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Original Research

Standardization of stocking density for maximizing biomass production of *Pangasius pangasius* in pond cage aquaculture

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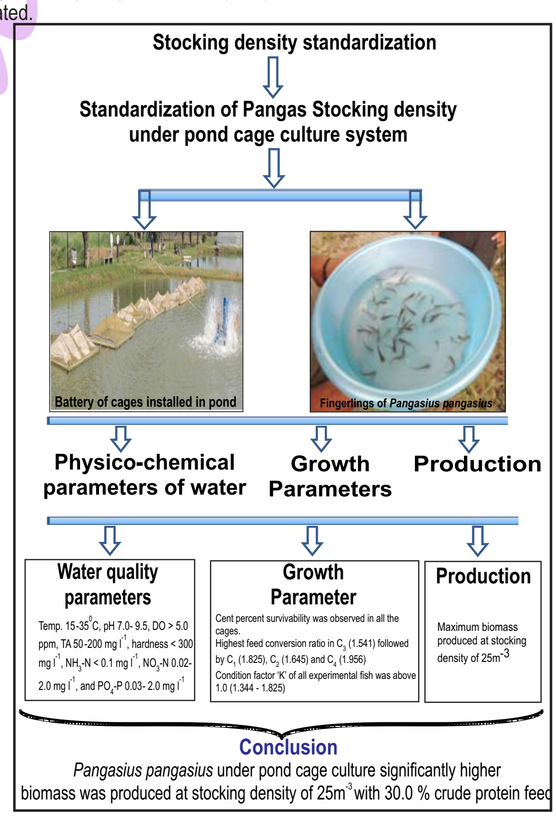
Abstract

Aim : *Pangasius pangasius,* a species of freshwater shark catfish native to Mekong River is highly resistant to crowding and low oxygen therefore, can be a suitable species for cage aquaculture. The objective of the present study was to standardize the stocking density of catfish *P. pangasius* in pond cage aquaculture for maximizing biomass per unit water area.

Methodology : A comparative study was conducted in fish cages (2.0 m×2.0 m ×1.0 m) installed in earthen pond of 600 m^{-2} area and depth of 2.0 m. Stocking density of *P. pangasius* fingerlings was maintained @ 15 m^{-3} (C_1), 20 m^{-3} (C_2), 25 m^{-3} (C_3) and 30 m^{-3} (C_4), respectively. Fish seed was procured from, Kolkata, West Bengal. The experiment was conducted for 120 days. Fish (in cages) were fed with formulated diets (30.0 % crude protein on dry weight basis) @ 3% body weight twice a day. Physico–chemical parameters of water and growth parameters were calculated.

Results : Maximum biomass was produced in C_3 which was 60.14 %, 22.72 % and 12.21 % higher than C_1, C_2 and C_4, respectively, with cent percent survivability in all the cages. Better feed conversion ratio was noted in C_3 (1.541) as compared to C_1 (1.825), C_2 (1.645) and C_4 (1.956), indicating higher the stocking density better feed conversion ratio up to stocking density of 25m^{-3}, and afterwards (@ 30 m^{-3}) food conversion efficiency decreased. The experimental fish recorded the value of exponent 'b' in the range of 2.868 to 3.063 revealing both negative, as well as positive allometric growth pattern and condition factor 'K' of all experimental fish was above 1.0 (1.344 - 1.825) indicating robustness or well-being of experimented fish.

Interpretation : The appreciable growth rate exhibited by fish during rearing period indicated that the species is suitable for cage culture at higher stocking density as food fish to meet the increasing nutritional demand.



Introduction

Population growth in developing nations is a leading cause for major changes in demand and supply for animal protein, from both livestock and fish (Delgado *et al.*, 2003). In last 20 years cage aquaculture has grown rapidly and the growth is still occurring in response to increasing demand of aquatic products from developed and developing nations (Halwart *et al.*, 2007). Pond cage aquaculture can play a major role in meeting this demand in areas away from coast line. In pond cage culture, fish in cages are fed with high-protein diet, while fish in pond depend on leftover feed and excreta of fish reared in cages (Yi and Lin, 2001). Pond cage aquaculture with high stocking density provides higher production, if crowding resistant species are used in cages (Datta *et al.,* 2014). Pangasius, a well-known catfish is one of the major fish species in the Mekong River and regarded as one of the largest and most important inland fishery resource in the world. It is now considered as the 3rd most important freshwater fish group within the aquaculture sector. It is considered as economically important food fish because of its fast growth, versatile feeding habit and hardiness. Pangasius is considered as low risk aquaculture species since it cannot reproduce naturally, fingerlings are produced by local hatcheries through induced spawning. Different species of *Pangasius* are highly resistant to stocking density (Hung *et al.,* 2001; Chattopadhyay *et al.,* 2002). *Pangasius pangasius* (Hamilton, 1822) is native to fresh and brackish waters of Bangladesh, India, Myanmar and Pakistan (Pal, 2010). Other species of *Pangasius* are used for commercial aquaculture but *P. pangasius* is cultured at limited scale (Pilay and Kutty, 2005) so the standardization of culture practices become necessary for economic benefits. This paper deals with the objective of standardizing the stocking density of *P. pangasius* in pond cage aquaculture for maximizing biomass per unit area.

Materials and Methods

The study was conducted at the Fish Farm of College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (Punjab), India (30.54° N latitude, 75.48 °E longitude and an altitude of 247 m above mean sea level). The experiment (in triplicate) was conducted in fish cages (2.0 m×2.0 m ×1.0 m cage bag size and 3 mm knot-less HDPE) installed in earthen pond of 600 m^{-2} area and depth of 2.0 m. Stocking density of *P. pangasius* fingerlings was maintained @ 15 m^{-3} (total 60 number) (C_1), 20 m^{-3} (total 80 number) (C_2), 25 m^{-3} (total 100 number) (C_3) and 30 m^{-3} (total 120 number) (C_4), respectively. *P. pangasius* (average length 2.93$\pm$ 0.2 cm and weight 0.25 $\pm$ 0.05g) collected from fish seed hatchery, Kolkata, West Bengal. The experiment was conducted for 120 days.

Formulated diets : Supplementary diet (30.0 % crude protein on dry weight basis) was formulated using agro-industrial by-products i.e. rice bran (30%), de-oiled ground nut (30%), de-oiled soybean (25%), fish meal (13 %) and vitamin – mineral mixture (2%). For preparation of diet, all dry feed ingredients were first grounded to a small particle size in a laboratory electric grinder and sieved through an approximately 250 µm sieve. Ingredients were thoroughly mixed in a commercial food mixer for 15 min. Stiff dough was prepared by adding water. The wet mixture was steamed for 5 min. and the diets were extruded with the help of a pelletizer of 2.0 mm in diameter. The pelleted diets were dried overnight at 55°C, afterwards were broken up and sieved into appropriate pellet sizes. Proximate composition of feed ingredients and formulated diets (Table 1) was determined as per the standard methods of AOAC (2005).

Feeding of fish : Fish (in cages) were fed with formulated diets @ 3% of body weight in total (two meals of 1.5 % body weight) twice a day (9.00 am and 5.00 pm). Check trays were maintained in each cage to calculate the amount of feed provided and amount of uneaten feed in each treatment for accurate calculation of Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER). The feed quantity was regulated based on the fortnightly sampling of 30 fish each from all the replications of each treatment. The following formulae were used to calculate FCR and PER:

FCR = Weight of feed given (g)/Fish weight gain (g)

PER = Fish weight gain (g)/ Protein intake (g).

Water analysis : Physico–chemical parameters of water samples including water temperature, pH, dissolved oxygen, total alkalinity, hardness, ammonia-nitrogen (NH_3-N), nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N) and phosphate-phosphorous (PO_4-P) were measured at weekly interval from the four corners of the experimental pond following standard methods (APHA, 2012).

Growth analysis : Random sampling of 30 fish from each of the replicate of each treatment were measured for total length (TL) and total weight (TW) at fortnightly interval basis to analyze the

Table 1 : Proximate composition (%) of feed ingredients and experimental diet

Ingredients	Moisture	Crude protein	Ether extract	Crude fiber	Ash	Nitrogen free extract
Rice bran	14.20	16.73	1.4	8.89	12.08	45.80
Groundnut cake	13.50	41.59	2.90	7.81	4.30	29.90
Soybean meal	12.10	42.50	3.60	6.80	5.90	29.10
Fish meal	14.65	48.35	3.0	3.10	21.75	9.15
Experimental diet	13.20	30.0	2.75	5.52	9.65	38.87

growth of fish. The following parameters were calculated to determine the growth response of the fish to the diets:

Daily Weight Gain (DWG) (g/day) = W_f-W_i (g) / Culture period (days)
Relative Growth Rate (RGR) (%) = (W_f-Wi) x 100 / W_f

Where W_f = Final average weight at end of experiment and Wi = Initial average weight at beginning of experiment.

Specific growth rate (SGR) = [Log_e (Final weight in gram) – Log_e (Initial weight in gram)] X 100 /Culture days

Survival Rate (%) = Number of fish that survived X 100/Number of fish stocked

Length-weight relationship : The length-weight (log-transformed) relationships were determined by linear regression analysis and scatter diagrams of length and weight. The length-weight relationship of the experimented fish is worked out as per Le Cren (1951).

$W = aL^b$

where, W is weight of fish (g), L is total length (cm), 'a' is the regression intercept and 'b' is the regression slope.

The logarithmic transformation of the above formula is-

Log W = log a + b log L

Fulton's condition factor (K): Fulton's condition factor (K) was calculated according to Htun-Han (1978) equation as per formula given below:

$K = W \times 100 / L^3$

Where, W = weight of fish (g), L= Length of fish (cm).

Statistical analysis : The analysis of covariance was performed to determine variation in 'b' values following the method of Snedecor and Cochran (1967). The statistical significance of Isometric exponent (b) was analyzed by a function: ts = (b-3) / Sb (Sokal and Rohlf, 1987) where, ts is t- test value; 'b' is the slope and Sb is the standard error of 'b' and comparison between obtained values of t-test and the respective critical values determined that 'b' values statistically significant and their inclusion in the isometric range (b=3) or allometric range (negative allometric; b<3). Statistical software SPSS 16 and PAST Ver. 1.8 was used in this study.

Results and Discussion

All mean values of water quality parameters viz. temperature, pH, dissolved oxygen (DO), total alkalinity (TA), hardness, NH_3-N, NO_3-N, and PO_4-P were within the acceptable range (Temp. 15-35^{0}C, pH 7.0- 9.5, DO > 5.0 ppm, TA 50-200 mg l^{-1}, hardness < 300 mg l^{-1}, NH_3-N < 0.1 mg l^{-1}, NO_3-N 0.02-2.0 mg l^{-1}, and PO_4-P 0.03-2.0 mg l^{-1}) as suggested by Bhatnagar and Devi, (2013) for general aquaculture in pond (Table 2). A cent percent survival was observed in all the cages indicating that survival rate remained unaffected upto stocking density of 30 m^{-3}. Similar results were observed in *Pangasius bocourti* with survival rate higher than 90% in stocking densities 12, 25, 50, 100, and 200 fish m^{-3} (Jiwyam, 2011). Constant survival rate with changing the stocking density was also observed in *Pangasius sutchi* reared in cages by Rahman *et al*. (2006). Thus it can be said that *P. pangasius* can sustain the crowding environment in fish cages. Maximum weight gain was recorded in C_3 followed by C_4, C_2 and C_1. Maximum biomass produced per unit area was observed in C_3 (60.14%, 22.72% and 12.21% more biomass produced then C_1, C_2 and C_4, respectively) (Table 3). Less biomass production was observed in

Table 2 : Physico-chemical parameters of water in the experimental pond

Water parameters	Mean±SE values	Range
Temperature (ºC)	26.69±0.17	13.0-31.9
pH	8.54±0.22	8.43-10.4
Dissolved oxygen (mg l^{-1})	6.60±0.24	5.0-8.8
Alkalinity (mg l^{-1})	166.80±0.44	148-296
Hardness (mg l^{-1})	195.62±0.12	154-308
Ammonia - nitrogen (NH_3-N (mg l^{-1})	0.0824±0.18	0.0616-0.3993
Phosphate- phosphorus PO_4-P (mg l^{-1})	0.4562±0.56	0.0179-0.9440
Nitrate – nitrogen NO_3^-N (mg l^{-1})	0.5586±0.66	0.1271-0.8291

Table 3 : Minimum, maximum growth (length and weight) and biomass produced in different treatments of *P. pangasius* in cages (Initial average length 2.93± 0.2 cm and wt. 0.25 ± 0.05g)

Treatments	Final minimum length (cm)	Final minimum weight gain (g)	Final maximum length (cm)	Final maximum weight gain (g)	Final average weightgain (g)	Biomass produced (kg)^1
C_1	22.733±0.057^d	73.500±2.50^b	24.333±0.057^d	79.00±2.00^c	77.616± 144^a	4.657±0.144^a
C_2	21.400±0.300^c	72.500±1.500^b	22.433±0.152^c	77.333±0.577^c	75.962± 546^b	6.077±0.033^b
C_3	19.066±0.057^b	72.333±0.577^b	21.633±0.152^b	75.000±1.000^b	74.580±776^d	7.458±0.113^d
C_4	16.633±0.152^a	53.833±0.288^a	17.633±0.152^a	56.166±0.288^a	55.38±322^c	6.646±0.132^c

Table 4 : Growth performance of *P. pangasius* in cages reared at difference stocking densities

Treatments	Feed conversion ratio	Daily weight gain (g day^{-1})	Relative growth rate (%)	Protein efficiency ratio
C_1	1.818 ± 0.007^{c}	0.647 ± 0.0005^{d}	99.78 ± 0.0007^{c}	1.825 ± 0.0007^{b}
C_2	1.646 ± 0.003^{b}	0.6285 ± 0.0013^{c}	99.77 ± 0.0010^{b}	2.026 ± 0.0010^{c}
C_3	1.548 ± 0.006^{a}	0.611 ± 0.0003^{b}	99.76 ± 0.0124^{b}	2.164 ± 0.0015^{d}
C_4	1.958 ± 0.002^{d}	0.4591 ± 0.0005^{a}	99.78 ± 0.0010^{a}	1.702 ± 0.0015^{a}

Each value is represented as mean±SE; Means followed by same superscript in a column are not significantly different ($p<0.05$)

Table 5 : Length-weight relationship of fish reared in cages

Treatments	Stocking density (m^{-3})	Specific growth rate (% day^{-1})	Logarithmic equation Log W = log a + b log L	Correlation coefficient (r)	Coefficient of determination (r^2)	Condition factor (K)	Slope 'b'
C_1	15	4.729	Log W = log 0.0103 + 2.868 log L	0.997	0.995	1.344	2.868
C_2	20	4.671	Log W = log 0.009 + 2.959 log L	0.998	0.997	1.569	2.959
C_3	25	4.658	Log W = log 0.0089 + 2.977 log L	0.9989	0.997	1.595	2.977
C_4	30	4.438	Log W = log 0.0083 + 3.063 log L	0.9994	0.998	1.825	3.063

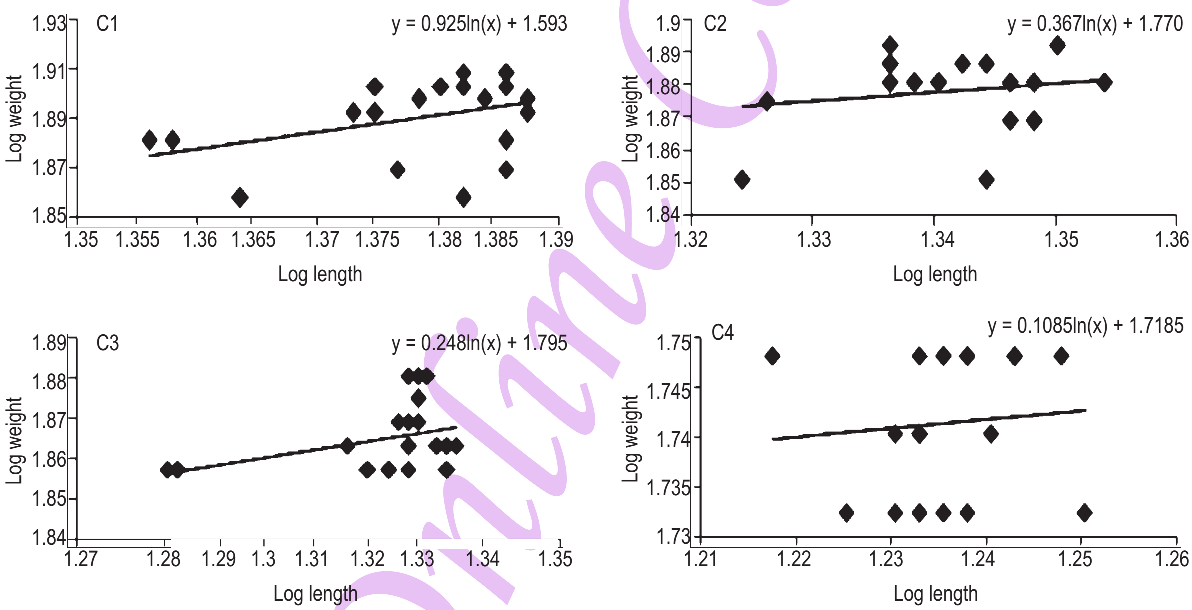


Fig. 1 : Log length and Log weight relationship of *Pangasius pangasius* in different treatment (C_1, C_2, C_3 and C_4)

C_4 (at stocking density of 30m^{-2}) which might be due to the maximum carrying capacity of unit volume of water reached highest at stocking density of 25m^{-3} after the biomass production per unit area had decreased. Fish kept at high densities normally have slow growth (El-Sayed, 2002). Individual growth and population density are known to be closely linked, and the growth of the larger sized fish is dramatically affected by stocking density (Coulibaly *et al.*, 2007).

Significant variation in feed conversion ratio (FCR) was observed among different treatments. Better feed conversion values were noted in C_3 (1.541) as compared to C_1 (1.825), C_2 (1.645) and C_4 (1.956), indicating higher stocking density having positive relation upto stocking density of 25m^{-3}, however FCR was decreased afterwards (Table 4). The FCR of *Pangasius hypophthalmus* was reported in the range of 1:1 to 1:3 by farmers in India in open pond (Singh and Lakra, 2012). Daily weight gain (DWG) was highest in C_1 (0.6475 g) in comparison to C_2 (0.6296 g), C_3 (0.6113 g), and C_4 (0.4596 g), respectively (Table 4). Specific growth rate (SGR) was observed maximum in C_1 (4.729) followed by C_2 (4.671), C_3 (4.658) and C_4 (4.438), respectively (Table 5) indicating that DWG and SGR decreased with increasing the stocking density. The results are with conformity of the findings of Ali *et al.* (2005) and Rahman *et al.* (2006) that DWG and SGR decreased with increasing the stocking density in *P. sutchi*. Length – weight relationship of fish stocked in different cages, values of regression co-efficient 'b' and logarithmic relationship between length and weight with regression equation are given in Table 5 and Fig. 1. Growth is said to be negative allometric when length increased more than weight (b<3) and positive allometric when b>3 (Froese, 2006). When total length was regressed with body weight, final 'b' varied between 2.868 to 3.063. The slope value was significantly lower than critical isometric value i.e., 3, in three treatments (C_1, C_2, C_3) indicating negative alometric growth, whereas C_4 showed positive allometric growth pattern; thus species become slender with increase in length. The results of the present study is in conformity with the views of Nehemia *et al*. (2012) that a fish normally does not retain same shape or body outline throughout their lifespan and specific gravity of tissue may not remain constant, the actual relationship may depart significantly from the cube law. Negative allometric growth pattern was observed by Yusof *et al.* (2011) and Mortuza and Al-Misned (2015). Variation in slope may be attributed to stocking density, carrying capacity and environmental factors (Deka and Gohain, 2015).

Condition factor (K) of a fish reflects physical and biological circumstances and fluctuations by interaction among feeding conditions, parasitic infections and physiological factors (Ndiaye *et al.,* 2015). This also indicates the changes in food reserves and therefore, an indicator of general fish condition. Moreover, body condition provides an alternative to the expensive invitro proximate analyses of tissues (Sutton *et al.,* 2000). Therefore, information on condition factor can be vital for culture system management because they provide the producer with information of the specific condition under which organisms are developing (Araneda *et al.,* 2008). The values of condition factor 'K' recorded in the present study were 1.344, 1.569, 1.595 and 1.825 in C_1, C_2, C_3 and C_4, respectively. Condition factor showed well being of fish fed with experimental diet, suggesting that formulated diet was preferred by *P. pangasius* and digested well. The K value >1.0 also indicates that the culture environment and conditions were suitable. Thus, *P. pangasius* is a suitable candidate species for cage culture also. The co-efficient of determination (r^2) values explained proper fit of the model for growth. In the present study, lowest value of r^2 of *P. pangasius* was recorded as 0. 995 (i.e. 99.5 % variability) in C_1 and highest were recorded as 0.998 (99.8% variability) in C_4 (Table 5), indicating more than 99.5% variability by the model and good fitness.

In the present study, growth rate, condition factor and co-efficient of determination value recorded for *P. pangasius* under pond cage culture indicated that maximum biomass can be produced at stocking density of $25m^{-3}$ in a sustainable basis with farm produced feed. Survival rate was recorded constant with different stocking density. The appreciable growth rate exhibited by fish during rearing period indicated that the species is suitable for cage culture at higher stocking density as food fish to meet the increasing nutritional demand of small and marginal farmers with small land holding may adopt these culture technologies for more income generation per unit area.

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