Effect of Stocking Density and Fixed-feeding Level on Growth and Survival Rates of *Pangasius bocourti* Sauvage, 1880 Reared in Hapas, Suspended in Fertilized Ponds

Wirat Jiwyam[1*], Thongchai Champasri[2] and Nisarat Tippayadara[1]

ABSTRACT

Feasible and flexible techniques of fish culture are readily adopted by farmers. The efficiency of each technique should be determined and optimized. Therefore the main objective of this study was to establish an appropriate rearing technique for juvenile Asian river catfish *Pangasius bocourti* in hapas suspended in fertilized ponds. A factorial experiment was carried out in 1 m^3 hapas (27 units), with 3 stocking densities (50, 100 and 150 fish $cage^{-1}$) and 3 fixed-feeding levels (4, 8 and 12% body weight per day, $BWday^{-1}$), over a period of 56 days. At the end of the experiment, all fish from each cage were weighed. Survival rate, specific growth rate, feed conversion ratio and yield were calculated. The survival rates for all treatments were more than 95%. The highest specific growth rate of the fish was 4% day^{-1} in fish which were fed at 12% of initial body weight per day. The specific growth rate of the fish from all stocking densities showed a significant asymptotic relationship ($SGR = - 0.014FL^2 + 0.370FL + 1.608$, $n = 27$, $R^2 = 0.952$, $P = 0.000$) with the feeding level. The natural food available in the culture system could not meet the feed requirement for fish which were fed at low fixed-feeding levels. Gross yield significantly increased with increasing stocking density during the rearing period. Increasing stocking density to 150 fish m^{-3} in a hapa suspended in a fertilized pond did not affect the growth and survival of juvenile *P. bocourti* initially weighing about 20 g. To increase fish yield m^{-3}, higher stocking densities should be tested.

Key words: Asian river catfish, *Pangasius bocourti,* stocking density, ration, feeding rate, cage, natural food

INTRODUCTION

The use of nylon netting made into complete suspended cages or hapas appears to be a flexible technique for nursing small fry since they provide a more easily managed, predator-free environment and obviate the need for pond preparation (Little *et al.* 1991). In the northeast of Thailand, where a large proportion of freshwater aquaculture production comes from small-scale farms, the use of a hapa suspended in a fertilized

[1] Fisheries Program, The School of Science and Technology, Nong Khai Campus, Khon Kaen University, Nong Khai Province 43000 Thailand
[2] Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Khon Kaen Province 40002 Thailand
* Corresponding author e-mail address: wirjiw@kku.ac.th

fish pond is widely practiced in nursing fish fry to fingerling or juvenile stages. Optimal growth of fish fry such as tilapia in hapa can be achieved by using supplementary feeds, as the fish cannot depend completely on suspended plankton to meet their nutrient requirements (Keshavanath *et al*. 2001). Pond fertilization is a management protocol that enhances biological productivity by using both organic manure and inorganic chemical fertilizers (Bhakta *et al*. 2004). The role of fertilizer in increasing fish production has been emphasized in many studies under tropical and temperate conditions (Barash *et al*. 1982; Schroeder *et al*. 1990; Milstein *et al*. 1995; Egna and Boyd 1997; Bhakta *et al*. 2004) hence the addition of fertilizers to nursery ponds is common practice among many fish culture systems (Mischke and Zimba 2004).

The use of high stocking densities as a technique to maximize water usage and thus increase stock production has been shown to have adverse effects on the survival, growth, and food conversion ratio of Nile tilapia in cages in stagnant ponds (Yi *et al*. 1996). The rearing of fish under lower densities results in good growth and a high survival rate, but the total yield is low (Gomes *et al*. 2000). Stocking density also has an effect on production, normally with lower costs at higher stocking rates (Huguenin 1997) hence total harvest and production are directly related to stocking density. However, studies concerning the relationship between stocking density and growth have shown that the optimal stocking for obtaining the highest possible fish yields depends upon the amount and quality of food available (Zonneveld and Fadholi 1991). However, it has also been noted that optimum feeding frequency and feeding rates vary depending on fish species, size and rearing system (Cho *et al*. 2003).

The Asian river catfish *Pangasius bocourti* is an omnivorous fish (Vidtayanon 1993), and is cultured in cages and earthen ponds in the Mekong River Delta in Vietnam (Hung *et al*. 2003) as well as in Thailand. To increase culture efficiency, juveniles having an individual weight of about 30 g are suitable to stock in these systems, particularly in cages. The first rearing stage of the fish from 1-3 g fingerlings to 20-40 g juveniles is essential for a successful grow-out period in cages, as reported by Silva *et al*. (2007) for tambaqui *Colossoma macropomum*. Furthermore, for each growth phase to be successful it is necessary to establish optimal parameters for growth, production and costs (Silva *et al*. 2007). The optimum stocking density for a species may be a critical factor in designing an efficient culture system (Hengsawat *et al*. 1997; Gomes *et al*. 2006). However, it is also important to establish an appropriate feeding management program. Meanwhile, partial or complete compensatory growth following food restriction occurs in many fishes (Russell and Wootton 1992; Jobling *et al*. 1993; Jobling *et al*. 1994; Kim and Lovell 1995; Hayward *et al*. 1997; Qian *et al*. 2000; Wang *et al*. 2000; Zhu *et al*. 2001; Tian and Qin 2003). Fixed-ration feeding is more feasible than adjusted-ration feeding considering labor requirements and handling of the fish. If the fish which were fed at restricted-ration yielded compensatory growth during grow-out, or if there were no effects on the subsequent growth of the fish, then fixed-ration feeding for each growth phase, particularly the first growth phase of 6-8 weeks, could be practiced. Recently, complete compensatory growth was reported in juvenile *Pangasius bocourti* (Jiwyam, 2010).

Therefore, the aim of the present study was to determine the optimum stocking density and feeding level for the first growth-phase rearing of juvenile *P. bocourti* in hapas, suspended in a fertilized pond. The role of natural food on growth of the fish in hapa is also discussed.

MATERIALS AND METHODS

Experimental site and design

To investigate the effect of stocking density and feeding levels on the growth and survival rates of *Pangasius bocourti* Sauvage, 1880, a 3 x 3 factorial design with 3 levels of stocking density (50, 100 and 150 fish m^{-3}) as the first factor and three feeding levels (4, 8 and 12% of body weight day^{-1}) as the second factor, was carried out at the Nong Khai campus of Khon Kaen University, Nong Khai Province, Thailand, for a period of 56 days during June and July 2009. Treatments were executed in triplicate and assigned randomly among the hapas. Commercial feed pellets containing high crude protein (40% by weight) manufactured by Chareon Pokpand Ltd were used to feed the fish three times per day (0800, 1200 and 1600 h). Hatchery-produced *P. bocourti* fingerlings with an initial mean weight of 2.17 g were conditioned in 24 m^2 nylon hapas suspended in an adjacent pond for 1 week prior to stocking in the experimental hapas. All fish were weighed at stocking and at harvest.

Experimental unit preparation

The experimental pond was drained to eradicate all aquatic animals including fish. Lime ($CaCO_3$) was applied in powdered form at the rate of 250 kg ha^{-1}, and one day later, the pond was filled with water from a nearby pond. After another day, semi-decomposed chicken manure was applied at 2,000 kg ha^{-1}, and weekly thereafter at 500 kg ha^{-1}. The manure was applied at the four corners of the pond. Twenty seven square-shaped nylon hapas (1 x 1 x 1.2 m) with a mesh size of 1 mm were suspended in the pond with a submerged volume of 1 m^3. After the first fertilization and before fish stocking, the pond was left alone for one week to allow plankton development in the water column.

Water quality monitoring

Monitoring of water quality was performed between 0900 and 1000 h on each sampling day. Water temperature (YSI Model 52), dissolved oxygen (YSI Model 52), pH (IQ Scientific Instruments) and chlorophyll *a* concentration (acetone extraction; APHA *et al*., 1989) inside and outside the hapas were monitored weekly. The following water analyses were conducted at biweekly intervals outside the hapas (open pond water) according to methods described by APHA *et al*. (1989): total alkalinity, total hardness, and nutrients; total ammonia nitrogen (phenate method), nitrite-N (diazotization), nitrate (cadmium reduction and diazotization), total phosphorus (persulfate digestion and ascorbic acid finish), and soluble reactive phosphorus (ascorbic acid method). Water transparency in the pond was also measured using a

Secchi disc. At the end of experiment, the plankton count of the pond water was done by using a Sedgewick-Rafter counting cell as described in APHA *et al*. (1989).

Fish harvest and growth calculation

At the end of the experiment, the total bulk weight and number of fish collected from each hapa were recorded. Thirty fish from each hapa (replicate) were randomly selected and individually measured for body weight and total length to calculate their condition factors. Specific growth rate (SGR), feed conversion ratio (FCR), net yield, survival and condition factor were calculated as follows:

SGR = [(ln final weight – ln initial weight) x 100]/days of rearing

FCR = feed applied (dried weight)/live weight gain

Survival rate = (number at harvest/ number at stocking) x 100

Condition factor was calculated as: $k = 100 \times \text{final weight}/(\text{total length})^3$; with weight in grams and total length in centimeters.

Statistical analysis

Data were analyzed using 3x3 factorial analysis to determine the effects of three stocking densities and three feeding levels. Analysis of variance (ANOVA) was used for the fish growth parameters. If a main effect was found significant, the ANOVA was followed by a Tukey-HSD test. Least square regression was performed to evaluate the relationships between specific growth as well as feed conversion efficiency and ratio, and judged by the coefficient of determination (r^2). Variations of treatment means are presented as mean ± standard error and were tested at the 5% probability level using SPSS (Statistical Package for Social Science).

RESULTS

The basic results on survival, growth, and feed conversion efficiency

Stocking and harvest data for the rearing of *P. bocourti* fingerlings with three stocking densities and three feeding levels are given in Table 1. Stocking density had no effect on the final weight, specific growth rate and survival of *P. bocourti* at their respective feeding levels ($P > 0.05$), however, feeding levels had a significant effect on growth parameters ($P < 0.05$). Survival rates were not significantly different ($P > 0.05$) among all treatment groups, ranging from 95 to 99%. The specific growth rate was lowest at the 4% feeding level (2.75 % day^{-1}), and increased as feeding level increased ($P < 0.05$). The highest specific growth rate (4.09% day^{-1}) was reached at 12% feeding level. Condition factors of fish for all stocking densities at different feeding levels were significantly different (Table 1). There was no significant interaction effect of stocking density and feeding level on the growth performance of *P. bocourti* ($P > 0.05$; Table 2).

Table 1. Production of *Pangasius bocourti* fingerlings reared for 56-days in hapas suspended in a fertilized pond at different stocking densities with different feeding levels (mean ± S.E., *N* = 3).

	Parameter					
	Average final weight (g)	**Specific growth rate (% day^{-1})**	**Survival rate (%)**	**Feed conversion ratio**	**Feeding level at harvest (% BWday^{-1}) ***	**Condition factor (k)**
Feeding level 4%						
50	10.72±0.62^{a}	2.99±0.11^{a}	95.3±2.7	0.56±0.06^{a}	0.79±0.07^{a}	0.82±0.02
100	11.13±0.28^{a}	2.87±0.03^{a}	96.3±1.8	0.59±0.02^{a}	0.83±0.03^{ab}	0.81±0.00
150	10.19±0.24^{a}	2.75±0.03^{a}	96.9±0.8	0.64±0.01^{ab}	0.89±0.01^{abc}	0.79±0.01
Mean	10.68±0.25^{X}	2.87±0.05^{X}	96.2±1.1	0.60±0.02^{X}	0.84±0.03^{X}	0.80±0.01^{X}
Feeding level 8%						
50	17.63±0.52^{b}	3.78±0.05^{bc}	96.7±0.7	0.64±0.02^{ab}	1.00±0.03^{bc}	0.82±0.02
100	16.87±0.12^{b}	3.68±0.03^{b}	96.7±0.7	0.68±0.02^{ab}	1.06±0.02^{cd}	0.80±0.02
150	16.06±0.39^{b}	3.61±0.02^{b}	98.9±0.8	0.69±0.01^{ab}	1.07±0.02^{cde}	0.80±0.03
Mean	16.85±0.30^{Y}	3.69±0.03^{Y}	97.4±0.5	0.67±0.01^{Y}	1.04±0.02^{Y}	0.81±0.01^{X}
Feeding level 12%						
50	22.14±0.48^{c}	4.09±0.09^{d}	96.7±1.8	0.79±0.04^{c}	1.26±0.05^{e}	0.86±0.02
100	21.45±0.52^{c}	4.05±0.09^{cd}	99.0±0.0	0.79±0.05^{c}	1.26±0.06^{e}	0.86±0.03
150	21.69±0.58^{c}	4.07±0.04^{cd}	99.3±0.4	0.77±0.02^{c}	1.24±0.03^{de}	0.83±0.02
Mean	21.76±0.28^{Z}	4.07±0.04^{Z}	98.3±0.7	0.78±0.02^{Z}	1.25±0.03^{Z}	0.85±0.01^{Y}

Notations a, b, c, d, e and f compare among the means within the same column, and notations X, Y and Z compare means of each growth parameter among feeding levels. Values with the same superscripts are not significantly different ($P > 0.05$) * Feeding level at harvest means weight of feed delivered to each hapa on the day before harves divided by final biomass in each cage, and then multiplied by 100.

Table 2. The interaction effect of feeding level and stocking density on growth performance of juvenile *Pangasius bocourti* reared in hapas, suspended in a fertilized pond for 56 days by two-way ANOVA.

Parameters/Factors	**MS**	***F***	***P*-value**	**R Squared**
Final weight				
Feeding	277.442	464.168	0.000	
Stocking	1.64	2.744	0.091	
Feeding * Stocking	0.619	1.036	0.416	0.981
Specific growth rate				
Feeding	3.385	295.752	0.000	
Stocking	0.046	4.060	0.035	
Feeding * Stocking	0.010	0.894	0.488	0.971
Survival rate				
Feeding	10.517	2.013	0.163	
Stocking	10.246	1.961	0.170	
Feeding * Stocking	1.373	0.263	0.898	0.333
Feed conversion ratio				
Feeding	0.081	26.493	0.000	
Stocking	0.004	1.155	0.337	
Feeding * Stocking	0.002	0.676	0.617	0.763
Feeding level at harvest				
Feeding	0.388	77.568	0.000	
Stocking	0.005	1.061	0.367	
Feeding * Stocking	0.003	0.628	0.649	0.899
Condition factor (k)				
Feeding	0.005	4.267	0.030	
Stocking	0.002	1.321	0.292	
Feeding * Stocking	0.000	0.243	0.910	0.403

The specific growth rate of the fish from all stocking densities showed a significant asymptotic relationship ($y = -0.014FL^2 + 0.370FL + 1.608$, $n = 27$, $R^2 = 0.952$, $P = 0.000$; Figure 1) with feeding levels, and indicated that feeding levels higher than 12% may not further improve the specific growth rate of fish. The feed conversion ratio showed a significant accelerating relationship with feeding level, and was described as $y = 0.001FL^2 + 0.005FL + 0.556$, $n = 27$, $R^2 = 0.697$, $P = 0.000$ (Figure 2), which indicated that the feed conversion ratio tended to increase rapidly as the fish were fed at higher feeding levels. The gross yields of fish for the different feeding levels were significantly affected by stocking density, and significantly increased linearly with increasing stocking density ($P < 0.05$), reaching 3.2 kg m^{-3} at the highest stocking density (150 fish m^{-3}) (Figure 3).

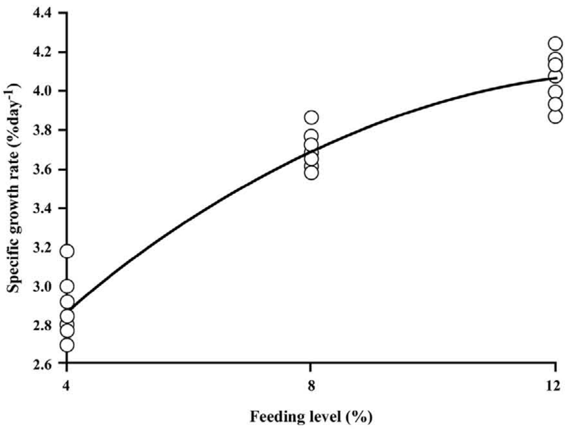

Figure 1. Relationship between specific growth rate (SGR) and feeding level (FL) of juvenile *Pangasius bocourti*, grown in hapas suspended in a fertilized pond for a period of 56 days.

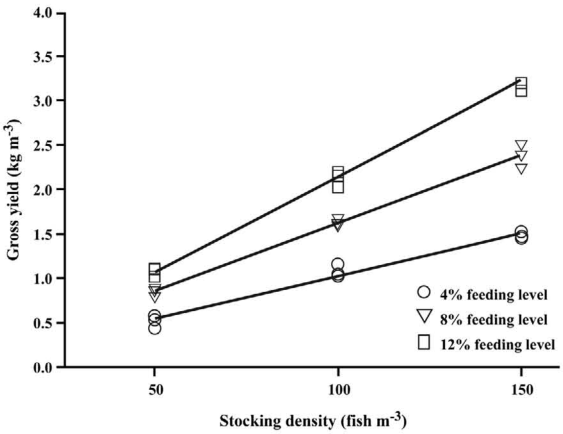

Figure 3. Linear relationship between stocking density and gross yield for the juvenile *Pangasius bocourti* fed at 4, 8 and 12% feeding levels: y = 0.0097x + 0.0545, R^2 = 0.975, P = 0.000; y = 0.0153x + 0.0911, R^2 = 0.988, P = 0.000; and y = 0.0216x + 0.0204, R^2 = 0.991, P = 0.000, respectively

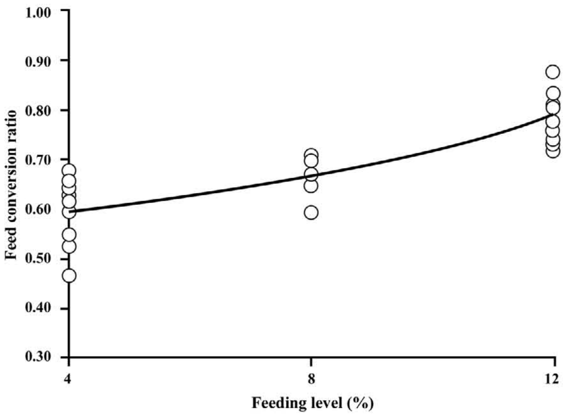

Figure 2. Relationship between feed conversion ratio and feeding level of juvenile *Pangasius bocourti* reared in hapas, suspended in a fertilized pond for a period of 56 days.

Water quality

Water quality parameters such as total ammonia nitrogen (0.013 to 0.017 mg l^{-1}), chlorophyll *a* (61.28 to 122.46 µg l^{-1}), dissolved oxygen (6.39 to 7.11 mg l^{-1}), pH (8.01 to 8.16) and temperature (33.21 to 33.53ºC) during the experiment were not different (P > 0.05) among the different treatments in the hapas. Water quality parameters outside the hapas (open pond water), i.e. dissolved oxygen, pH, temperature, total alkalinity, total hardness, soluble reactive phosphorus, total phosphorus, total ammonia nitrogen, nitrite, nitrate and Secchi disc depth were 5.50-8.60 mg l^{-1}, 6.05-9.06, 30.70-34.80 ºC, 148.4-166.8 mg l^{-1}, 100.1-154.2 mg l^{-1}, 0.30-1.78 mg l^{-1}, 0.77-5.70 mg l^{-1}, 0.01-0.04 mg l^{-1}, 0.09-0.25 mg l^{-1}, 0.02-0.85 mg l^{-1} and 33.50-75.00 cm, respectively.

Plankton composition

The plankton composition in the experimental pond at the end of the experiment is given in Table 3. With respect to phytoplankton, the Chlorophyceae was the dominant group in terms of number of cells or colony-forming units, followed by Cyanophyceae, Euglenophyceae and Bacillariophyceae. Two groups of zooplankton, namely, Crustacea and Rotifera were also present in the plankton community.

Table 3. Plankton communities in pond water fertilized with chicken manure at a rate of 2000 kg ha^{-1} per month, and used in the suspended experimental hapas.

Classification	Cells or colony-forming units or individuals l^{-1}
Phytoplankton	
Bacillariophyta	
Coscinodiscus	$50x10^3$
Navicula	$5x10^3$
Nitzschia	$30x10^3$
Pleurosigma	$20x10^3$
Chlorophyta	
Scenedesmus	$36x10^3$
Chlorella	$264x10^3$
Oocystis	$96x10^3$
Cyanophyceae	
Microcystis	$168x10^3$
Euglenophyceae	$160x10^3$
Zooplankton	
Rotifera	$9x10^3$
Crustacea	
Copepods	$14x10^3$

DISCUSSION

Fish growth was not significantly influenced by stocking density in this experiment. As observed in the present study, the mean survival rate for all treatments was > 95%, confirming that juvenile *Pangasius bocourti* is well adapted to the hapa rearing system. This finding parallels the results of previous studies on other species. The survival of large tilapia, starting with an initial weight of between 141 to 152 g in cages suspended inside ponds for a 90-day period decreased from 91 to 57% when stocking densities were increased from 30 to 70 fish m^{-3} (Yi *et al.*, 1996). In an 8-week culture of African catfish in cages in ponds, from an initial mean weight of 32 g, the final mean weight ranged from 346 (at 200 fish m^{-3}) to 385 g (50 fish m^{-3}), with survival rates varying from 85 to 95% (Hengsawat *et al.* 1997). Yi and Lin (2001) observed a survival rate of >95% for large Nile tilapia *Oreochromis niloticus*, initially weighing between 91 to 103 g that had been reared over a 90 day period in cages inside ponds. Cage culture of tambaqui (*Colossoma macropomum*) in a central Amazon floodplain lake gave a survival rate of >95% (Gomes *et al.* 2006). The maximum survival rate of African catfish *Heterobranchus longifilis*, reared over a 90-day period and starting with an initial weight 0.8 g in fine mesh-netting cages in a man-made lake was 68% with a stocking density of 50 fish m^{-3} (Coulibaly *et al.* 2007).

In the present study, stocking densities had no significant effect on final weight and specific growth rate of *P. bocourti* for their respective feeding levels ($P > 0.05$). The specific growth rate was lowest at the 4% feeding level (2.75 % day^{-1}), and increased with feeding level ($P < 0.05$) to the highest rate of 4.1% day^{-1} at the 12% feeding level. This value was in the range of 5.20 to 3.66% day^{-1} observed by Hung *et al.* (2003) in *P. bocourti* fingerlings (initial mean weight of 4.7g) from a 4-week feeding trial in a closed culture system.

An asymptotic relationship between specific growth rate and feeding level indicated that a feed input of about 12% of the initial weight per day was the maximum for this growth phase, and could yield good growth in the fish. At lower feeding levels (4 or 8%), the specific growth rates are likely to decrease with increasing stocking density, while feed conversion ratios are the inverse. When the fish were fed at the 12% level, the FCR is likely to decrease with increasing stocking density. The FCR presented an inverse relationship with stocking density, with lower FCRs at higher densities being observed in the grow-out cage culture of tambaqui *Colossoma macropomum* (Chellapa *et al.* 1995; Gomes *et al.* 2006). The fish in the previous study were fed with commercial pellets containing 34% crude protein at apparent satiation, and the results might suggest that natural food also has an impact on fish growth when they are fed restrictively. However, those fish did not attain the optimum growth which was indicated by the significant higher specific growth rate of the fish that were fed at the 12% feeding level. The condition factor also indicated that only the fish fed at the 12% feeding level tended to attain normal growth, as fish could not completely depend on suspended plankton to meet their nutrient requirements (Keshavanath *et al.* 2001). This result could be confirmed by the suggestion that the best feeding strategy for tambaqui during the first growth phase in cages are 10% of body weight day^{-1} divided into 3 meals day^{-1} (Silva *et al.* 2007).

Homogenous water quality among hapas indicated that the different rates of growth and survival of the experimental fish were not affected by being inside the hapas. Therefore suspended organisms in the pond water were also available for fish inside the hapas. All the water quality parameters were within the acceptable ranges, as recommended for tropical aquaculture (Boyd 1982; Boyd and Tucker 1992; Beveridge 1996). Phytoplankton standing crops in terms of total mean chlorophyll *a* concentrations in the hapa-water throughout the experimental period ranged from between ~70 to >~100 $\mu g\ l^{-1}$ with a total mean of about 87.5 $\mu g\ l^{-1}$, and stable throughout the experimental period in all treatments except during the 5^{th} week. The Secchi disc depth measured in open-pond water ranged from 33 to 75 cm during the experimental period. Chlorophyll *a* concentration was similar to the results of a previous study in Thailand by Diana *et al.* (1991), in which the ponds, which were fertilized with chicken manure at a rate of 2,142 kg ha^{-1} per month, had an average chlorophyll *a* concentration of 96 $\mu g\ l^{-1}$. The plankton communities in the present study were similar to those observed in a polyculture trial conducted in earthen ponds in Bangladesh (Azim *et al.* 2002). Zooplankton, rotifers and copepods were also observed in the experimental pond and indicated that natural food was available in the culture system. Mischke and Zimba (2004) suggested that in channel catfish nursery ponds, high concentrations of copepods, cladocerans and ostracods would be desirable from the time of fry stocking through about five weeks of production. The availability of zooplankton in the pond during the present study may have been limiting for the fish in the hapas, as it is well known that the diet requirements are stringent for fish cultured in cages and that the availability of natural food could decrease at high densities, possibly resulting in nutrition deficiencies (Coulibaly *et al.* 2007). Hence, rearing in open pond may result in higher growth than rearing in hapas due to the greater availability of natural food in the former.

The lower FCR at high stocking density when fish were fed at the highest feeding level (12%, considered optimum in the present study), may have resulted from an increase in competition for the available diet and may have led to more efficient consumption (Gomes *et al.* 2006). However, it has been documented that increasing stocking density results in stress (Leatherland and Cho 1985) which in turn leads to enhanced energy requirements causing reduced growth and food utilization (Hengsawat *et al.* 1997). Therefore, the crowding tolerance, tolerance to low dissolved oxygen level of the fish, and maximum carrying capacity of the culture system should be taken into consideration for FCR interpretation.

In the present study, fish attained an average individual weight of over 20 g, which is the suitable size for stocking in cage culture. In Thailand, the juveniles used to stock the cages for the grow-out period are usually bought by number; however, after the grow-out period they are sold based on the weight of the marketable fish. Therefore, the potential average individual weight of over 20 g clearly shows an opportunity for high profitability. A feed conversion ratio of 0.8 with a rather high specific growth rate of the fish (4 % day^{-1}) in hapas at 150 fish m^{-3}, and fed with a fixed-ration of 12% of their initial weight could be economically feasible in to produce *Pangasius bocourti* seed for cage culture. Due to its apparent practicality, this rearing technique may be a viable alternative to traditional rearing techniques, and could easily be adapted by rural farmers. The results show that the maximum yield for juvenile *P. bocourti* during an 8-week rearing cycle was not reached, since the relationship between fish density and yield remained linear. Therefore, to increase fish yield per m^3, higher stocking densities should be tested. Furthermore, to maximize growth of *P. bocourti* in hapas, adjusted feeding based on the results of the present study should be tested.

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