



Research Note

Early Growth Performance of an Endangered Barb, *Sahyadria denisonii* (Day 1865) Fed with Different Diets

T. V. Anna Mercy and Sajan Sajeevan*

Department of Fisheries Resources Management and Harvest Technology, Faculty of Fisheries, Kerala University of Fisheries and Ocean Studies (KUFOS), Panangad, Kerala - 682 506, India

Investigation on the nutritional physiology of larval stages provide the basis for defining the length of larval period and for understanding the quantitative and qualitative feed requirements of larvae (Faruque et al., 2010). Most important live food organisms used in larval rearing of freshwater species are micro algae, brine shrimp larvae or micro worms (Leger et al., 1986). Artificial dry foods are the other alternative food source for larval rearing (Appelbaum & Van Damme, 1988; Legendre et al., 1995). Commonly observed effects of commercial starters on fish are low growth or survival rate, poor food conversion, high variability of individual size (Wolnicki et al., 2003) and body deformities (Rennert et al., 2000; Mercy & Sajan, 2014). Egg production from brood stocks is simple but larval rearing management is important and difficult for fish production (Shelbourne, 1964). Success of larval rearing depends mainly on the availability of suitable diets that are readily consumed and that provide the required nutrients to support higher growth and health.

Sahyadria denisonii (Day, 1865) is a endemic ornamental fish that belongs to the family Cyprinidae distributed in eleven rivers of the Western Ghats of India (Mercy et al., 2010). Many reports are available on the feeding frequency and growth of edible fishes viz., *Labeo rohita* (Gangadhara et al., 1997); *Labeo fimbriatus* (Sherly, 1997); *Puntius parrah* (Bindhu et al., 2002); *Tor putitora* (Islam, 2002); *Puntius sarana* (Chakraborty et al., 2003); *Horabagrus brachysoma*

(Jasmine & Prasad, 2006); *Labeo gonius* (Rahman et al., 2008); *Catla catla* (Manjapaa et al., 2009; Priyadarsini et al., 2011) *Cirrhinus ariza* (Rahman et al., 2009) and *Sperata seenghala* (Rahman et al., 2014), but information about freshwater ornamental fish is less (James & Sampath, 2003). Larval development of *S. denisonii* has not been previously reported, despite the importance of such studies for the management of natural fisheries in Western Ghats of India and to conserve this endemic, endangered species. The aim of the present study is to know the growth performance and survival of *S. denisonii* larvae fed with different diets under controlled conditions.

S. denisonii larvae were obtained by artificial propagation carried out at the indigenous fish hatchery of Department of Fisheries Resource Management, Kerala University of Fisheries and Ocean Studies (KUFOS), Panangad, Kerala (India) (Mercy et al., 2010; 2014). Newly hatched larvae of *S. denisonii* feed on its own yolk for 3 to 4 days soon after hatching. After 3 days, they were fed with pure culture of *Paramecium* (Infusoria) as the first exogenous feed up to 9 days after hatching as per Mercy et al. (2014). Feeding experiments were conducted in glass tanks (60 x 30 x 30 cm) with three replicates per diet. Four different types of diets such as, micro worms (MWD), artemia flakes (AFD) of OSI Feeds, USA, Higashi feed (HFD) of Higashi Aqua feeds Pvt. Ltd, Kerala and Varna feed (VFD) of Central Marine Fisheries Research Institute (CMFRI), Cochin, Kerala were used in the experiment. Fifteen larvae were stocked in each experimental tank and reared for a period of 45 days.

For the bread culture of micro worms, a layer of white bread was placed in a plastic container and

Received 12 August 2013; Revised 15 May 2014; Accepted 16 May 2014

* E-mail: sajanpolayil@gmail.com

soaked with sterilized water or milk. The inoculum of micro worms was pasted over the soaked bread, covered with muslin cloth and kept at room temperature. The moisture level of the bread was checked daily and kept in wet condition. After 4 to 6 days of inoculation, worms were found crawling along the sides of the container. It was harvested by simply sweeping out the sides by hand or a brush.

Initial average weight of the larvae was 1.48 ± 0.47 mg and length 18.5 ± 0.25 mm (9 days old). Larvae were fed *ad libitum* at 09.00, 13.00 and 18.00 h throughout the study period. Satiation was determined based on visual observation of acceptance and refusal of feed. The unconsumed feed and excrements were siphoned out from the tanks and 2/3 portion of the water in each tank was exchanged before feeding every day. Water quality parameters were monitored daily by following standard procedures (APHA, 1994). Dead larvae were removed and counted to estimate the percentage of survival rate. After 45 days of larval rearing, the increase in the length and weight of the larvae was measured to determine the growth rate, specific growth rate, daily growth rate and weight gain. Growth parameters were determined as described by Sveier et al. (2000) as follows:

Weight gain (%) =

$$\frac{(\text{Final weight} - \text{Initial weight})}{\text{Initial weight}} \times 100$$

Average Daily growth (%) = $\frac{\text{Weight gain (\%)}}{\text{Time interval in days}}$

Survival (%) = $\frac{\text{Final number}}{\text{Initial number}} \times 100$

Specific growth rate (SGR) =

$$\frac{(\text{Final weight (mg)} - \text{Initial weight (mg)})}{\text{Time interval in days}} \times 100$$

Nutritional composition of the four feeds were analysed according to the standard method of AOAC (1990) for protein, fat and fibre. Nitrogen content was measured using a microkjeldahl apparatus and crude protein was estimated by multiplying nitrogen content by 6.25. Total lipid content was determined by ether extraction for 16 h and ash was determined by combusting samples in a muffle furnace at 550°C for 6 h. Crude fibre was estimated according to Goering & Van Soest (1970). The results were analysed with one-way ANOVA to determine

significant difference among the treatments using SPSS 16.0 software.

Nutritional composition of the diets is given in Table 1. Growth and survival rate of the larvae of *S. denisonii* fed with different diets are summarised in Table 2. The results showed that young ones of *S. denisonii* could be reared successfully in controlled conditions with locally available diets. Average higher larval survival rates (94.31%) in the present experiment indicate the hardy nature of the larvae of *S. denisonii*. Morphological developments in larvae of *S. denisonii* during experimental period are presented in Fig. 1. Larvae fed with MWD were found to have significantly lower survival (93.3%) than larvae fed on AFD (100%), ($p < 0.05$). Larvae of *S. denisonii* fed with AFD recorded an average weight of 6.21 ± 0.05 mg which was higher than the weight of larvae fed with other diets. Specific growth rate, weight gain, daily growth rate and survival rate of larvae fed with AFD were 1.25 ± 0.24 , 272.83 ± 93 , 6.06 ± 2.08 and 100% respectively.

From Table 2, it is evident that specific growth rate (SGR) was highest ($1.25 \pm 0.24\%$) in larvae fed with AFD, followed by VFD ($0.84 \pm 0.14\%$) and MWD ($0.81 \pm 0.10\%$), but was the lowest in HFD fed larvae ($0.62 \pm 0.01\%$). The slower growth of the larvae on the dry foods in early larval rearing of fishes is reported (Crooks et al., 2013). Wee & Ngamsnal (1978) obtained SGR in the range of 1.27-1.85% in *Puntius gonionotus* fed with varying dietary protein (15-55%). Dietary protein has primary importance in

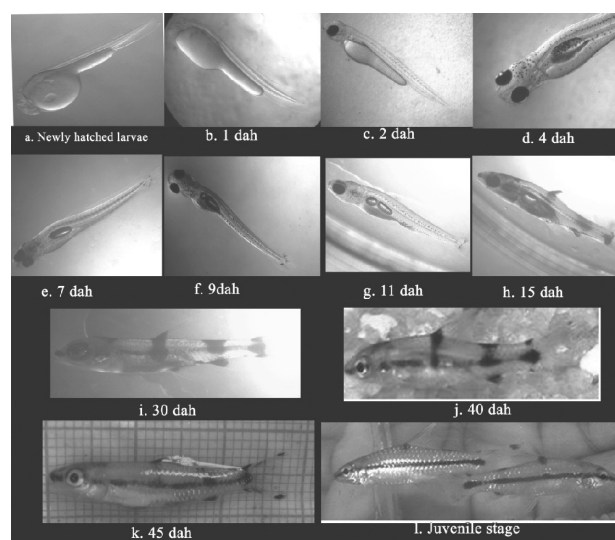


Fig. 1. Early larval morphological and colour developments in *Sahyadria denisonii*

Table 1. Nutritional characteristics of the diets used in the experiment

Dietary composition (%)	MWD	AFD	HFD	VFD
Crude protein	47.0	50.0	39.0	38.0
Crude fat	21.0	6.0	12.5	9.0
Crude fibre	19.5	2.0	12.2	2.0

mm), MWD (26.0 ± 0.14 mm) and lowest with HFD (21.5 ± 0.07 mm). Larvae fed with micro worms obtained less growth even though this feed was readily accepted. Similar observations were recorded by Jasmine & Prasad (2006). The colour, odour and palatability of artemia flakes might have stimulated the feeding by *S. denisonii*. According to James et al. (1993) feed intake by fish depends on quality, density physical attractiveness and mode of presentation of food as well as size of the prey and predator.

Table 2. Growth performance and survival rate of *S. denisonii* larvae fed with different diets

Parameters	MWD	AFD	HFD	VFD
Final mean length (mm)	26.0 ± 0.14^c	33.3 ± 0.07^d	21.5 ± 0.07^a	35.5 ± 0.14^b
Final mean weight (mg)	2.45 ± 0.07^c	6.21 ± 0.05^d	2.13 ± 0.04^a	4.90 ± 0.71^b
Specific growth rate (%/day)	0.81 ± 0.10^c	1.25 ± 0.24^d	0.62 ± 0.01^a	0.84 ± 0.14^b
Average daily growth (%)	2.91 ± 0.52^c	6.06 ± 2.08^d	2.02 ± 0.02^a	3.12 ± 0.77^b
Weight gain (%)	130.90 ± 23^c	272.83 ± 93^d	91.07 ± 02^a	140.56 ± 34^b
Survival rate (%)	93.3 ± 1.4^c	100 ± 0.0^d	86.6 ± 3.6^a	96.6 ± 1.9^b

Values are mean $\pm$ SD. Numbers in the same row with the same superscript letter are not significantly different ($P > 0.05$). AFD: Artemia flakes diet, MWD: Micro worm diet, VFD: Varna feed diet, HFD: Higashi feed diet.

different stages (35 to 56%) of fish feeding (Jauncey & Ross 1982; Lovell, 1989). Furthermore, Wilson (1989) found that dietary protein requirements decreased with increasing size and age of fish. In the present study, highest weight gain was recorded in AFD having 50% protein and lowest in larvae fed with HFD (39% protein). The results of the present study clearly showed the effect of dietary protein on the growth of *S. denisonii*. Similar results were recorded in *Tilapia* sp. (Nayak et al., 1996); *Labeo fimbriatus* (Sherly, 1997) and *Puntius parrah* (Bindhu et al., 2002), when fed on 50% protein diets. In nature, post-larval stages of *S. denisonii* exhibit a bottom dwelling habit and they rely on bottom deposited diets such as detritus, algae and diatom that grow on pebbles and stones. Similar finding was noticed by Costa & Fernando (1967).

Water quality parameters did not show significant variation from the average value during the course of experiment (Table 3).

Significantly higher weight gain (6.21 ± 0.05 mg) was observed in larvae fed with AFD ($p < 0.05$) followed by VFD (4.90 ± 0.71 mg), MWD (2.45 ± 0.07 mg) and lowest with HFD (2.13 ± 0.04 mg) (Table 2). Significantly higher increase in length ($p < 0.05$) was observed in VFD (35.5 ± 0.14 mm) followed by AFD (33.3 ± 0.07

In this study, a higher survival rate was observed in larvae fed with artemia flakes (AFD) with no mortality. No significant difference of survival was observed between larvae fed with MWD and VFD ($p > 0.05$), but it was significantly ($p < 0.05$) different from HFD. Similar results regarding survival rates were noticed in striped bass (*Morone saxatilis*) larvae that only consumed dry feed (Baragi & Lovell, 1986). Nevertheless, there have been some successes for larval production from feeding micro-diets after feeding live diets (Curnow et al., 2006). In the present study, larval size variability increased over the course of the experiment in all the groups except in the fish fed with artemia flakes. Backiel (1986)

Table 3. Water quality parameters observed in rearing tanks

Parameter	Mean $\pm$ SD
Water temperature ($^{\circ}\text{C}$)	27 ± 1.16
pH	7.0 ± 0.85
Dissolved oxygen (ppm)	5.5 ± 1.8
Total alkalinity (ppm)	35.6 ± 6.0
Hardness (ppm)	55.46 ± 10.28
Ammonia (ppm)	< 0.02

suggests that the high variability of individual size could be resulted from food competition as well as diet acceptability.

It can be concluded that *S. denisonii* larvae fed with artemia flake diets (AFD) exhibited significantly better growth and survival rate than other three experimental diets (MWD, VFD and HFD). However, *Paramecium* is essential in the initial stage of larval rearing of *S. denisonii* as live feed, because larvae are usually very small and fragile with an undeveloped digestive system (Singh et al., 2012). Absorption of endogenous food reserves, or more accurately the transition from endogenous to exogenous nutrition, is also a critical developmental and ecological transition for fish larvae (Kamler, 2008). Present findings also emphasize the potentiality of micro worms as an alternative for artemia naupli in larval rearing of *S. denisonii* like other ornamental fish such as *Carrassius auratus* L. and *Poecilia reticulata* (Parameshwaran et al., 2001; Schlechtriem, 2004) and thereby production cost can be minimised. This study represents an important step for successful rearing optimization of the *S. denisonii* under controlled conditions and the live feeds were found to significantly affect early survival rate in this species. These findings could be beneficial for the development of better larval rearing techniques for *Sahyadria denisonii* under hatchery conditions.

Acknowledgements

The authors thank the Marine Products Export Development Authority for funding Project (2007-2010) on 'stock assessment and development of captive breeding technology of *Puntius denisonii*, an endemic indigenous ornamental fish of Western Ghats of India'. Sajan. S thanks the Government of Kerala for the doctoral research fellowship during 2010-2013. Authors thank the authority, College of Fisheries, Kerala University of Fisheries and Ocean Studies, Ernakulam, India for providing the necessary facilities to carry out this work. We are grateful to anonymous reviewers for their critical comments on an earlier draft of the manuscript.

References

- Appelbaum, S. and Van Damme, P. (1988) The feasibility of using exclusively artificial dry feed for the rearing of Israeli *Clarias gariepinus* (Burchell, 1822) larvae and fry. *J. Appl. Ichthyol.* 4: 105-110
- AOAC (1990) Official Methods of Analysis, 15th edn., (Kenneth, H., Ed) Association of Official Analytical Chemists, 1298p, Arlington, VA, USA
- APHA (1992) Standard Methods for the Examination of Water and Waste-water. 18th edn., 1288p, American Public Health Association, Washington DC, USA
- Backiel, T. (1986) Masking effect of variability of growth on its estimation in juvenile tench, *Tinca tinca* (L), reared at different temperatures. *Pol. Arch. Hydrobiol.* 33: 69-95
- Baragi, V. and Lovell, R. T. (1986) Digestive enzyme activities in striped bass from first feeding through larval development. *Trans. Amer. Fish. Soc.* 115: 478-484
- Bindhu, R., Ramanujan, N. and Sambhu, C. (2002) Impact of dietary protein on growth, feed utilization and body composition of *Puntius parrah*. *Fish. Technol.* 39(2): 88-93
- Chakraborty, B. K., Miah, M. I., Mirza, M. J. A. and Habib, M. A. B. (2003) Rearing and nursing of local Sarpunti, *Puntius sarana* (Hamilton), at different stocking densities. *Pak. J. Biol. Sci.* 6: 797-800
- Costa, H. H. and Fernando, E. C. M. (1967) The food and feeding relationship of the common meso and macrofauna in the Maha Oya a small mountaimous stream at Peracdeiya. *Ceylon J. Sci.* 7: 74-90
- Crooks, N., Rees, W., Black, A., Hide, D., Britton, J. R. and Henshaw, A. (2013) Influence of live and dry diets on growth and survival of Chub (*Leuciscus cephalis*) larvae. *Fish. Aquacult. J.* 62: 1-6
- Curnow, J., King, J., Partridge, G. and Kolkovski, S. (2006) Effects of two commercial micro-diets on growth and survival of barramundi (*Lates calcarifer* Bloch) larvae within various early weaning protocols. *Aquacult. Nutr.* 12(4): 247-255
- Day, F. (1865) The Fishes of Malabar. 209 p, Bernard Quaritch, London, UK
- Faruque, M. M., Ahamed, M. K. and Quddus, M. M. A. (2010) Use of live food and artificial diets supply for the growth and survival of African catfish (*Clarias gariepinus*) larvae. *World J. Zool.* 5(2): 82-89
- Gangadhara, B., Nandeesh, M. C., Varghese, T. J. and Keshavanath, P. (1997) Effect of varying protein and lipid levels on the growth of rohu, *Labeo rohita*. *Asi. Fish. Sci.* 10(2): 139-147
- Goering, H. K. and Van Soest, P. G. (1970) Forage Fiber Analysis (Apparatus, Reagent, Procedures, and Some Applications) 379 p, US Dept. Agric. Handbook, Washington DC, USA
- Islam, M. S. (2002) Evaluation of supplementary feeds for semi-intensive pond culture of mahseer, *Tor putitora* (Hamilton). *Aquacult.* 212: 263-276
- James, R. and Sampath, K. (2003) Effects of animal and plant diets on growth and fecundity of ornamental fish, *Beta splendens* (Regan). *J. Aqua. Trop.* 55: 39-52

- James, R., Muthukrishnan, J. and Sampath, K. (1993) Effects of food quality on temporal and energetics cost of feeding in *Cyprinus carpio* (Cyprinidae). J. Aqua. Bamid. 8: 47-53
- Jasmine, H. and Prasad, G. (2006) Growth of the threatened yellow catfish *Horabagrus brachysoma* (Gunther, 1864) fed with different diets. Zoos print jour. 21(1): 2120-2122
- Jauncey, K. and Ross, B. (1982) A Guide to Tilapia Feed and Feeding. University of Stirling, Scotland, UK
- Kamler, E. (2008) Resource allocation in yolk-feeding Fish. Review. Fish Biol. Fish. 18: 143-200
- Legendre, M., Kerdchuen, N., Gorraze, G., and Bergot, P. (1995) Larval rearing of an African cat fish *Heterobranchius longifilis* (Teleostei, Claridae) effect of dietary lipids on growth, survival and fatty acid composition of fry. Aquat. Living Resour. 8: 355-363
- Leger, P., Bengtson, D. A., Simpson, K. L. and Sorgeloos, P. (1986) The use and nutritional value of artemia as a food source. Oceanogr. Mar. Biol. Annu. Rev. 24: 521-623
- Lovell, T. (1989) Nutrition and Feeding of Fish. 260 p, An AVI Book, Van Nostrand Reinhold, New York
- Manjappa, K., Keshavanath, P. and Gangadhara, B. (2009) Performance of *Catla catla* (Ham.) fingerlings fed with carbohydrate-rich diets in manured tanks. Asi. Fish. Sci. 22(3): 971-984
- Mercy, T. V. A., Malika, V. and Sajan, S. (2010) Breakthrough in breeding of *Puntius denisonii*. Infotish Internat. 3(4): 14-17
- Mercy, T. V. A., Sajan, S. and Malika, V. (2014) Captive breeding technology and developmental biology of *Sahyadria denisonii* (Cyprinidae)- an indigenous ornamental barb of the Western Ghats of India. Indian J. Fish. (In press)
- Mercy, T. V. A. and Sajan, S. (2014) Deformities in hatchery-bred redline torpedo barb, *Sahyadria denisonii* (Day 1865) (Teleostei, Cyprinidae). J. Threatened Taxa. (In press)
- Nayak, N. P., Tripathi, S. D., Jain, K. K. and Srivastava, P. P. (1996) Proc. Fourth Indian Fish. Forum (M. M. Joseph Ed) Mangalore, Karnataka, India
- Parameshwaran, K., Edirisinghe, U., Dematawewa, C. M. B. and Nandasena, K. G. (2001) Effects of live and formulated feeds on larval growth and survival of guppy (*Poecilia reticulata*) reared in indoor tanks. Trop. Agri. Res. 13. 421-430
- Priyadarshini, M., Manissery, J. K., Gangadhara, B., Rao, L. M. and Keshavanath, P. (2011) Growth response of *Catla catla* (Actinopterygii: Cypriniformes: Cyprinidae) to soya and maize supplemented traditional feed mixture. Acta Ichthyol. Piscat. 41(3): 159-164
- Rahman, M. A., Zaher, M. and Azimuddin, K. M. (2008) Evaluation of growth, survival, and production of an endangered fish, *Labeo gonius* (Hamilton) fingerlings in earthen nursery ponds. J. Appl. Aquacult. 20: 62-78
- Rahman, M. A., Zaher, M. and Azimuddin, K. M. (2009) Development of fingerling production techniques in nursery ponds for the critically endangered Reba Carp, *Cirrhinus ariza* (Hamilton, 1807). Turk. J. Fish. Aquatic Sci. 9: 165-172
- Rahman, M. A., Arshad, A., Yusoff, F. M., Amin, S. M. N., Marimuthu, K. and Araea, R. (2014) Development of captive breeding and seed production techniques for Giant river catfish *Sperata seenghala*. North Amer. J. Aquacul. 76: (2), 97-103
- Rennert, B., Kohlman, K. and Hack, H. (2000) A performance test with five different strains of tench (*Tinca tinca*) under controlled warm water conditions-Proc: 3 International Workshop on Biology and Culture of the Tench (*Tinca tinca* L.), Machern
- Schlechtriem, C. (2004) The suitability of the free-living nematode *Panagrellus redivivus* as alternative live food for first feeding fish larvae. Ph.D. Thesis, University of Hohenheim/ Shaker Verlag, Aachen
- Shelbourne, J. E. (1964) The artificial propagation of marine larvae. Advan. Biol. 2: 1-83
- Sherly, (1997) The effect of hormones and growth promoters on the growth responses of fringed lipped carp, *Labeo fimbriatus* (Day). Ph D. Thesis, University of Kerala, Thiruvananthapuram, India
- Singh, S.K., Mishra, U., Roy, S. D., Chadha, N. K. and Venkateshwarlu, G. (2012) Effect of feeding enriched formulated diet and live feed on growth, survival and fatty acid profile of deccan mahseer, *Tor Khudree* (Sykes) first feeding fry. J. Aquacult. Res. Develop. 3: 143
- Sveier, H., Raae, A. J. and Lied, E. (2000) Growth and protein turnover in Atlantic salmon (*Salmo salar* L.); the effect of dietary protein level and protein particle size. Aquacult. 185: 101-120
- Wee, K. L and Ngamsnal, D. (1978) Production of *Puntius gonionotus* (Bleeker) in ponds. Indian J. Fish. 21(2): 77-83
- Wilson, R. P. (1989) Protein and amino acid requirements of fishes, In: Progress in Fish Nutrition, (Shiau, S., Ed) 51-76p, National Taiwan Ocean University, Keelung, Taiwan
- Wolnicki, J., Kaminski, R. and Myszkowski, L. (2003) Effect of supplementation of a dry feed with natural food on juvenile tench *Tinca tinca* (L.) growth, condition, size distribution and food conversion. J. Appl. Ichthy. 19(3): 157-160