface area ($cm^2$) of bamboo poles in cages of the two fish species. Values are means (± SD) of three replicated cages per sampling date (n = 3)

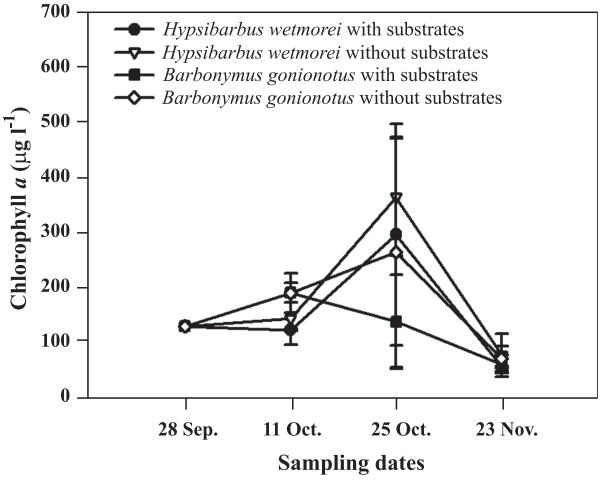


Figure 2. Average chlorophyll *a* concentrations of water in different treatments during the experimental period. Values are means (± SD) of three replicated cages per sampling date (n = 3).

## DISCUSSION

All the water quality parameters were within the acceptable ranges, as recommended for tropical aquaculture (Boyd, 1982; Boyd and Tucker, 1992; Beveridge, 1996). There was no significant difference in growth and survival between the added-substrate and no-added-substrate cages for each species ($p > 0.05$). The absence of significant difference in condition factors of each species were stocked in substrate added and no-added cages may reflect the adequate feeding in both conditions. However, this primarily indicated that both species feed mainly on plankton and that periphyton may not play a significant contribution on fish growth. Although the high fluctuation chlorophyll *a* concentration ($156.82 \pm 103.74$ µg $l^{-1}$) showed that the sampling resolution in time and space was totally insufficient to capture the dynamics of primary productivity. However, it is interesting to note that the chlorophyll level is higher in cages of both species without added substrate compared to the cages with added substrate (in October). This could mean that the bamboo poles do have already an effect on the hydrodynamics, reducing the current speed (or deflecting the current speed) so that somewhat less inflow into the cages takes place, replenishing the stock on which in part the fish may have been feeding. Feeding on small planktonic algae is unlikely to fully cover the energy demands for most carp and tilapia species has been mentioned by Dempster *et al.* (1995). Furthermore, fish generally require larger food sources such as benthic algae, algal detritus or plant fodder, which can be consumed by the fish more efficiently (Dempster *et al.*, 1993; Yakupitiyage, 1993). But the reason why adding substrates did not show significant effect on growth of the two species may be due to filter feeding on plankton which could support the nutritional requirement of the fish in substrate-free cages. Because by getting more water through the gills, the smaller fish could filter proportionally more phytoplankton and gather more metabolizable energy for growth than the larger ones (Huchette and Beveridge, 2003).

The results from an earlier periphyton -based cage experiment showed that the average AFDM of periphyton in the uppermost 50 cm of water column was 0.36 mg $cm^{-2}$ (Huchette and Beveridge, 2003) which is in agreement with average AFDM of 0.33 mg $cm^{-2}$ in the present study. Increased levels of periphyton biomass just after fish stocking indicated low grazing pressure during the 1st week of rearing period. This may be caused by the fish still becoming acclimatized to the culture system where the juveniles seem to be grazing on the periphyton – in which case grazing pressure is less than periphyton growth rates or, no feeding was observed in which case the likely explanation is that the fish were suffering from stress which lowered their feeding rate while acclimation occurred. However, the reduction of periphyton biomass after the 2nd week, as well as observation of feeding behaviour, indicated that both species also consume periphyton. The rather constant levels of periphyton biomass after the 4th week of the experiment indicated periphyton grazing by the fish in the substrate-added cages throughout the rearing period. Lamberti and Moore (1984) noted that productivity per unit substrate

area is highest at intermediate grazing levels, and Huchette *et al*. (2000) stated that grazed periphyton communities were younger, healthier and more productive. So substrate area that was provided in the present study may not be a major constraint for periphyton production. Periphyton productivity could also be reduced by shading (Keshavanath *et al*., 2004), but in the present study, shading by planktonic organisms may not be a limiting factor on growth of periphyton which was indicated by average Secchi disc depths (41.31 ± 7.75 cm) of water column and the depth at substrates installed in cages. Furthermore, there was an indicator that cyprinid species may prefer to consume periphyton on artificial substrates. It was noted that wild fish of various genera, the biggest and most abundant being *Puntius* species, were found in the cage containing additional periphyton substrates (Huchette and Beveridge, 2003).

The results in which periphyton did not show significant effect on growth of Nile tilapia was obtained in pond culture by Shrestha and Knud-Hansen (1994). This indicated that the mixed results for the same fish species in periphyton-based culture systems may result from various factors, i.e. feeding behaviour of fish, quality and quantity of plankton, and the biomass of fish in a system. A previous study that was conducted in substrate-free cages suggested that the availability of natural food could decrease at high densities and possibly result in nutrition deficiencies (Coulibaly *et al*., 2007). This indicated that plankton in the present study was available in sufficient amounts and quality for the fish in the substrate-free cages. Therefore, the results of the present study suggest that when plankton can fully support the nutritional requirements of the fish, then adding substrates will not further improve the growth of the fish. Additionally, in added-substrate cages, the fish were either mostly feeding on plankton or feeding preferentially on the periphyton growing on the substrate surfaces. Consequently, as the fish grew further or at a higher stocking biomass, the contribution of periphyton to plankton may be increased and become significant and as such, periphyton can be an alternative or a complement for natural feed in fingerling production of *Barbodes gonionotus* and *Hypsibarbus wetmorei*.

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