Original Paper   http://indexmedicus.afro.who.int

Optimum stocking density for *Parachanna obscura* larvae fed at its optimum ration

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ABSTRACT

Two experiments were conducted during 15 days respectively in re–circulating system composed of 15 plastic tanks containing 20L of water each. This study aims at estimating optimum feeding rate and stocking density for efficient growth, food utilization and survival rate of *Parachanna obscura* larvae (initial body weight = 0.1 ± 0.0 g and 0.15 ± 0.13 g). In the first experiment, five food rations (5, 10, 20, 25 and 30% of body weight per day) were tested in triplicate. Specific Growth Rate (SGR) and Feed Efficiency (FE) of larvae fed different rations varied significantly (P<0.05). Best SGR and FE were obtained with rations from 5 to 10% of body weight. Two mathematical models were used to analyse the relationships between feeding level and SGR. Optimum and maximum rations are 8.13% and 25% of body weight respectively. In the second experiment, five stocking densities (5, 20, 35, 50 and 65 larvae/L) were assigned in triplicate. Fish were fed manually with ration 8.13% of body weight. Results showed that SGR and FE obtained with different densities varied significantly (P<0.05). Highest final body weight and survival rate of fish were obtained with stocking density 20/L of water. Moreover, best SGR and FE were observed in larvae stocked at density 20 larvae/L (P<0.05). Thus, optimum stocking density of *P. obscura* fry (initial body weight = 0.15 ± 0.13 g) is 20/L.
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Keywords: *Parachanna obscura*, rations, stocking density, growth, Feed Efficiency.

INTRODUCTION

Parachanna obscura is the most common African Channidae (Bonou and Teugels, 1985). It has a remarkable growth potential, a high economic value and represents a good potential for African aquaculture (Victor and Akpocha, 1992). Because of its tasty flesh, with only few bones, *P. obscura* is favourite food fish and constitute an extremely important part of the staple food for African people (O 'Bryen and Lee, 2007). Thus, the population of *P. obscura* has declined considerably due to increased fishing pressure and various anthropogenic activities leading to siltation, aquatic pollution and loss of natural habitat for spawning and growth (O 'Bryen and Lee, 2007). These factors not only destroyed the breeding grounds but also caused havoc to the availability of brood fish including fry and fingerlings. To maintain this fish population as well as its conservation and rehabilitation,

development of a suitable technology for rearing of fry in nursery ponds is urgently needed. Efforts at culturing *P. obscura* merely start and end at collecting them from the wild. Also, culture of this species will boost the number of species currently cultivated through aquaculture and will contribute to the much desired increase of the supply of fish protein. In intensive larvae and fry culture, several factors influence survival rate, welfare, growth and production for example feeding level (El-Sayed, 2002) and stocking density (Sahoo et al., 2004; Rahman et al., 2005; Schram et al., 2006). Determination of appropriate rations for cultured fish is important to achieving maximum productivity because ration size and feeding frequency determine feed intake, food utilization, and growth (Andrews and Page, 1975; Grayton and Beamish, 1977). The use of high stocking density as a technique to maximize space usage to increase stock production has been shown to have an adverse effect on growth (Trzebiatowski et al., 1981). In some species, the increase of stocking density usually results in stress which leads to enhanced energy requirements causing reduced growth, food utilization and net yield (Grant, 1997; Begout-Anras and Lagardere, 2004). The economic success of production control in aquaculture depends, to a large extent, on reasonable feeding costs. Indeed, the relationship between growth rate and ration in fish is very important because feed accounts for 70% of the cost of the intensive fish culture (Pillay, 1990). One way of reducing feeding costs is to estimate appropriate daily ration and stocking densities for optimum growth, food utilization and survival rate of fry (Fiogbe and Kestemont, 2003). This may have the effect not only of reducing the cost of feeding but also the biological loading of recirculation systems and effluent production in flow-through systems (Woods, 2005). No work has yet been undertaken on stocking density and ration size of *P. obscura* for rearing in nurseries. Webber and Riordan (1976) stated that one of the main obstacles in the development of aquaculture is the availability of fry. Those works were therefore designed to determine optimum feeding rate and stocking density for well growth, food utilization and survival rate of *P. obscura* larvae.

MATERIELS AND METHODS
Experimental facilities and fish

Larvae of *P. obscura* (initial weight = 0.04 ± 0.2 g) were collected from a swamp " Dra" in Takon (South – Est - Benin). Water temperature, pH and dissolved oxygen in the swamp were 27.7 °C, 6.1 and 2.1 mg/L respectively. Once collected larvae were immediately shipped to the experimental station to the Research Unit in Wet Land of the Department of Zoology and Genetics of the Faculty of Sciences and Technology (University of Abomey-Calavi, Benin) and put in circular tank. Before the beginning of each experiment, larvae were fed with only live food for four days, mainly zooplankton (*Brachionus spp*) collected in a pond with planktonic net and newly hatched *Artemia nauplii* (EG grade, INVE, Dendermonde, Belgium). The food substitution was progressive and was 25, 50, 75 and 100% of artificial food according to the feeding plan reported in Table 1. High-energy starter crumbles (Coppens CATCO Crumble Excellent, 0.5 - 0.8 mm, 56% protein, 15% fat) were used as feed. Two experiments were conducted in a re-circulated system composed of 15 plastic tanks containing 20L of water each. All tanks were linked to a 225 L re-circulated system, which received water from the experimental tanks. Water was re-circulated through mechanical and biological filter system before being pumped into each tank at a flow rate of 0.5 L/min.

Experimental procedures
Experimental 1

This experiment aims at determining the optimum dietary ration for *P. obscura* larvae. Five feeding levels (5, 10, 20, 25 and 30% of fish body weight) were tested in

Table 1: Feeding plan of *P. obscura* larvae.

Duration (days)	Food
1	100% zooplancton (rotifères : *Brachionus spp*)
1	*Artemia nauplii* 75% + zooplancton 25%
1	*Artemia nauplii* 50% + zooplancton 50%
1	*Artemia nauplii* 100%
1	*Artemia nauplii* 75% + artificial food 25%
1	*Artemia nauplii* 50% + artificial food 50%
1	*Artemia nauplii* 25% + artificial food 75%
8	artificial food 100%

Artificial food =Coppens 0,5 - 0,8 mm, 56% proteine and 15% lipide.

triplicate on larvae (initial mean weight = 0.1 g) for 15 days. Food was distributed manually every hour from 08:00 AM to 08:00 PM.

Experimental 2

Five stocking densities (5, 20, 35, 50 and 65 larvae/L) were tested in triplicate on larvae (initial mean weight = 0.15 g) for 15 days in order to estimate the optimum stocking density. Larvae were fed daily manually with ration 8.13% of body weight every hour from 08:00 AM to 08:00 PM.

Rearing procedure

Each tank was daily finely siphoned in order to find uneaten food and likewise remove faeces and dead fish. After siphoning, the water volume (approximately 25%) was adjusted in each plastic and dead fish were counted and checked under a binocular microscope to assess signs of cannibalism and malformation.

Water quality parameters such as temperature, dissolved oxygen and pH were daily measured. These parameters were respectively 28 ± 0.15 °C, 6.46 ± 0.24 mg/L and 6.64 ± 0.11 in experiment 1 and 27.7 ± 0.08 °C, 5.12 ± 0.37 mg/L and 6.23 ± 0.03 in experiment 2.

At the beginning of each experiment, 30 larvae were weighed individually. All fish were counted and weighed every 3 days before being released into their corresponding

plastic and food ration adjusted accordingly. No feed was offered to the fish on the day the measurements were taken.

At the end of each experiment, all fish were counted and fish body weights per plastic as well as individual weight were taken. The Specific Growth Rate (SGR), the Feed Efficiency (FE) and survival rate were calculated on the basis of the initial and final body weight, according to the duration of the experiment (number of days = d) as followed : SGR (%/d) = 100 x [ln(final weight) – ln(initial weight)]/d. FE was calculated on the basis of the total food distributed (FD, g), the Initial and Final Biomasses [IB and FB, respectively (g)], and Biomass of Dead fish (DB, g) as followed: (FB+DB-IB)/FD. Survival rate (%) = 100 x FN/IN (IN, FN = Initial and Final Number of fish respectively).

Data analysis

The mean values of final weight, SGR, FE and survival rate were compared between treatments by one – way analysis of variance (ANOVA 1) after verifying the homogeneity of variance using "Hartley' s test" for each experiment. Significant differences between treatments means ($p<0.05$) were determined using a Fisher's test (Saville, 1990). Results are given as means ± standard deviation.

Two mathematical (dose – reponse) models were used to assess the effect of

feeding level on specific growth rate of *P. obscura* fry.

The general equation of the broken line model (Robbins et al., 1979) is $y = L + U(R-X_{LR})$ where L is the ordinate and R, the abscissa of the breakpoint. R is taken as the estimated requirement (feeding level that guarantees the maximum specific growth rate). X_{LR} means X less than R and U is the slope of the line for X_{LR}. By definition, $R-X_{LR}$ is zero when X>R.

The model of Brett and Grove (1979) was applied to the second order polynomial regression between feeding level and specific growth rate. This model allows determination of:

- The maximum feeding level (corresponding to the maximum specific growth rate and calculated by taking the first derivative of the second order polynomial equation),
- The optimum feeding level (obtained graphically and corresponding to the best feed conversion ratio).

RESULTS

Experiment 1

The growth performance, feed utilization and survival rate of fish are presented in Table 2. Survival rate varied between 91.03 ± 9.09 and 96.80 ± 2.22% and were not significantly affected by the food ration (P>0.05).

Final body weight and specific growth rate were significantly influenced by feeding level. The increase of food ration led to an increase of growth performance. Final body weight and SGR of fish fed the 5% ration was significantly the lowest (P<0.05).There were no significant differences (P>0.05) between growth performances for fish fed from 10% to 30% rations.

FE was significantly influenced by feed rations. Feed Efficiency increased with increasing ration up to 10%. From 20% to 30% feeding level, Feed Efficiency decreased. The significantly lowest Feed Efficiency were obtained with 25% and 30% rations of body weight (P<0.05).

Relationships between feeding level and specific growth rate have been used to determine the optimum and maximum feeding rate. After semi-logarithmic transformation [SGR = f[log (ration)]] (Bourbonnais, 2005), a second degree polynomial regression analysis (Brett and Grove, 1979) was used to interpolate the data (Figure 1). Values of optimum and maximum feeding levels are shown in Table 3. Using the broken line model of Robbins et al. (1979) on the specific growth rate data (Figures 2-1 and 2-2), the maximum ration was estimated to be 25% or 25.12% without or after semi-logarithmic transformation respectively.

Experiment 2

Survival rate of fish under experimental conditions ranged from 93.33 ± 11.55 (5 larvae/L) to 98.33 ± 2.89 (20 larvae/L) without any significant difference between treatments (P>0.05) (Table 4).

Final body weight of fish were affected by stocking densities (Figure 3) without significant difference (P>0.05). SGR differed significantly (P<0.05) with stocking density. The SGR of fish stocked at 35/L density were not significantly different (P>0.05) from that of fish stocked at 5, 20, 50 and 65 larvae/L densities. The SGR obtained with 20 larvae/L stocking density was significantly (P<0.05) higher than those of fish stocked at 5, 50 and 65/L.

FE obtained with 5, 35, 50 and 65 larvae/L were not significantly different (P>0.05). Best FE and highest SGR were obtained for fish stocked at 20/L density.
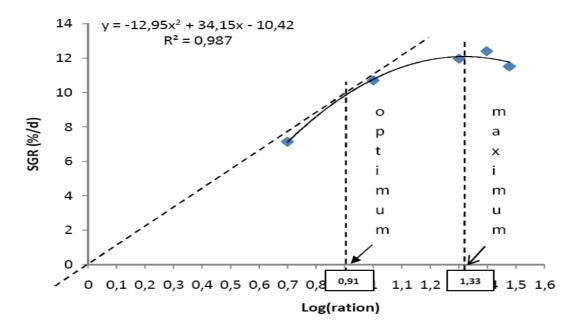
Figure 1: Variation in the specific growth rate of *P. obscura* larvae reared at different daily rations according to the model of Brett after semi logarithmic transformation.
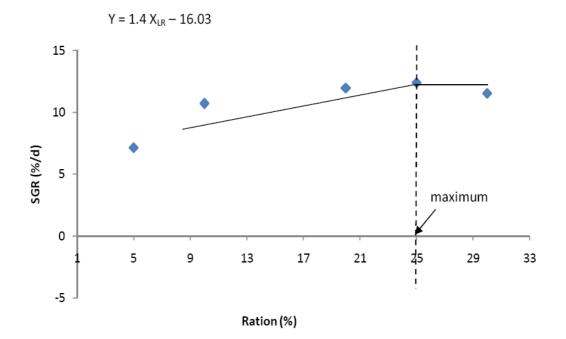

Figure 2-1: Variation in the specific growth rate of *P. obscura* larvae reared at different daily rations according to the broken line model.
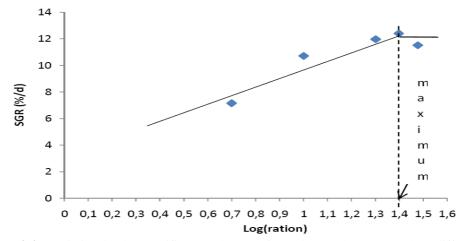
Figure 2-2: Variation in the specific growth rate of *P. obscura* larvae reared at different daily rations according to the broken line model after semi logarithmic transformation.
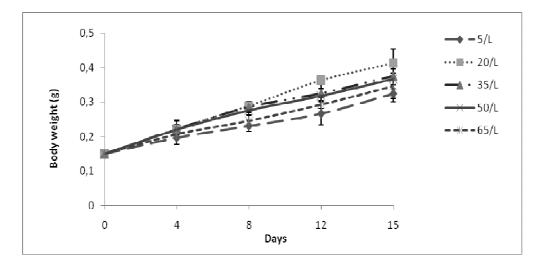

Figure 3: Variation of body weight of *P. obscura* larvae reared at different densities.

Table 2: Growth performance, Feed Efficiency and survival rate of *P. obscura larvae* (0.1 g) reared at different feeding levels.

Rations	Initial body weight (g)	Final body weight (g)	SGR (%/d)	Feed Efficiency	Survival rate (%)
5%	0.10±0.00	0.18±0.02 a	7.15±0.61 a	2.11± 0.28 a	91.03±9.09
10%	0.10±0.00	0.24±0.02 b	10.71±1.24 b	2.32± 0.05 a	91.67±4.84
20%	0.10±0.00	0.25±0.00 b	11.97±0.07 b	1.19± 0.09 b	94.87±4.84
25%	0.10±0.00	0.28±0.00 b	12.24±0.25 b	0.87 ± 0.02 c	96.15±1.92
30%	0.10±0.00	0.26±0.00 b	11.53±0.26 b	0.74 ± 0.02 c	96.80±2.22

Means on the same line followed by different superscripts are significantly different (P<0.05).

Table 3: Values of optimum and maximum feeding levels of *P. obscura* larvae.

Optimum		Maximum (Brett and Grove, 1979)		Maximum (Robbins et al., 1979)		Maximum (Robbins et al., 1979)
Log (ration)	Ration *	Log(ration)	Ration *	Log(ration)	Ration *	Ration
0.91	8.13	1.33	21.88	1.4	25.12	25

Log(ration) = X → Ration* = 10^X

Table 4: Growth performances, Feed Efficiency and survival rate of *P. obscura* larvae (0.15 g) reared at different stocking densities.

Density	Initial body weight (g)	Final body weight (g)	SGR (%/d)	Feed efficiency	Survival rate(%)
5/L	0.15±0.13	0.32±0.02	7.01±0.70 a	0.67± 0.11 a	93.33±11.55
20/L	0.15±0.17	0.41±0.04	9.18±0.89 b	0.78 ± 0.08 b	98.33±2.89
35/L	0.15±0.06	0.39±0.22	8.51±0.77ab	0.72± 0.11 ab	98.09±1.65
50/L	0.15±0.25	0.37±0.03	8.11±0.70a	0.69± 0.09 a	96.67±3.06
65/L	0.16±0.08	0.35±0.25	7.58±0.99 a	0.68 ± 0.10 a	95.90±0.89

Means on the same line followed by different superscripts are significantly different ($P < 0.05$).

DISCUSSION
Experiment 1

It is apparent from the results of this study that growth of larvae *P. obscura* fed different rations varied significantly. Significant growth improvement was recorded with increasing rations up to 10% and was not significant from 20 to 30% rations of body weight per day. Similar observations were reported by Brett and Grove (1979), De Silva and Anderson (1995), Fiogbe and Kestemont (2003), Imtiaz (2007). Indeed, as food availability increases, the quantity consumed by the fish will also increase, giving a linear increase in SGR up to the point of maximum voluntary food intake (Peres and Oliva-Teles, 2005). Overfeeding does not necessarily result in higher growth. Beyond a certain level, overfeeding has no effect on growth, and results in a poor growth (De Silva and Anderson, 1995). According to Brett and Grove (1979), when the feeding level moves towards the maximum daily consumption, the digestion efficiency decreases and limits the supply of energy destined to growth. The decrease of growth rate below the maximum ration can easily be explained by an increase of fish spontaneous activities to the detriment of growth (Kerr, 1971). This study indicated that feeding fish in the range of 5 to 10% of body weight results in optimum utilization of food for growth. Growth rate and ration interact to determine FE and are used to estimate the daily ration.

Best FE was obtained with rations 5 and 10% of body weight. Significantly low FE for higher rations could be the result of loss of nutrients and wastage of food, because fish took longer time to consume food to reach satiation (Tvenning and Giskegjerde, 1997), Hassan and Jafri (1994).

Optimum ration estimated (8.13%) is near to data recommended for other larvae of carnivorous fish: 7% (*Solea vulgaris*, Lagardere, 1987), 7.4 (*Perca fluviatilis*, Fiogbe and Kestemont, 2003), 7.7 (*Clarias gariepinus*, Hogendoorn, 1983), 5–10% (*Pleuronectes platessa*, Basimi and Grove,

1985) and 10% (*Chrysichtys nigrodigitatus*, Hem et al., 1995). Maximum Specific Growth Rate obtained in this study agreed with 10.77 ± 1.5, 11.6 and 12.0%/d obtained by Fiogbe and Kestemont (2003) for *P. fluviatilis* larvae and Nlewadin et al. (2004) for *Heterobranchus longifilis* and *C. gariepinus* larvae respectively.

Experiment 2

Survival rates of *Parachanna obscura* larvae were not affected by stocking density. This result is consistent with Islam et al. (2006) who reported that mortality of catfish "*Pangasius sutchi*" was not dependent on stocking density. However, care should be taken with that result, as the effects of density on survival rate will be entirely dependent on the range of stocking densities.

Stocking density is one of the main factors determining the growth (Engle and Valderrama, 2001; Rahman et al., 2005) and the final biomass harvested (Boujard et al., 2002). Consequently, identifying the optimum stocking density for a species is a critical factor not only for designing an efficient culture system (Leatherland and Cho, 1995), but also for optimum husbandry practices (Aksungur et al., 2007). In this study, best Feed Efficiency (FE) and highest SGR were obtained for fish stocked at 20/L density. SGR and FE obtained with 5, 35, 50 and 65/L densities are not significantly different (P>0.05). This is probably due to social interactions for food and space. Indeed, in many cultured fish species, growth is inversely related to stocking density and this is mainly attributed to social interactions (Siddiqui et al., 1989; Irwin et al., 1999; Ma et al., 2006). Social interactions through competition for food and/or space can negatively affect fish growth. According to Suziki et al. (2001) and Begout-Anras and Lagardere (2004), increase in stocking density results is increasing stress (aggressive behavior, dominance), which leads to higher energy requirements, causing a reduction in growth rate and feed efficiency. Contrarily, in case of low stocking densities, fish may not form shoals and feel comfortable. Moreover, in aquaculture, high stocking density induced stress reducing fish appetite for feed, which could result in feed not being utilized, one could explain the decrease in FE and SGR when stocking density increase (Pankhurst and Van der kraak, 1997).

The optimum stocking density (20/L) found in this study was ranging from 10 – 25 larvae/L recommended for *C. gariepinus* fry (mean weight < 1 g) (Imorou et al., 2008). The SGR obtained with density 20/L (9.18%/ d) agreed with 9.7%/d observed with 25 Larvae/L by Imorou et al. (2008). This similarity can be explained by presence of accessory breathing organs which allowing *P. obscura* and *C. gariepinus* to live in water with low dissolved oxygen and high ammonia levels (Bonou and Teugels, 1985).

In conclusion, those works showed that for best growth and Feed Efficiency when *P. obscura* larvae are reared in re-circulating system, optimum ration and stocking density are respectively 8.13% and 20 larvae/L.

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