



Standardisation of Weaning Protocol for Larvae of *Clarias magur* (Hamilton, 1822)

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Abstract

Clarias magur (Hamilton, 1822) is a highly priced commercially important species targeted for aquaculture diversification in India and South-East Asian countries. Weaning from live to formulated feed during larval rearing is critical for successful seed production. Hence, the present study aimed to standardise the effective weaning age to achieve high growth and survival of *C. magur*. The 4 days post-hatch (dph) larvae with mean initial length and weight of 7.4 ± 0.83 mm and 4.97 ± 0.35 mg, respectively, were selected, and 2100 larvae were randomly stocked into 21 plastic tubs (100 larvae per tub) for six weaning treatments (W4, W7, W9, W11, W13 and W15 dph) and the control in triplicates. The study was conducted for 21 days and 4 to 24 dph. All larvae in the treatments groups were fed *Artemia* nauplii *ad libitum* and a weaning diet fed to apparent satiation according to W4 to W15 schedules. The control group larvae were fed with *Artemia* nauplii alone. Results revealed that final length and weight, weight gain per cent, daily weight gain and specific growth rate were significantly ($P < 0.05$) higher in the *Artemia* nauplii fed control group followed by W15 dph larval group. The survival rate was significantly ($P < 0.05$) higher for W15 larvae, followed by the control group. The present study indicates that the ideal protocol for *C. magur* larval fed with *Artemia* nauplii from 4 dph for weaning to formulated diet was at 15 dph until 24 dph for good growth and survival rates.

Keywords: catfish, hatchery management, larval nutrition, larviculture, live feed

Introduction

The unavailability of proper larval diet and feeding strategies remains a major bottleneck in the mass-scale larval production of catfish. The first feeding of fish larvae requires live feed to provide energy for growth and physiological function (Hamre et al., 2013; Palińska-Żarska et al., 2014; Radhakrishnan et al., 2020). Live feed such as rotifers, copepods and *Artemia* nauplii are the best larval feeds due to their nutrient profile, ability to remain alive in the rearing environment, and easy digestion and assimilation by the larvae (Damle and Chari, 2011). Previous studies revealed that larvae fed with live feed could achieve better digestive tract development than those fed with artificial feed (Vanhaecke et al., 1990; Harzevili et al., 2004; Stejskal et al., 2021). However, the mass

production of live feed has several challenges as it requires a dedicated facility, high maintenance cost, labour-intensive and tedious (Faulk and Holt, 2009; Herath and Atapaththu, 2013). Furthermore, the live feed production cost accounts for more than 50 % of the hatchery operation expenditure (Drossou et al., 2006). Gradual weaning from live feed to formulated feed is essential to minimise the production cost and improve larval survival and quality. The loss of larvae at the early stage substantially affects the larval production and profitability of a hatchery (Engrola et al., 2010). Improper or early weaning leads to higher mortality and reduced growth of fish larvae (Kestemont et al., 2003; Engrola et al., 2010), attributed to small mouth size, inadequate development of digestive tract and insufficient production of digestive enzymes (Hamza et al., 2007;

Alvarez-González et al., 2008; Pradhan et al., 2014). The ideal age for larval weaning of different catfishes and other species are provided in Table 1.

Clarias magur (Hamilton, 1822) is commercially valuable, widely distributed and cultured in India, Bangladesh, Pakistan, Indonesia, and other Asian countries (Sahoo et al., 2016; Ferosekhan et al., 2021a). *Clarias magur* is a neotype of *Clarias batrachus* (Linnaeus, 1758) belongs to the Clariidae family (Ng and Kottelat, 2008; Mir et al., 2018). The fish has great importance for aquaculture in India owing to higher market price (USD6–8 kg⁻¹), and it grows 150 g in 1 year and biomass yield of 2–3 tonnes.ha⁻¹year⁻¹ with higher profit margin as compared to carp culture in India. The fish has fewer pin bones which more consumers prefer and it is considered an alternative species for aquaculture diversification in India (Sahoo and Ferosekhan, 2018). The broodstock development, captive breeding and seed production management of *C. magur* and *C. batrachus* was standardised for commercial scale production (Sahoo et al., 2004; Srivastava et al., 2012; Sahoo et al., 2016; Ferosekhan et al., 2021a). According to Yasmin et al. (1998) *C. batrachus* larvae solely fed with formulated diet had significantly reduced growth and survival compared to the larvae fed with the live feed. Kumari et al. (2021), when studying, the ontogeny of the digestive tract and its accessory organs of *C. magur*, reported that the major organ or structural development was accomplished within 10–12 dph.

During the early feeding stages of magur larvae, the digestive enzymes (trypsin and pepsin) are in low quantities causing improper digestion and reduced growth (Mir et al., 2018). The larval weaning at the

correct age is paramount to achieving higher growth, survival, and health condition in the seed rearing phase. But there is no standardised feeding protocol available for the proper weaning of *C. magur* larvae. Hence, the present study was undertaken to standardise the ideal weaning strategies for early transition from live feed to formulated feed for *C. magur* larvae.

Materials and Methods

Ethical statement

The experiment was conducted with the consent of the ethical committee of the ICAR–Central Institute of Freshwater Aquaculture, Bhubaneswar. All protocols involving the use of fish were in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines, Ministry of Environment and Forests, Government of India.

Broodstock management and seed production

The *Clarias magur* broodfish were reared in the earthen pond (400 m²) at Catfish Unit, ICAR–Central Institute of Freshwater Aquaculture, Bhubaneswar, India. Broodfish were fed with a commercial diet (40 % crude protein and 10 % lipid) at the rate of 2 % of the body weight.day⁻¹. Male and female brood fish were identified through secondary sexual character and the maturity status was assessed to select matured brooders for the induced breeding programme. Male and female broodstock of 130–150 g (n = 5) were selected and induced with the synthetic hormone,

Table 1. Ideal weaning age for different fish species reported by various researchers.

Sl. No.	Species	Weaning age	References
1	Butter catfish, <i>Ompok bimaculatus</i> (Bloch, 1794)	7 dph	Pradhan et al., 2014
2	Chinese longsnout catfish, <i>Tachysurus dumerili</i> (Bleeker, 1864) (syn. <i>Leiocassis longirostris</i> Günther, 1864)	10 dph	Liu et al., 2012
3	Basa catfish, <i>Pangasius bocourti</i> Sauvage, 1880	6 dph	Hung et al., 2002
4	Bighead catfish, <i>Clarias microcephalus</i> (Günther, 1864)	4 dph	Fermin et al., 1996
5	African catfish, <i>Clarias gariepinus</i> (Burchell, 1822)	5 dph	Verreth and Van Tongeren, 1989
6	Striped snakehead, <i>Channa striata</i> (Bloch, 1793)	17 dph	Hien et al., 2017
7	Crucian carp, <i>Carassius Carassius</i> (Linnaeus, 1758)	30 dph	Łączyńska et al., 2016
8	Common carp, <i>Cyprinus carpio</i> Linnaeus, 1758	14 dph	Minya et al., 2018
9	Pike-perch, <i>Sander lucioperca</i> (Linnaeus, 1758)	19 dph	Kestemont et al., 2007
10	Barbel, <i>Barbus barbus</i> (Linnaeus, 1758)	12 dph	Nowosad et al., 2021
11	Greenback flounder, <i>Rhombosolea tapirine</i> Günther, 1862	23 dph	Hart and Purser, 1996
12	Peled, <i>Coregonus peled</i> (Gmelin, 1789)	23 dph	Matoušek et al., 2020

Ovatide™ (Hemmo Pharma Pvt. Ltd., Mumbai, India) at 0.1 mL per 100 g body weight brooders. The larvae were produced following the standard seed production protocol (Sahoo et al., 2007, 2016; Ferosekhan et al., 2021b).

Experimental design and weaning protocol

For the weaning experiment, hatchlings of 4 days post-hatch (dph) (7.40 ± 0.83 mm and 4.97 ± 0.35 mg) were used. Healthy larvae (2100 larvae) were stocked into 21 circular plastic tubs (25 L; 4 larvae.L⁻¹) under a completely randomised design and maintained in seven experimental treatment groups in triplicates. *Clarias magur* larvae were subjected to seven feeding schedules and the weaning was studied at different days of post-hatch such as 4 (W4), 7 (W7), 9 (W9), 11 (W11), 13 (W13) and 15 (W15) dph and feeding of *Artemia* nauplii alone was the control (C) treatment as shown in Figure 1. The experiment was conducted for 21 days (4–24 dph) under the natural photoperiod, and all the experimental tubs were provided with continuous aeration. The uneaten excess feed and excreta were removed daily before feeding the larvae. Two-thirds of water was exchanged daily to maintain the ideal water quality in larval rearing tanks.

Formulation and preparation of larval feed

The experimental feed for *C. magur* larvae was formulated (Table 2) and prepared for the weaning study. All the feed ingredients were purchased from the local market at Bhubaneswar, Odisha, India. The feed ingredients were weighed as per the formulation and mixed well with the required volume of water to prepare the feed dough. The dough without the addition of vitamin and mineral mix, fish oil, sunflower oil and carboxy methyl cellulose (CMC) was steam-cooked in a pressure cooker for 25 min and cooled at room temperature. The oils, vitamin and mineral mix and CMC were added and uniformly mixed. The feed dough was pelletised through hand pelletiser and the feed pellets were dried at 40 °C to obtain diet moisture of less than 10 %. The dried pellets were grounded and sieved to obtain particle size of less than 300 microns for feeding *C. magur* larvae. Weaning diet was prepared with 40 % inclusion of fish meal and 3 % oil sources (fish oil and sunflower oil) and the diet had 40.60 % crude protein and 7.80 % crude lipid. The larvae were fed thrice daily to satiation level in all the experimental groups.

Artemia nauplii production

Artemia cysts (OSI Red Ring, USA) were hatched in saltwater (30 ppt) under vigorous aeration in *C. magur* hatchery and the freshly hatched *Artemia* nauplii (Instar I) were fed *ad libitum* to the larvae throughout the experimental period.

Water quality parameters

The physico-chemical parameters were measured in the morning before feeding the larvae. Water temperature (Digital thermometer, Hanna, India), dissolved oxygen (Wrinkler's method), carbon-di-oxide (Titration method) and pH (Elico pH meter, India) were measured daily and total ammonia, nitrites total alkalinity and total hardness (Titration method) were measured in the laboratory with the use of a spectrophotometer (Varian 50 Bio UV-visible spectrophotometer, USA) analysed twice a week in all the experimental tanks as per the standard procedure (APHA, 2005).

Proximate composition

The proximate composition of the magur larval diet was analysed according to the standard procedures of AOAC (1995). The diet's moisture content (%) was determined by drying the feed in a hot air oven at 105 °C overnight. The crude protein was determined by Kjeldahl method (nitrogen $\times$ 6.25) using the Kjeldahl distillation systems (Vapodest, Gerhardt Analytical System, Germany). The crude lipid content of the diet was estimated by solvent extraction method (SOCS plus, SCS 08 AS, PELICAN Instruments, India). The total ash content of the diet was calculated by incinerating a known weight of the sample in a muffle furnace at 550 °C for a period of 6 h.

Growth performance and survival

At the end of the experiment, 20 larvae were collected from each treatment tank and measured for the total length (nearest to 1 mm) and body weight (nearest to 1 mg). Mortality was recorded daily, and the dead larvae were removed immediately to calculate the larval mortality. The growth parameters and survival rate were calculated as per the standard formula (Ferosekhan et al., 2020, 2021b).

$$\text{Weight gain (mg)} = \text{Final weight (mg)} - \text{Initial weight (mg)}$$

$$\text{Weight gain (\%)} =$$

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

$$\text{Daily weight gain (mg. day}^{-1}\text{)} =$$

$$\frac{\text{Final weight (mg)} - \text{Initial weight (mg)}}{\text{Duration of rearing period (days)}}$$

$$\text{Specific growth rate (\% day}^{-1}\text{)} =$$

$$\frac{[\text{Ln (Final weight (mg))} - \text{Ln (Initial weight (mg))}]}{\text{Duration of rearing period (days)}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Number of survived larvae}}{\text{Number of larvae stocked}} \times 100$$

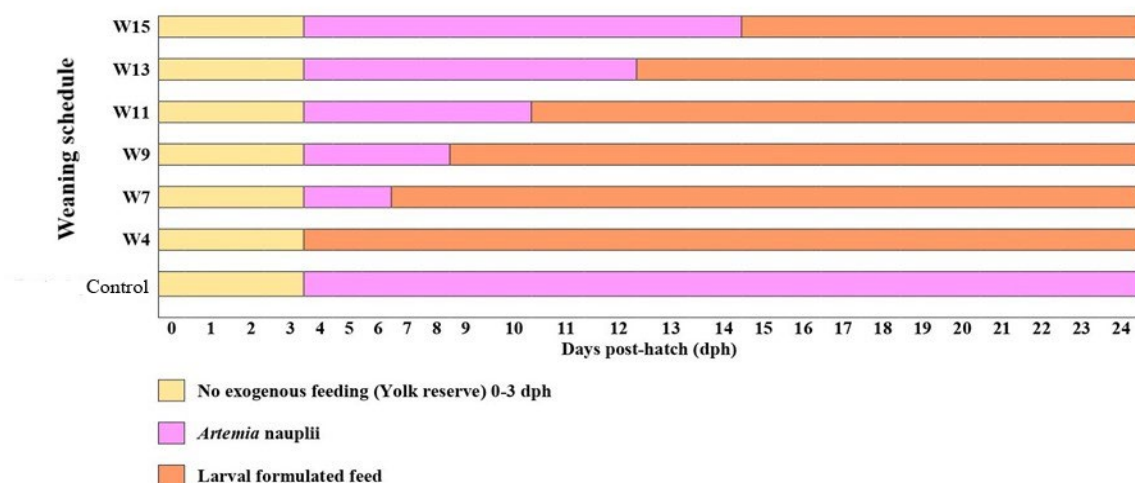


Fig. 1. Schematic representation of weaning protocol for *Clarias magur* larvae.

Table 2. Ingredient composition and proximate analyses of the *Clarias magur* larval weaning diet (% of dry matter basis).

Ingredients (%)	Weaning diet
Fish meal	40.00
Soya flour	20.00
Groundnut oil cake	20.00
Maize	5.00
Wheat flour	6.00
De-oiled rice bran	3.00
Vitamin and mineral mix*	2.00
Fish oil	1.50
Sunflower oil	1.50
CMC binder	1.00
Proximate composition	
Crude protein (% dry matter, DM)	40.60
Crude lipid (% DM)	7.80
Ash (% DM)	6.40
Moisture (%)	8.50

*Vitamin and mineral mix: Each 1 kg contains Vitamin A - 5000 IU; Vitamin D3 - 1000 IU; Vitamin B1 - 10 mg; Vitamin B2 - 10 mg; Vitamin B6 - 5 mg; Vitamin B12 - 15 mcg; Vitamin B3 - 75 mcg; Vitamin B5 - 10 mcg; Vitamin C - 150 mg; Vitamin E - 25 mg; Vitamin H - 5 mg; Vitamin B9 - 5 mg; Ca - 225 mg; Co - 20 mg; Mn - 60 mg; Fe - 30 mg; Cu - 2 mg; Zn - 2 mg; K - 20 mg; Mg - 2 mg; Choline chloride - 50 mg.

Statistical analysis

All the data were expressed as mean $\pm$ standard deviation (SD) and data variables were checked for normality (Kolmogorov-Smirnoff test) and homogeneity of variance (Levene's test). The data without normal distribution were arcsine transformed to perform the statistical analysis. All the parameters were analysed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests to determine the significant differences between the means using IBM-SPSS statistics version 20. $P < 0.05$ were considered to represent the significance level

between the treatment groups.

Results

Water quality parameters

The measured water quality parameters such as water temperature, dissolved oxygen, carbon-di-oxide, pH, total alkalinity as CaCO_3 , total water hardness as CaCO_3 ranged between 26-27.5 $^{\circ}\text{C}$, 4.8-5.5 mg.L^{-1} , 1.5-2.0 mg.L^{-1} , 7.6-7.9, 95-115 mg.L^{-1} , 90-125 mg.L^{-1} , respectively. The total ammonia and nitrites were found to be less than 0.03 and 0.04 mg.L^{-1} ,

respectively.

Growth performance and survival

The growth performance of *C. magur* larvae weaned at different days post-hatch (dph) stages is shown in Table 3. Complete mortality was observed in W4 larval group in 4 days; hence this treatment group was abandoned, and the data is not presented in the results. The final length of *C. magur* larvae (17.64 ± 0.34 mm) was found to be significantly higher ($P < 0.05$) for *Artemia* nauplii (control) fed group than those of other treatments. The final weight (59.04 ± 2.29 mg)(Fig. 2), weight gain per cent (1087.95 ± 46.05) and daily weight gain (2.70 ± 0.12 mg) were significantly greater ($P < 0.05$) for *Artemia* nauplii fed larval group (control) followed by W15 treatment group. The specific growth

rate was significantly higher ($P < 0.05$) for the control group followed by W15 treatment group (Fig. 3). It was observed that the weaning schedule followed for *C. magur* larvae fed (W15) with 11 days (4 to 14 dph) *Artemia* and 10 days (15 to 24 dph) formulated feed showed significantly higher ($P < 0.05$) survival (76 %) than the control group fed only *Artemia* (62 %)(Fig. 4).

Discussion

Optimising the weaning protocol for larval rearing is an essential step for the successful production of quality seeds. The present study described the different weaning strategies and the ideal age or time for successful weaning from live feed to formulated feed for *C. magur* larvae in captive conditions. The water quality parameters of larval rearing tanks were

Table 3. Growth performance of *Clarias magur* larvae weaned at different days post-hatch (dph).

Growth parameters	C	W7	W9	W11	W13	W15	P value
Final length (mm)	17.64 ± 0.34^a	11.44 ± 0.31^e	13.65 ± 0.47^d	13.77 ± 0.15^{cd}	14.28 ± 0.24^c	15.99 ± 0.39^b	< 0.05
Weight gain (%)	1087.95 ± 46.05^a	184.35 ± 15.09^d	151.10 ± 23.59^d	326.62 ± 40.86^c	398.39 ± 37.46^c	681.94 ± 95.81^b	< 0.05
Daily weight gain (mg.day ⁻¹)	2.70 ± 0.12^a	0.46 ± 0.04^d	0.38 ± 0.06^d	0.81 ± 0.10^c	0.99 ± 0.10^c	1.69 ± 0.24^b	< 0.05

Initial length: 7.4 ± 0.83 mm; Initial weight: 4.97 ± 0.35 mg. C – Control; W – Weaning at 7, 9, 11, 13, 15 dph.

Values are mean $\pm$ SD for each parameter ($n = 3$). Different superscripts in the same row indicate significant differences among different weaning ages for a given parameter ($P < 0.05$, One-way ANOVA, Duncan Post-Hoc).

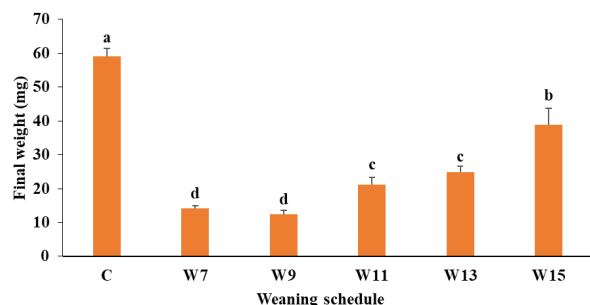


Fig. 2. Final body weight of *Clarias magur* larval fish weaned at different days of post-hatch (dph). C – Control; W – Weaning at 7, 9, 11, 13, 15 dph. Different alphabets above the bars indicate significant differences among different treatment groups ($P < 0.05$, One-way ANOVA, Duncan Post-Hoc).

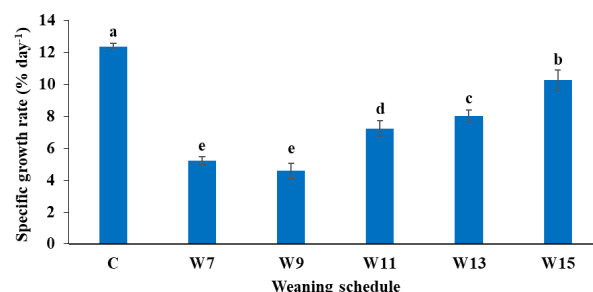


Fig. 3. Specific growth rate of *Clarias magur* larvae weaned at different days of post-hatch (dph). C – Control; W – Weaning at 7, 9, 11, 13, 15 dph. Different alphabets above the bars indicate significant differences among different treatment groups ($P < 0.05$, One-way ANOVA, Duncan Post-Hoc).

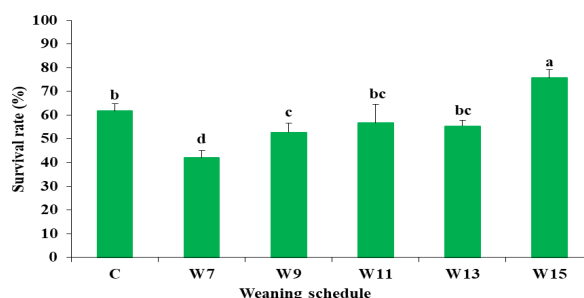


Fig. 4. Survival rate of *Clarias magur* larvae weaned at different days of post-hatch (dph). C – Control; W – Weaning at 7, 9, 11, 13, 15 dph. Different alphabets above the bars indicate significant differences among different treatment groups ($P < 0.05$, One-way ANOVA, Duncan Post-Hoc).

regularly monitored as it is recognised that in many species that, water quality conditions directly influence the larval performance (Boyd, 2017). Water temperature is a crucial parameter that determines larvae' growth and health status. The water temperature of larval rearing tank was between 26.0–27.5 °C throughout the experimental period, which was also reported as an optimal temperature range during the larval culture of *C. batrachus* (Sahoo et al., 2007, 2008, 2016). The dissolved oxygen level was observed between 4.8 to 5.5 mg.L⁻¹ and this range seem to be at the optimum level for *C. magur* larval rearing. The ideal pH range for *C. batrachus* larval rearing was reported as 7.0 to 8.5 (Sahoo et al., 2007, 2016) and the same range was recorded in the present study. The variations of alkalinity, hardness, ammonia, and nitrite between the experimental tanks followed a similar pattern reported for the *C. batrachus* larval rearing (Sahoo et al., 2008; Srivastava et al., 2012; Sahoo et al., 2016).

The provision of live feed to larvae improves the growth performance and survival and also triggers the digestive system development in fish larvae (Kolkovski et al., 1993). It has been reported that *Artemia* nauplii and other live feeds could survive longer in larval tanks and this makes the live feeds more accessible to fish larvae (Merchie, 1996), aiding improved growth in fish larvae. It is well documented that fish larvae initially have a low secretion of endogenous digestive enzymes. The provision of live food acts as an exogenous source of digestive enzymes that stimulate pancreatic secretion, which further improves the digestion and absorption of food (Kolkovski et al., 1993; Noori et al., 2012). Feeding of live feed to fish larvae stimulates the secretion of digestive enzymes such as trypsin, chymotrypsin and amylase for easy digestion and absorption by the larvae. In the turbot, *Scophthalmus maximus* (Linnaeus, 1758), larvae live feed consumption showed increased secretion of digestive enzymes (43–60 % protease, 78–88 % esterase and 89–94 % amylase), which supported higher digestibility and feed conversion efficiency (Munilla-Moran et al., 1990).

In the present study, the highest growth was observed in the control group fed with *Artemia* nauplii and this might be due to the improved digestion and assimilation by the fish larvae. It was also noted that withdrawal of live feed after 7 or 9 dph and feeding only with formulated feed showed reduced growth. When weaned early, several catfish species larvae always led to reduced growth and poor survival rate (Hung et al., 2002; Liu et al., 2012; Pradhan et al., 2014). In the present study, a gradual increase of growth was observed while feeding formulated feed from 15 dph or beyond, which might be due to the increased secretion of endogenous enzymes, which helps in digestion and absorption of formulated feed. The inability of larvae to digest the formulated diet at early weaning has

been attributed to improper development of the stomach and lack of digestive enzyme secretion (Cahu and Infante, 2001).

Successful weaning was reported for different catfishes such as *Pangasius bocourti* Sauvage, 1880, larvae after 6 days (Hung et al., 2002), *Leiocassis longirostris* (Bleeker, 1864) larvae after 10 days (Liu et al., 2012), *Ompok bimaculatus* (Bloch, 1794) larvae after 7 days (Pradhan et al., 2014) and *Clarias gariepinus* (Burchell, 1822) larvae after 5 days (Verreth and Van Tongeren, 1989). It is obvious from the earlier reports and the present study that weaning time is species-specific and depends on ontogeny and development of the functional digestive system in the larvae (Kestemont et al., 1995; Kestemont et al., 1996; Cuvier-Pères and Kestemont, 2001; Dabrowski and Portella, 2005). The highest value of weight gain, daily weight gain and specific growth rate were observed for larvae fed with *Artemia* nauplii. The survival rate was reported significantly higher for the W15 treatment group, where larvae fed with *Artemia* nauplii up to 15 dph and later fed with the formulated feed. The survival rate in control was lower than W15, but it was higher than in other treatment groups. This indicates that *Artemia* nauplii alone will not be sufficient for the magur larval growth and survival. Larvae need other vital nutrients in addition to the live feed.

The reduced growth and survival observed in all other treatment groups (W4, W9, W11 and W13) fed with formulated diet during early stages was probably due to the underdeveloped digestive system of *C. magur* larvae at 13 dph. In *C. magur* larvae, the peak level of mRNA expression pattern of trypsin and pepsin were detected only after 11 and 16 dph, respectively (Mir et al., 2018). The suitable weaning time for *C. magur* larvae could be 16 days, as both enzymes significantly increased after this age (Mir et al., 2018). The current study also showed that *C. magur* larvae weaned to formulated feed after 15 dph had a better growth performance and survival rate.

Conclusion

The present study reveals that the ideal age for weaning *Clarias magur* larvae from live feed to formulated feed was 15 dph. The initial feeding of *C. magur* larvae with *Artemia* nauplii from 4 to 14 dph and starting formulated feed from 15 dph onwards was the best weaning strategy to improve the survival and growth rate in *C. magur* seed production. Further studies are being conducted to support these findings through analysing the growth and metabolism-related gene expression, physiological and biochemical changes and ontogenic development of the digestive system. These will provide additional information on the digestive enzymes secretion and key gene expression pattern in relation to feeding live and formulated feed to optimise the weaning protocol.

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Author contributions: S. Ferosekhan: Experiment design, conducted the study, analysed the data, wrote the manuscript. S.K. Sahoo: Experiment design, sample analysis, wrote the manuscript. K. Radhakrishnan: Conducted the study, analysed the data, revised the manuscript. M. Gokulakrishnan: Sample analysis, data collection and analysis. S.S. Giri: Experiment design, revised the manuscript. B.R. Pillai: Experiment design, supervised the study, data analysis, manuscript correction.

References

- Alvarez-González, C.A., Moyano-López, F.J., Civera-Cerecedo, R., Carrasco-Chávez, V., Ortiz-Galindo, J.L., Dumas, S. 2008. Development of digestive enzyme activity in larvae of spotted sand bass *Paralabrax maculatofasciatus*. 1. Biochemical analysis. *Fish Physiology and Biochemistry* 34:373–384. <https://doi.org/10.1007/s10695-007-9197-7>
- APHA. 2005. Standard methods for the examination of water and wastewater. American Water Works Association/American Public Works Association/Water Environment Federation. 1504 pp. <https://doi.org/https://doi.org/10.2105/AJPH.51.6.940-a>
- Boyd, C.E. 2017. General relationship between water quality and aquaculture performance in ponds. In: *Fish diseases: Prevention and control strategies*. Elsevier, pp. 147–166. <https://doi.org/10.1016/B978-0-12-804564-0.00006-5>
- Cahu, C., Infante, J.Z. 2001. Substitution of live food by formulated diets in marine fish larvae. *Aquaculture* 200:161–180. [https://doi.org/10.1016/S0044-8486\(01\)00699-8](https://doi.org/10.1016/S0044-8486(01)00699-8)
- Cuvier-Péres, A., Kestemont, P. 2001. Development of some digestive enzymes in Eurasian perch larvae *Perca fluviatilis*. *Fish Physiology and Biochemistry* 24:279–285. <https://doi.org/10.1023/A:1015033300526>
- Dabrowski, K., Portella, M.C. 2005. Feeding plasticity and nutritional physiology in Tropical fishes. *Fish Physiology* 21:155–224. [https://doi.org/10.1016/S1546-5098\(05\)21005-1](https://doi.org/10.1016/S1546-5098(05)21005-1)
- Damle, D.K., Chari, M.S. 2011. Performance evaluation of different animal wastes on culture of *Daphnia* sp. *Journal of Fisheries and Aquatic Science* 6:57.
- Drossou, A., Ueberschär, B., Rosenthal, H., Herzig, K.H. 2006. Ontogenetic development of the proteolytic digestion activities in larvae of *Oreochromis niloticus* fed with different diets. *Aquaculture* 256:479–488. <https://doi.org/10.1016/j.aquaculture.2006.01.038>
- Engrola, S., Dinis, M.T., Conceição, L.E.C. 2010. Senegalese sole larvae growth and protein utilization is depressed when co-fed high levels of inert diet and *Artemia* since first feeding. *Aquaculture Nutrition* 16:457–465. <https://doi.org/10.1111/j.1365-2095.2009.00682.x>
- Faulk, C.K., Holt, G.J. 2009. Early weaning of southern flounder, *Paralichthys lethostigma*, larvae and ontogeny of selected digestive enzymes. *Aquaculture* 296:213–218. <https://doi.org/10.1016/j.aquaculture.2009.08.013>
- Fermin, A.C., Bolivar, M., Edna, C. 1996. Weaning of the Asian catfish, *Clarias macrocephalus* Gunther, larvae to formulated dry diet. In: *Feeds for small-scale aquaculture*. Proceedings of the national seminar-workshop on fish nutrition and feeds, Santiago, C.B., Coloso, R.M., Millamena, O.M., Borlongan, I.G. (Eds.). pp. 83–86. SEAFDEC Aquaculture Department, Iloilo, Philippines.
- Ferosekhan, S., Sahoo, S.N., Giri, S.S., Sahoo, S.K. 2021a. Scenario of captive production of *Clarias magur* in India. *Aquaculture Asia* 25(1):10–13.
- Ferosekhan, S., Sahoo, S.K., Radhakrishnan, K., Velmurugan, P., Shamna, N., Giri, S.S., Pillai, B.R. 2020. Influence of rearing tank colour on Asian catfish, magur (*Clarias magur*) and pangas (*Pangasius pangasius*) larval growth and survival. *Aquaculture* 521:735080. <https://doi.org/10.1016/j.aquaculture.2020.735080>
- Ferosekhan, S., Giri, A., Sahoo, S., Radhakrishnan, K., Pillai, B., Shankar Giri, S., Swain, S. 2021b. Maternal size on reproductive performance, egg and larval quality in the endangered Asian catfish, *Clarias magur*. *Aquaculture Research* 52:5168–5179. <https://doi.org/10.1111/are.15385>
- Hamre, K., Yúfera, M., Rønnestad, I., Boglione, C., Conceição, L.E., Izquierdo, M. 2013. Fish larval nutrition and feed formulation: knowledge gaps and bottlenecks for advances in larval rearing. *Reviews in Aquaculture* 5:S26–S58. <https://doi.org/10.1111/j.1753-5131.2012.01086.x>
- Hamza, N., Mhetli, M., Kestemont, P. 2007. Effects of weaning age and diets on ontogeny of digestive activities and structures of pikeperch (*Sander lucioperca*) larvae. *Fish Physiology and Biochemistry* 33:121–133. <https://doi.org/10.1007/s10695-006-9123-4>
- Hart, P.R., Purser, G.J. 1996. Weaning of hatchery-reared greenback flounder (*Rhombosolea tapirina* Günther) from live to artificial diets: Effects of age and duration of the changeover period. *Aquaculture* 145:171–181. [https://doi.org/10.1016/S0044-8486\(96\)01343-9](https://doi.org/10.1016/S0044-8486(96)01343-9)
- Harzevili, A.S., Vught, I., Auwerx, J., De Charleroy, D. 2004. Larval rearing of ide (*Leuciscus idus* (L.)) using decapsulated artemia. *Fisheries and Aquatic Life* 12:191–195.
- Herath, S. S., Atapaththu, K.S.S. 2013. Sudden weaning of angel fish *Pterophyllum scalare* (Lichtenstein) (Pisces: Cichlidae) larvae from brine shrimp (*Artemia* sp.) nauplii to formulated larval feed. *SpringerPlus* 2:102. <https://doi.org/10.1186/2193-1801-2-102>
- Hien, T.T.T., Tam, B.M., Tu, T.L.C., Bengtson, D.A. 2017. Weaning methods using formulated feeds for snakehead (*Channa striata* and *Channa micropeltes*) larvae. *Aquaculture Research* 48:4774–4782. <https://doi.org/10.1111/are.13298>
- Hung, L.T., Tuan, N.A., Cacot, P., Lazard, J. 2002. Larval rearing of the Asian Catfish, *Pangasius bocourti* (Siluroidei, pangasiidae): Alternative feeds and weaning time. *Aquaculture* 212:115–127. [https://doi.org/10.1016/S0044-8486\(01\)00737-2](https://doi.org/10.1016/S0044-8486(01)00737-2)
- Kestemont, P., Fiogbé, E.D., Parfait, O., Micha, J.C., Mélard, C. 1995. Relationship between weaning size, growth, survival and cannibalism in the common perch larvae *Perca fluviatilis*: preliminary data. *Larvi* 95:285–288.
- Kestemont, P., Jourdan, S., Houbart, M., Mélard, C., Paspatis, M., Fontaine, P., Cuvier, A., Kentouri, M., Baras, E. 2003. Size heterogeneity, cannibalism and competition in cultured predatory fish larvae: Biotic and abiotic influences. *Aquaculture* 227:333–356. [https://doi.org/10.1016/S0044-8486\(03\)00513-1](https://doi.org/10.1016/S0044-8486(03)00513-1)
- Kestemont, P., Mélard, C., Fiogbé, E., Vlavanou, R., Masson, G. 1996. Nutritional and animal husbandry aspects of rearing early life stages of Eurasian perch *Perca fluviatilis*. *Journal of Applied Ichthyology* 12:157–165. <https://doi.org/10.1111/j.1439-0426.1996.tb00082.x>
- Kestemont, P., Xueliang, X., Hamza, N., Maboudou, J., Toko, I.I. 2007. Effect of weaning age and diet on pikeperch larviculture. *Aquaculture* 264:197–204. <https://doi.org/10.1016/j.aquaculture.2006.12.034>
- Kolkovski, S., Tandler, A., Kissil, G.W., Gertler, A. 1993. The effect of

- dietary exogenous digestive enzymes on ingestion, assimilation, growth and survival of gilthead seabream (*Sparus aurata*, Sparidae, Linnaeus) larvae. *Fish Physiology and Biochemistry* 12:203–209. <https://doi.org/10.1007/BF00004368>
- Kumari, R., Sharma, P., Sarma, D., Siddaiah, G.M., Dubey, M.K., Mir, I.N. Srivastava, P.P., 2021. Ontogeny and development of the gastrointestinal system in Indian walking catfish (*Clarias magur*) during its early development. *Fish Physiology and Biochemistry* 47:1033–1052. <https://doi.org/10.1007/s10695-021-00957-2>
- Łącznyńska, B., Palińska-Żarska, K., Nowosad, J., Biłas, M., Krejszeff, S., Müller, T., Kucharczyk, D., Żarski, D. 2016. Effect of age, size and digestive tract development on weaning effectiveness in crucian carp, *Carassius carassius* (Linnaeus, 1758). *Journal of Applied Ichthyology* 32:866–872. <https://doi.org/10.1111/jai.13100>
- Liu, B., Zhu, X., Lei, W., Yang, Y., Han, D., Jin, J., Xie, S. 2012. Effects of different weaning strategies on survival and growth in Chinese longsnout catfish (*Leiocassis longirostris* Günther) larvae. *Aquaculture* 364:13–18. <https://doi.org/10.1016/j.aquaculture.2012.04.051>
- Matoušek, J., Gebauer, T., Stejskal, V. 2020. Effect of weaning initiation time and feed pellet size on peled *Coregonus peled* (Gmelin 1789) larviculture. *Aquaculture Research* 51:2150–2154. <https://doi.org/10.1111/are.14533>
- Merchie, G. 1996. Use of nauplii and meta-nauplii. In: *Manual on the production and use of live food for aquaculture*, Lavens, P., Sorgeloos, P. (Eds.), FAO Fisheries Technical Paper 361. FAO, Rome, pp. 137–163.
- Minya, D., Kovács, L., Homoki, D., Fehér, M., Bársony, P., Stündl, L. 2018. Effect of different weaning time on the growth and survival of common carp (*Cyprinus carpio* L.) larvae. *Acta Agraria Debreceniensis* 75:55–59. <https://doi.org/10.34101/actaagrar/75/1646>
- Mir, I.N., Srivastava, P.P., Bhat, I.A., Muralidhar, A.P., Varghese, T., Gireesh-Babu, P., Jain, K. K. 2018. Expression and activity of trypsin and pepsin during larval development of Indian walking catfish (*Clarias magur*). *Aquaculture* 491:266–272. <https://doi.org/10.1016/j.aquaculture.2018.03.049>
- Munilla-Moran, R., Stark, J.R., Barbour, A. 1990. The role of exogenous enzymes in digestion in cultured turbot larvae (*Scophthalmus maximus* L.). *Aquaculture* 88:337–350. [https://doi.org/10.1016/0044-8486\(90\)90159-K](https://doi.org/10.1016/0044-8486(90)90159-K)
- Ng, H.H., Kottelat, M. 2008. The identity of *Clarias batrachus* (Linnaeus, 1758), with the designation of a neotype (Teleostei: Clariidae). *Zoological Journal of the Linnean Society* 153:725–732. <https://doi.org/10.1111/j.1096-3642.2008.00391.x>
- Noori, F., Van Stappen, G., Sorgeloos, P. 2012. Preliminary study on the activity of protease enzymes in Persian sturgeon (*Acipenser persicus* Borodin, 1897) larvae in response to different diets: effects on growth and survival. *Aquaculture Research* 43:198–207. <https://doi.org/10.1111/j.1365-2109.2011.02816.x>
- Nowosad, J., Kupren, K., Biegaj, M., Kucharczyk, D. 2021. Allometric and ontogenetic larval development of common barbel during rearing under optimal conditions. *Animal* 15:100107. <https://doi.org/10.1016/j.animal.2020.100107>
- Palińska-Żarska, K., Żarski, D., Krejszeff, S., Nowosad, J., Biłas, M., Trejchel, K., Kucharczyk, D. 2014. Dynamics of yolk sac and oil droplet utilization and behavioural aspects of swim bladder inflation in burbot, *Lota lota* L., larvae during the first days of life, under laboratory conditions. *Aquaculture International* 22:13–27. <https://doi.org/10.1007/s10499-013-9663-4>
- Pradhan, P.K., Jena, J., Mitra, G., Sood, N., Gisbert, E. 2014. Effects of different weaning strategies on survival, growth and digestive system development in butter catfish *Ompok bimaculatus* (Bloch) larvae. *Aquaculture* 424:120–130. <https://doi.org/10.1016/j.aquaculture.2013.12.041>
- Radhakrishnan, K.D., AkbarAli, I., Schmidt, B.V., John, E.M., Sivanpillai, S., Thazhakot Vasunambesan, S. 2020. Improvement of nutritional quality of live feed for aquaculture: An overview. *Aquaculture Research* 51:1–17. <https://doi.org/10.1111/are.14357>
- Sahoo, S.K., Ferosekhan, S. 2018. Seed production and culture of *Clarias magur*. In: *Training manual on mass breeding and culture technique of catfishes*. SAARC Agriculture Centre, Dhaka, Bangladesh. pp. 36–41.
- Sahoo, S.K., Ferosekhan, S., Giri, S.S., Swain, S.K. 2016. Recent trends in breeding and seed production of magur in India. *World Aquaculture* 47(2):59–62.
- Sahoo, S.K., Giri, S.S., Chandra, S. 2008. Rearing performance of *Clarias batrachus* larvae: Effect of age at stocking on growth and survival during fingerling production. *Aquaculture* 280:158–160. <https://doi.org/10.1016/j.aquaculture.2008.05.020>
- Sahoo, S.K., Giri, S.S., Chandra, S., Sahu, A.K. 2007. Spawning performance and egg quality of Asian catfish *Clarias batrachus* (Linn.) at various doses of human chorionic gonadotropin (HCG) injection and latency periods during spawning induction. *Aquaculture* 266:289–292. <https://doi.org/10.1016/j.aquaculture.2007.02.006>
- Sahoo, S.K., Giri, S.S., Sahu, A.K. 2004. Effect of stocking density on growth and survival of *Clarias batrachus* (Linn.) larvae and fry during hatchery rearing. *Journal of Applied Ichthyology* 20:302–305. <https://doi.org/10.1111/j.1439-0426.2004.00534.x>
- Srivastava, P.P., Raizada, S., Dayal, R., Chowdhary, S., Lakra, W.S., Yadav, A.K., Sharma, P., Gupta, J. 2012. Breeding and larval rearing of Asian catfish, *Clarias batrachus* (Linnaeus, 1758) on live and artificial feed. *Journal of Aquaculture and Research Development* 3:1000134.
- Stejskal, V., Gebauer, T., Sebesta, R., Nowosad, J., Sikora, M., Biegaj, M., Kucharczyk, D. 2021. Effect of feeding strategy on survival, growth, intestine development, and liver status of maraena whitefish *Coregonus maraena* larvae. *Journal of the World Aquaculture Society*, 52:829–842. <https://doi.org/10.1111/jwas.12785>
- Vanhaecke, P., Vrieze, L. De, Tackaert, W., Sorgeloos, P. 1990. The use of decapsulated cysts of the brine shrimp *Artemia* as direct food for carp *Cyprinus carpio* L. larvae. *Journal of the World Aquaculture Society* 21:257–262. <https://doi.org/10.1111/j.1749-7345.1990.tb00537.x>
- Verreth, J., Van Tongeren, M. 1989. Weaning time in *Clarias gariepinus* (Burchell) larvae. *Aquaculture* 83:81–88. [https://doi.org/10.1016/0044-8486\(89\)90062-8](https://doi.org/10.1016/0044-8486(89)90062-8)
- Yasmin, A., Mollah, M.F.A., Haylor, G.S. 1998. Rearing of catfish (*Clarias batrachus* Lin.) larvae with live and prepared feeds. *Bangladesh Journal of Fisheries Research* 2:145–150.