International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

International Journal of Fauna And Biological Studies

ISSN 2347-2677
IJFBS 2015; 2(5): 16-21
Received: 10-07-2015
Accepted: 12-08-2015

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Larval rearing of an endangered catfish, *Ompok bimaculatus* (Bloch, 1794) with live and artificial diets: A preliminary study in Tripura, India

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Abstract
An *ex-situ* experiment was conducted for 28 days in order to evaluate the survival and growth rate of the larvae of an endangered freshwater catfish *Ompok bimaculatus* (Bloch, 1794) for stock enhancement. 7 days old post-hatchlings were stocked in plastic tubs having 30 L capacity and fed five different diets; zooplankton, *Tubifex*, zooplankton plus tubifex, egg custards and compound feed. Fishes accepted all types of diets. The experiment revealed that specific growth rate (SGR) was relatively higher in post-hatchlings fed on zooplankton plus tubifex (4.79±0.58) followed by tubifex (4.11±0.52), zooplankton (3.94±0.14), egg custards (3.46±0.31) whereas minimum SGR was observed with compound feed (2.93±0.24). Significant difference ($p<0.05$) in SGR was observed in fish fed on live food. The final mean weight and weight gain showed significant differences ($p<0.05$) among feed groups. However, weight gain (%) was highest in diet of zooplankton plus tubifex (13.67±1.5) whereas lowest in compound feed (8.13±0.9). Highest mean survival rate was noticed in zooplankton plus tubifex (66.50±2.14%) and live tubifex (61.75±2.02%) whereas the lowest mean survival rate was recorded in compound feed (45.82±1.03%) on day 28. However, further investigations are required for improvement on the larval rearing in commercial scale of this delicious species.

Keywords: *Ompok bimaculatus*, larval rearing, specific growth rate, growth and survival.

1. Introduction
The successful large scale culture of any fish species for human consumption demands that the resource be easily renewable [1]. The indigenous catfish, *Ompok bimaculatus* (Bloch, 1794) occurs in rivers, lakes, floodplains and wetland and distributed in India, Bangladesh, Borneo, Java, Sumatra, Laos, Sri Lanka, Nepal, Malaya, Myanmar, Pakistan, Thailand, Cambodia and Vietnam [2]. It is a bony fish, belonging to the family *Siluridae* of the order *Siluriformes.* It is delicious, nutritious and highly priced food fish especially in the entire East and North East India and Bangladesh because of soft bony structure and rich lipo-protein content [3]. They are usually carnivorous and insectivorous in nature but occasionally feed on crustaceans and planktons and the fry and juveniles of this species exhibit cannibalistic tendency [4]. Over the few decades, the wild population of *O. bimaculatus* declined drastically in India due to indiscriminate fishing during breeding season, wide use of pesticide and insecticides, pollution, extensive habitat degradation and alteration, the proliferation of exotic fish species, disease, poisoning, dynamite and other destructive fishing and also due to climatic variability [5] [6]. It has been listed as an endangered (EN) species as per IUCN (International Union for the Conservation of Nature and Natural Resources, Gland) criterion due to reduced abundance and restricted distribution [7] [8]. Therefore, being a potential candidate species for aquaculture and its economic importance, conservation of this fish is up most important [9]. Owing to non-availability of fish juveniles in natural waters and complexity of larval rearing of this fish, the development of larval rearing technology is indispensable for conservation of this endangered catfish in its endemic region Tripura in a sustainable manner and mass scale fish juvenile's production for increase fish stocks in wild or species diversification in aquaculture. In the present study an attempt has been made to evaluate growth performances and survival rates of *O. bimaculatus* post-hatchlings fed on five different diets comprising live and dry feeds.

2. Materials and Methods

2 days old larvae of *O. bimaculatus* were collected from captive bred stock developed by the authors under hatchery conditions [3]. After complete yolk sac absorption of the larvae (3-4 days old), they were fed zooplankton, predominantly consisting of copepods, rotifers as well as cladocerans in a cemented tank (1500 L capacity) for the first 6-7 days at the beginning of the experiment. The larval rearing experiment was done with randomly selected 7 days post-hatchlings in plastic tubs (30 L capacity) provided by natural habitat like aquatic macrophyte *(Hydrilla verticillata)* and water hyacinth *(Eichornia carassipes)* with five different kinds of diets i.e. zooplankton, tubifex (*Tubifex tubifex*), zooplankton plus tubifex, egg custards and compound feed for a period of 28 days. The initial stocking density per tub was 100±10 larvae (length=10.84-11.14 mm; the body weight=0.60-0.72 g). Plastic tubs were used separately for each of the food and three replicates (feeding regimes) were applied for each treatment (3x5=15 plastic tubs). During the experiment, the modification of feeding ration was made as per intensity of the feed intake noticed from time to time. For supply to the larvae, zooplankton consisting of copepods, rotifers and cladocerans were cultured in a concrete cistern (3.4 x 0.8 x 0.5 m) using phased fertilization method [10]. The proximate compositions of zooplankton were 66.16% protein, 10.46% lipid and 6.31% ash. Zooplankton were collected with scoop net and washed thoroughly before feeding to the larvae. Tubifex were cultured in a concrete cistern making the culture bed by using a mixture of garden soil and digested sludge and a continuous water flow of 0.25-0.5 l/min. Mustard oil cake (100 g) and cattle dung (10 kg) were applied weekly as organic fertilizers. The required quantity of worms was harvested daily by using plastic sieve and washed thoroughly under tape water before feeding them to the post hatchlings [11]. Tubifex contain 64.8% protein, 14.0% lipid and 6.0% ash. The larval dry feed containing egg custards and other ingredients were prepared with 2 nos. whole hen eggs (albumin and yolk), 2 g Spirulina powder, 6 g Corn flour, 4 g Artemia flakes, 2 g Yeast, 6 g Milk powder, 10 ml Cod-liver oil and mixture were stirred and steamed for nearly 10 min., cooled and washed. The custard was passed through a 0.1 mm mesh sieve under pressure and then fed to the post-larvae. The proximate composition of egg custards was 54.47% protein, 11.08% lipid and 2.65% ash. The commercially formulated compound feed was a microparticulated feed (manufactured by Gold Coin Biotechnologies, Johor, Malaysia) formulated with the ingredients of marine and plant proteins, marine and plant lipids, sterols, carbohydrate, K, P, Ca, Mg, I, Se, Zn, Cu, Mn, Fe, choline, inositol, vitamins A, D, C, E, K, folic acid, biotin, pantothenic acid, pyridoxine, nicotinic acid, B_{12}, carotenoids and attractants. The nutritional profile of the feed was 50% protein, 10% fat, 18% ash, 2% HUFA and 10% moisture.

The test larvae were fed twice a day at 08: 00 hr and 20: 00 hr. Satiations were determined based on visual observations of acceptance and refusal of the supplied diets. A strict hygienic condition was maintained by removing the dead larvae, if any, unconsumed feed and excreta from the bottom region of the tub at regular interval by siphoning method. For accelerating survival rate of the larvae mechanical aeration was provided in each tub using aquarium aerators regularly. Various physico-chemical characteristics of water like temperature, pH, free carbon-dioxide (CO_2), dissolved oxygen (DO), alkalinity, hardness, conductivity, total dissolved solids, ammonia, phosphate, nitrate, dissolved organic matter were monitored twice a week [12]. Dead larvae were removed and counted twice a day to estimate the percentage of survival rate per 24 hrs. For determination of growth, weekly 10 larvae were randomly sampled from each tub on every 7th day and each larva was placed in paper towel in order to absorb water and weighed in electronic balance to the nearest 0.01 g. The length was measured by measuring scale to the nearest 0.01 mm and mean and Standard Deviation (SD) value were calculated daily. Each time after taking length and weight measurements, the fishes were dipped with 1 ppm $KMnO_4$ solution for 20 seconds and the larvae were carefully released back to their respective tub. The behaviour of the fish larvae was also observed during the course of the experiment, especially during feeding. To avoid any kind of environmental hazard, the tub water was treated with $KMnO_4$ (2.5%) twice a week. On the last day of the experiment, all the remaining larvae were individually counted for the calculation of actual survival rate. Survival rates were calculated by taking into account the remaining and discarded larvae. The specific growth rate (SGR) and the weight gained (%) were determined adopting the following calculation [13]:

$$SGR\ (\%\ /\ day) = 100\ (Ln\ W_2 - Ln W_1)\ /\ \Delta t$$

Where, W_1 and W_2 are the initial and final weights of the larvae respectively, Δt = time in days

$$Weight\ gain\ (\%) = \{ (W_2 - W_1) / W_1 \}\ 100$$

$$Survival\ rate = \{(No - Nt) / No\}\ 100;$$

Where, No = Number of larvae at the initiation of experiment and

Nt = Number of larvae surviving at the end of 28 days of experiment.

All the growth parameters such as mean initial weight, initial length, final weight, final length, specific growth rates, weight gain and survival rate were subjected to one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range to test significant difference among the feeding regimes. Differences were considered significant at $p<0.05$. All data were analyzed using SPSS (version 16.0) for windows software program for statistical analysis. Data are presented as treatment means ± SD.

3. Results

The rearing performance of the larvae of *O. bimaculatus* in different plastic tubs with five different diets has been presented in Fig. 1-2. The larvae were found to be active and acclimatize easily in captive environments and instantaneously accepted all feeds provided in the experiment. A typical preying habit of *O. bimaculatus* was noticed towards swallowing feed with moving upward and downward directions. Moreover, they exhibit good swimming nature and schooling behaviour with attacking tendencies to weak larvae during the experiment. However, the larvae became lethargic after feed intake and the rate of feed intake was higher in zooplankton, tubifex, and zooplankton plus tubifex in contrast to other diets evaluated. The experiment was also revealed that the egg custard was rapidly breakdown in the plastic tub resulted to enhancing fouling rates. The post-hatchling fed on zooplankton plus tubifex demonstrated a fast growth rate and achieved the highest final mean length and weight as compared to other feeds tested. The results of ANOVA showed a significant effect of feeds on growth. Significant difference ($p<0.05$) was observed in the final mean length and mean weight of the larvae fed on zooplankton plus tubifex. The SD of final mean length and mean weight differed greatly at the end of the experiment.

SGR was estimated to find out the growth performance of larvae during the experimental period and the findings are depicted in Fig. 3. In the trial, high value of SGR was noticed for the larvae fed on zooplankton plus tubifex showing an average of 4.79±0.58, followed by tubifex (4.11±0.52), zooplankton (3.94±0.14). However, SGR was lower for the larvae fed on egg custards (3.46±0.31) and compound feed (2.93±0.24). Significant variation ($p<0.05$) in SGR was observed in zooplankton, tubifex, zooplankton plus tubifex, egg custards and compound feed. During the experiment, the weight gain (%) was higher for the larvae fed on zooplankton plus tubifex (13.67±1.5) followed by tubifex (11.30±1.1), zooplankton (10.76±1.2), egg custards (9.41±0.8) and compound feed (8.13±0.9) and the results are shown in Fig.3. Significant difference ($p<0.05$) in weight gain (%) was noticed in all the diets tested. The mean survival rate of the larvae of *O. bimaculatus* fed on five different diets was ranged from 45.82 to 66.50% during the rearing period for 28 days and the results are illustrated in Fig.4. Here, highest mean survival rate was noticed for the larvae fed on zooplankton plus tubifex (66.50%), followed by tubifex (61.75%), zooplankton (59.36%), whereas 52.18% survivability was recorded on egg custards and low rate of survival was noticed in larvae fed on compound feed (45.82%).

DO play a vital role in rearing of larvae because larvae require optimum level of oxygen for sustaining their physiological condition. In the present study, the mean level of DO was 8.4±0.28 ppm at 8.00 AM, pH 7.9±0.4, whereas mean free CO_2 was 0.37±0.08 ppm. Temperature is another key factor in larval rearing and water temperature range in the experiment was 28.1±2.1 ^{0}C. The mean values of other critical water quality characteristics were as follows; alkalinity 86.0±1.20 ppm, hardness 128.0±1.34 ppm, conductivity 0.392±0.010 ms/cm, total dissolved solids 182.0±6.94 ppm, ammonia 0.006±0.002 ppm, phosphate 0.096±0.001 ppm, nitrate 0.041±0.004 ppm and dissolved organic matter 3.94±0.58 ppm.

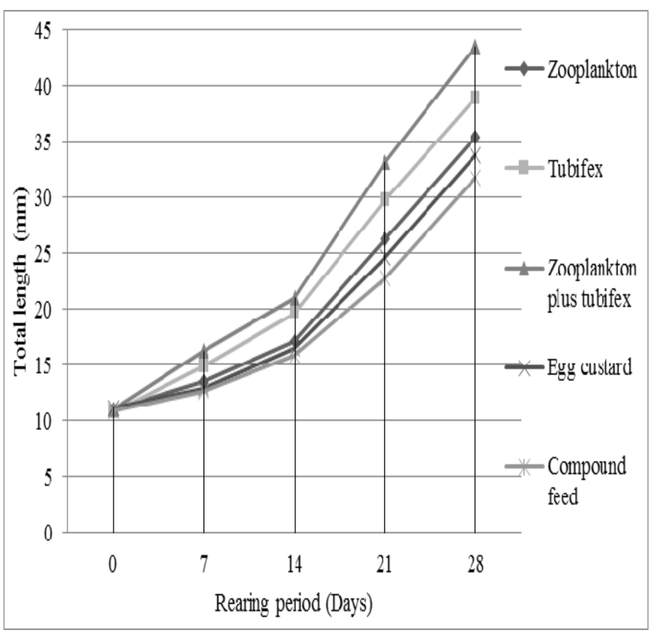

Fig1: Total length (mean±S.D.) of *O. bimaculatus* post hatchlings reared with five different diets for 28 days.

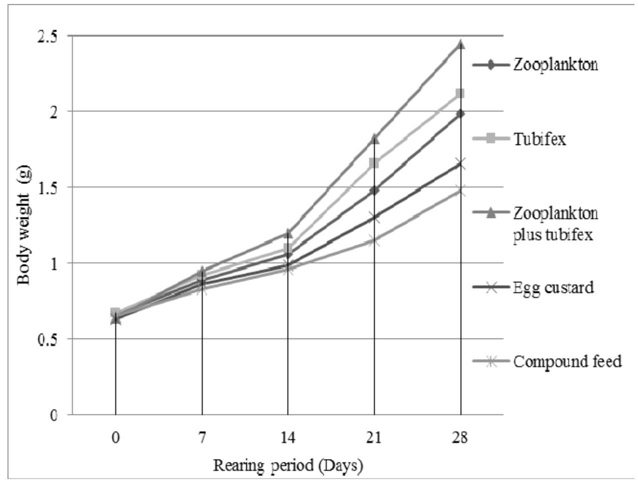

Fig 2: Body weight (mean±S.D.) of *O. bimaculatus* post hatchlings reared with five different diets for 28 days.

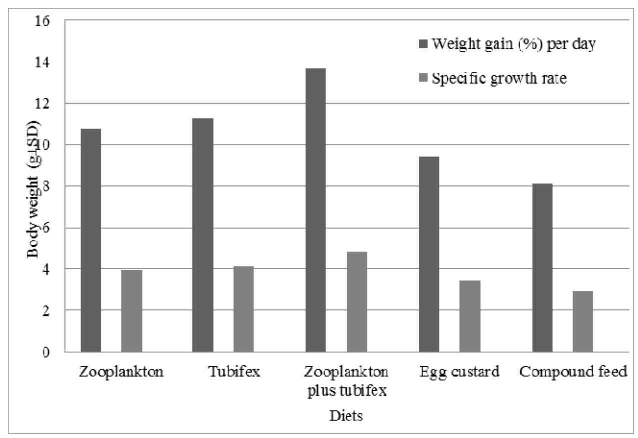

Fig 3: Weight gained (%) and Specific Growth Rate of *O. bimaculatus* fed with five different diets for 28 days.

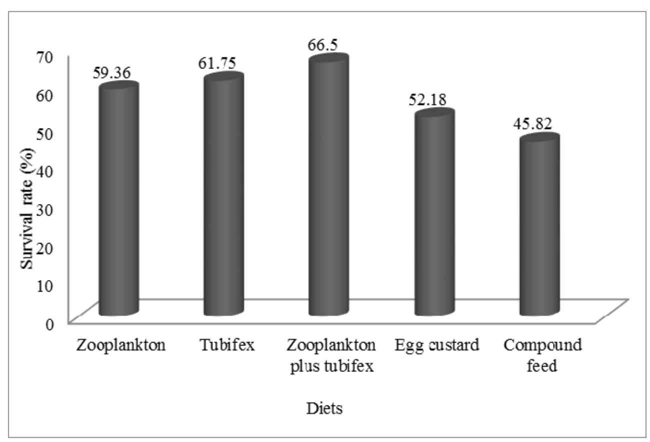

Fig 4: Survival rate (%) of *O. bimaculatus* fed with five different diets.

4. Discussion

Rearing of larvae under captive conditions depends mainly on the availability of suitable diets that are readily consumed and that provide the required nutrients to support higher growth and health. Studies so far revealed that fish larvae are in general physiologically immature with little or no capacity to produce certain hormones and digestive enzymes and that they are dependent to a greater or lesser extent on exogenous sources (mother and/or live food) [14, 15]. The present study revealed variations in the growth, SGR and survival of *O. bimaculatus* fed on the same levels of five different diets having similar environmental conditions. During the study, higher intake of feed was noticed for zooplankton plus tubifex as compared to other feed tested. The speedily breaking down and scattering of egg custard in the plastic tubs resulted to enhancing fouling rates. James *et al*. [16] pointed out that feed intake capability in the fish depend several important parameters such as size of the prey and predator, quality, density, physical attractiveness and mode of availability of the food substances. Fluchter [17] noticed that the different larval stages of fish have specific nutritional requirements.

The present study suggests that the post-hatchlings of *O. bimaculatus* can be reared on live as well as artificial diets on experimental level. In fact, the larvae prefer some particular diets over other feeds. All the live food (zooplankton, tubifex and zooplankton plus tubifex) and artificial diet (egg custards and compound feed) are found to be suitable diets for the rearing of post-hatchlings. Mgaya and Mercer [18] noticed that during rearing of fish larvae live feed play vital role in enhancing the survival rate. Pal *et al*. [19] also studied the effect of six different feed types such as wheat, flour, rice bran, soybean powder, prawn meal, zooplankton and cooked egg on the growth and survival rate of 2-days old larvae of climbing perch and reported that the fish larvae fed egg yolk show poor survival and growth rate, while feeding live organisms depicts relatively greater growth and survival rate. Degani [20] also found that when juvenile *Trichogaster trichopterus* are fed live feed they grew faster than those fed formulated feed because of the palatability, high consumption rate and chemical composition. Ronyai and Ruttkay [21] made an experiment on the survival rate of the larvae of European catfish (*Silurus glanis*) where live food organisms are noticed as efficient food. Wilson [22] observed relatively better performance in growth and survival of fish larvae fed live feed in turbot (*Scophthalmus maximus*), milkfish (*Chanos chanos*), stripped bass (*Morone* spp.) and barramundi (*Lates calcarifer*). Kanazawa [23] also reported that live feed is preferable for larval nutrition than non-live feeds.

Artificial dry diets are the other alternative food sources for larviculture and it has been reported that some of the freshwater fish species can be exclusively reared on dry diets from the starting of the exogenous feeding [24] [25] [26]. Although dry feeds have met the nutritional requirements of fish larvae and have been prepared in appropriate sizes with today's technology, the attractiveness and digestibility of these feeds for the larvae have not been improved completely [27]. Moreover, in the early larval stage, this low success of dry diets has been attributed to insufficient feed intake, digestion and absorption due to the absence of a functional digestive system. Further it has been shown that formulated compound diets do not provide optimal larval growth when used exclusively as larval food, especially during the early larval stages of cyprinids and catfish [28].

Successful rearing of fish larvae using zooplankton has been reported in several fish species [29]. *Cyprinus carpio* during larval stage prefers zooplankton and hence, showing greater survival rate in both fry and fingerling stages including earlier larval stage. Moreover, James *et al*. [16] noticed that due to presence of larger quantity of protein in zooplankton the survival rate as well as growth potential in *C. carpio* is relatively larger. Amongst various species of zooplankton, the Cladocera (*Moina*) are known to be suitable as an initial feed for *Chanos chanos* [30] and *Clarias macrocephalus* [31]. Red blood worms (Chironomus) and tubifex have also been used successfully for larval rearing of *S. glanis* [21]. Horvat *et al.* [32] reported that the tubifex was used as a live feed for nursing of *S. glanis* on a large scale in Hungary owing to its economic value.

In the present study, relatively lower growth rates of larvae was noticed in the artificial diets could be due to the physical property of the feeds like low effectiveness of the diet as the sole food supply for fish larvae [33, 34], while Bergot [35] reported that artificial feeds change the relation, which exists between the animal and its environment. The poor growth rate and survival of *O. bimaculatus* larvae fed with egg custards and compound feed could also be due to low digestibility at first feeding. In flatfish juvenile *Pleuronectes americanus,* the significant difference of the percentage of dry matter among the three pelleted diets was lost and considerably increased fouling rates due to rapid breakdown of dry feed in the tank [36]. Abi-Ayad and Kestemont [37] reported that the deterioration of water quality and tank cleanliness due to the use of formulated feeds also probably affected the growth and survival rate of larvae in the early stage.

In the present study, a higher mean survival rate (66.5%) was found for the post-hatchling fed on zooplankton plus tubifex which could be due to improved food intake by the larvae from live foods. While larvae fed with unconventional feed (egg custards and compound feed) had relatively lower survival rate (52.18% and 45.82% respectively) and this might be due to impaired feeding of larvae. Giri *et al*. [38] observed highest survival rate in the larvae of *Wallago attu* fed zooplankton in live and dry feed conditions as compared to live zooplankton alone in their different larval stages. Hung *et al.* [34] found 92.7% survival rate of the larvae of Mekong catfish (*Pangasius bicourti*) fed on tubifex. The impact of zooplankton and formulated artificial feed on *Clarias batrachus* larvae were evaluated for a period of five weeks and noticed live feed as suitable feed showing larger growth potential and survival rate [39] [26]. The present study revealed that the mean SGR was highest in live zooplankton plus tubifex (4.79±0.58g) in compared to artificial diets (2.93±0.24g). Similarly, Fermin and Boliver [31] reported that the SGR of *C. macrocephalus* larvae fed live feed is higher than those fed non-live food. Hung *et al.* [33] obtained a specific growth rate of 36.7±1.3% in a 9 days trial on Mekong catfish (*P bicourti*) larvae fed on tubifex. Rahman *et al.* [40] found that the growth and survival of the larvae of *C. batrachus* was better in larvae fed tubifex than those fed non-live feeds. According to Haque and Barua [41], non-live feeds (fish meal and wheat flour) were not at all suitable for the larvae of *Heteropneustes fossilis,* while live food (tubifex) resulted in the best growth and survival. Ghyeas [42] observed the effects of three feeds viz., tubifex, a formulated feed and a commercial nursery feed on growth and survival of *Heteropneustes fossilis* larvae and found that the growth and survival of larvae fed tubifex gave the highest growth and survival. Hirano and Hanyu [43] reported that all developmental stages of *C. gariepinus* can adapt to artificial feeds, however, the best growth was obtained by larvae fed on live feed.

The conclusion of the present study is that zooplankton plus tubifex are the best food among the live and inert foods examined as foods for *O. bimaculatus* post-hatchlings in terms of survival rate, health condition and growth performance. The larval preference of live food organisms is due to suitable size and slow jerky movements along with nutritional content.

Further investigations of this delicious species on its suitability for commercial culture are immediately wanted because of its fine adaptation to confinement and good tolerance to captive environment. The potential areas for further studies in order to conserve in its endemic region Tripura, India in a sustainable manner and mass scale fish juveniles production for increase fish stocks in wild or species diversification in aquaculture are the optimization of stocking density, improvement of diet composition, feeding level and development of feeding strategies.

5. Acknowledgements

The authors are sincerely thankful to the Department of Science and Technology, Government of India, New Delhi for the financial support (sanction no. DST: ST (Tripura)/LSR/246/486, dated 01/01/2008; and sanction order from Tripura State Council for Science and Technology: F. No. 10(19)/TSC (ST)/1574-85, dated 04/08/2010). The authors are also obliged to the Head, Department of Zoology, Tripura University (Central University) for providing laboratory facilities. Kind cooperation extended by Sri Kshitish Ch. Das, progressive fish farmer at Udaipur, Tripura, is gratefully acknowledged.

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