

An Evaluation of the Use of Egg Yolk, *Artemia* nauplii, Microworms and *Moina* as Diets in Larval Rearing of *Helostoma temmincki* Cuvier and Valenciennes

S.H. CHEAH, H.A. SHARR, K.J. ANG and A. KABIR

Department of Fish Biology and Aquaculture,
Faculty of Fisheries and Marine Science,
Universiti Pertanian Malaysia,
Serdang, Selangor, Malaysia.

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ABSTRAK

Larva *Helostoma temmincki* yang berumur lima hari mempunyai jumlah panjang 4.6 ± 0.1 mm dimasukkan ke dalam 12 tangki ternakan larva pada kadar pelepasan 1000 larva/tangki (10 larva/l). Larva ikan diberi makanan Diet I (telur kuning), Diet II (telur kuning + *Artemia nauplii*), Diet III (telur kuning + cacing mikro) dan Diet IV (telur kuning + *Moina*) selama 4 minggu. Selepas seminggu, larva yang diberi makanan Diet III mencapai saiz 8.4 mm panjang dan 7.0 mg berat. Saiz ini adalah lebih besar daripada saiz larva yang diberi makanan lain ($P < 0.05$). Pada akhir tempoh dua minggu larva yang diberi makan Diet II, III dan IV lebih besar daripada larva yang diberi makan Diet I ($P < 0.05$). Selepas minggu ketiga, didapati larva yang diberikan makanan Diet IV yang terbesar sekali ($P < 0.05$). Percubaan ini menunjukkan bahawa cacing mikro sangat sesuai sebagai makanan permulaan dan *Moina* adalah makanan yang sesuai untuk ternakan larva *Helostoma temmincki* selepas dua minggu di kawasan tropika.

ABSTRACT

Five day old *Helostoma temmincki* larvae measuring 4.6 ± 0.1 mm in total length were stocked into 12 larval culture tanks at the rate of 1000 larvae/tank (10 larvae/l). The larvae were fed Diet I (Egg yolk), Diet II (Egg yolk + *Artemia nauplii*), Diet III (Egg yolk + microworms), and Diet IV (Egg yolk + *Moina*) for a period of 4 weeks. After 1 week of rearing, larvae fed Diet III measuring 8.4 mm total length and 7.0 mg weight were larger than larvae fed the other diets ($P < 0.05$). At the end of two weeks, larvae fed Diets II, III and IV were larger than larvae fed Diet I ($P < 0.05$). From week 3 onwards, larvae fed Diet IV were consistently larger ($P < 0.05$). This experiment shows that microworms were very suitable as a starter feed and that *Moina* was suitable for feeding *Helostoma temmincki* larvae after week 2 under tropical conditions.

INTRODUCTION

Helostoma temmincki Cuvier and Valenciennes, commonly called kissing gourami and locally known as Temakang, Tembakang, Tebakang or Biawan is found in Peninsula Malaysia, Sumatra, Borneo, Thailand (Mohsin and Ambak, 1983) and Vietnam (Bardach *et al.*,

1972). This fish is of commercial importance both as a foodfish and as an ornamental fish.

Temakang is polycultured in Indonesia as the main species in combination with *Osteochilus hasselti*, *Cyprinus carpio* and *Puntius gonionotus* (Cholik, 1980). In Malaysia, it is cultured both in Peninsular Malaysia and Sarawak (Low, 1976).

The method of breeding and larval rearing of this fish using pond systems in Indonesia has been described by Ardiwinata (1981) and in Malaysia, Low (1976) reported that fish weighing 0.3 kg were suitable breeders and that infusoria was food for the young fry. Further information about this species could be obtained from Bardach *et al.* (1972).

In addition to the use of ponds for larval rearing, there is a need to establish larval rearing systems for fish breeders who do not have pond facilities at their disposal. When fish larvae are reared under tank conditions, the choice of larval feed is of prime importance. Several diets have been used in fin fish larval rearing such as strained boiled egg yolk, *Artemia* nauplii, microworms and *Moina*.

Strained boiled egg yolk has been used in rearing larvae of major Chinese carps (Chen *et al.*, 1969), *Cyprinus carpio* and *Puntius gonionotus* (Ahmad Tajuddin *et al.*, 1977) and *Pangasius sutchi* (Potaros and Sitasit, 1976; Thalathiah *et al.*, 1983). In the case of *Artemia* nauplii, they have been extensively used in larval rearing of the freshwater species 'Koi' carp (Tay, 1973), silver carp (Opuszynski, 1979) and *Hampala macrolepidata* (Ambak *et al.*, 1982).

Microworms serve the same purpose as newly hatched brine shrimp in the feeding of baby fishes which have outgrown the use of infusoria (Masters, 1975). The biology and method of culture of microworms have been described by Ivleva (1973) and Masters (1975) as well as in several aquarium books. Five genera have been identified namely *Panagrellus*, *Turbatrix*, *Cephalobus*, *Rhabditis* and *Diplogaster* where *Panagrellus* was thought to be probably the main form in the original cultures (Masters, 1975). *Moina* have been used in larval rearing of 'Koi' carp (Tay, 1973), *Clarias macrocephalus* (Carreon *et al.*, 1976; Mollah, 1983) and *Pangasius sutchi* (Potaros and Sitasit, 1976).

Presently there is lack of documented information on larval rearing of *Helostoma temminckii* under tank conditions in Malaysia and as such an experiment was conducted at the

hatchery of the Faculty of Fisheries and Marine Science, Universiti Pertanian Malaysia, to evaluate the effect of enriching the basic diet of boiled egg yolk with live food such as *Artemia* nauplii, microworms and *Moina*.

MATERIALS AND METHODS

Experimental Tanks

Twelve fibre glass tanks with dimensions 72 cm × 55 cm × 33 cm and a total capacity of 130 l were used for larval culture of *Helostoma temminckii*. Four such tanks were serviced by a biological filter which had similar tank dimensions and as such 3 biological filter tanks were set up to service 12 culture tanks. In order to prevent loss of larvae from culture tanks during filtration, water was moved through a 0.24 mm nylon cloth from the culture tank to the biological filter and back by means of 2.0 cm diameter pipes by way of airlift pumps. The flow rates are given in Table 1. All the tanks were filled with chlorine free freshwater and conditioned for 3 days prior to the commencement of experiments.

Experimental Design

Eventhough the mouth structure was completely formed by the third day, the larvae began to consume exogeneous food on the fifth day. As such, five-day-old larvae measuring 4.6 ± 0.1 mm which had completed yolk sac absorption were randomly stocked at the rate of 1000 larvae/tank (10 larvae/l) into the culture tanks. The feed used were boiled egg yolk (E), *Artemia* nauplii (A), microworms (MW), *Moina* (M) and they were given in the following combinations: Diet I (E), Diet II (E + A), Diet III (E + MW), Diet IV (E + M). There were 3 replicates per diet combination.

Feeding and Preparation/Culture of Food

Feeding was done 3 times a day *ad libitum* throughout the 4 week study period and the feeding schedule is given in Table 2.

TABLE 1
Summary of water quality data in culture tanks of *Helostoma temmincki* for week 1 to 4

Diet	R ¹	Flow rate (l/min)	Temperature (°C)				pH				D.O. (mg/l)				NH ₃ - N (mg/l)				CO ₂ (mg/l)				Alkalinity (mgCaCO ₃ /l)			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
E*	1	1.00	26.0	25.0	26.0	27.0	7.6	7.4	7.5	7.4	8	7	4	5	0.04	—	0.04	0.04	2	2	2	2	39.5	63.0	71.5	72.0
	2	1.10	26.5	25.0	26.0	27.0	7.6	7.6	7.5	7.4	9	8	6	7	0.02	—	0.04	0.04	2	2	2	2	38.5	62.0	64.5	66.0
	3	0.88	26.0	25.5	26.0	27.0	7.6	7.3	7.4	7.5	9	8	5	7	0.02	—	0.04	0.04	2	2	2	2	37.8	65.0	74.0	71.0
E + A*	1	1.01	26.5	25.5	26.0	27.0	7.6	7.6	7.6	7.8	8	7	7	7	0.02	—	0.02	0.04	2	2	2	2	38.1	70.0	74.0	71.0
	2	1.30	26.5	25.5	26.0	27.0	7.6	7.7	7.6	7.7	8	7	7	7	0.02	—	0.02	0.04	2	2	2	2	41.7	67.0	72.5	70.0
	3	0.60	26.5	25.5	26.0	27.0	7.6	7.6	7.3	7.4	8	9	7	8	0.02	—	0.02	0.04	2	2	2	2	39.3	64.0	66.0	64.0
E + MW*	1	0.80	26.0	25.5	26.0	27.0	8.0	7.8	7.5	7.5	8	7	7	7	0.02	—	0.04	0.04	2	2	2	2	37.0	65.0	73.0	70.0
	2	0.31	26.0	25.0	26.0	27.0	7.7	7.7	7.4	7.6	8	9	7	7	0.02	—	0.04	0.04	2	2	2	2	35.3	64.0	80.0	71.0
	3	0.40	26.0	25.0	26.0	27.0	7.8	7.8	7.6	7.8	8	9	8	8	0.02	—	0.04	0.04	2	2	2	2	34.0	61.0	80.0	60.0
E + M*	1	0.87	26.0	25.0	26.0	27.0	7.8	7.8	7.6	7.8	8	7	7	8	0.02	—	0.02	0.04	2	2	2	2	38.7	66.0	74.0	72.0
	2	0.23	26.5	25.0	26.0	27.0	7.7	7.9	7.6	7.5	8	8	8	8	0.02	—	0.02	0.04	2	2	2	2	38.5	61.0	65.0	66.0
	3	0.58	26.0	25.0	26.0	27.0	7.8	8.0	7.4	7.6	9	8	7	7	0.02	—	0.02	0.04	2	2	2	2	40.5	65.0	72.5	70.0

* for explanation refer to Table 2.

R¹ Replicate

TABLE 2
Feeding schedule and the diets used for larval rearing
of *Helostoma temmincki*

WEEK	TIME (Hours)	DIET			
		E ¹	E + A ²	E + MW ³	E + M ⁴
1	0830	E	E	E	E
	1200	E	E	E	E
	1530	E	A	MW	M
2-4	0830	E	A	MW	M
	1200	E	E	E	E
	1530	E	A	MW	M

Note:

- E¹ — Egg yolk
 E + A² — Egg yolk + *Artemia* nauplii
 E + MW³ — Egg yolk + microworm
 E + M⁴ — Egg yolk + *Moina*

A chicken egg was first hard boiled and the yolk was then passed through sieves of mesh sizes 0.075 mm, 0.15 mm, 0.2 mm and 0.4 mm to feed the larvae in week 1, 2, 3 and 4 respectively. *Artemia* cysts which were imported from China (Greatwall Brand) were hatched in 25 l aerated jars containing saltwater at 20%. Microworm seedstock* were cultured in the laboratory using a broth of cooked bread and milk. The worms were sieved through a plankton net of mesh size 0.065 mm prior to feeding fish larvae in week 1 and unsieved worms were used for subsequent weeks. The water circulation in tanks fed microworms was stopped for 30 minutes during feeding to prevent loss of worms from the culture tanks. *Moina* were purchased from an aquarium shop and cultured in the hatchery following the method reported by Ang (1973). The fish in week 1, 2 and 3 were fed *Moina* that had been passed through but retained on sieves with the mesh sizes of 0.15 mm – 0.075 mm, 0.40 mm – 0.15 mm and 0.6 mm – 0.4 mm respectively; however, fish in week 4 were fed unsieved *Moina*.

Fish Sampling and Data Analysis

Twenty fish larvae were randomly netted out from each tank and they were returned to their respective tanks after length-weight measurements were taken at weekly intervals. The lengths of fish were initially determined using a micrometer but as the fish grew larger a measuring board was used instead. The weights of fish were determined using an analytical balance Sartorius model FABR-2842. All length and weight data were analysed using Duncan's Multiple Range Test which was preceded by one way analysis of variance.

Water Quality Monitoring

The water quality in all tanks was monitored at weekly intervals. The water temperature was measured using a mercury thermometer Globe brand with a range of -10°C to 100°C whereas pH, dissolved oxygen and carbon dioxide were determined using a Hach Kit model

*Imported from Sri Lanka and graciously donated by Dr. M. W. R. N. De Silva of UPM.

DR/EL2. The ammonia-nitrogen and alkalinity were determined using a La Motte Ammonia-Nitrogen test kit model NANR and the titration method using hydrochloric acid and methyl orange indicator.

RESULTS AND DISCUSSION

Environmental Conditions of Culture Tanks

The water quality data of the culture tanks are presented in Table 1. There was very little variation in the water quality of tanks receiving different larval feeds. The flow rates ranged from as low as 0.23 l/min to as high as 1.10 l/min. The water temperature recorded at 1000 hours ranged from 25.0°C to 27.0°C during the culture period. The values determined were within the normal temperature range for tropical conditions.

The pH in the culture tanks ranged from 7.4 to 8.0. These values are within the safe levels of pH 6.5 to 9.0 which are recommended for fish production (Ellis, 1937 in Boyd, 1979). The dissolved oxygen values ranged from 4 mg/l to 9 mg/l but most tanks recorded values above 6 mg/l. Such high dissolved oxygen levels would be most suitable for aquaculture as the recommended level is more than or equal to 5 mg/l (Hora and Pillay, 1962). All the culture tanks recorded low ammonia-nitrogen values which ranged from 0.02 mg/l to 0.04 mg/l. Healthy growth of fishes can be expected in waters containing less than 2 ppm of dissolved ammonia (Hora and Pillay, 1962). This was indicative that the diets used did not cause any serious organic pollution of the water or that the biological filter was very efficient in removing nitrogenous wastes from the culture water. The free carbon dioxide content was constant in all tanks at 2 mg/l. Water bodies that supported good fish populations normally contained less than 5 mg/l of free carbon dioxide (Ellis, 1937 in Boyd, 1979). The total alkalinity ranged from 34.0 mg/l to 41.7 mg/l in week 1. This trend progressively increased and stabilized in week 3. The high alkalinity could be attributed to the cockle shells and limestone chips which were used for the filter bed material. These values fall within the range of 30

mg/l to 200 mg/l for alkalinity in freshwater aquaculture systems (Stickney, 1979).

Generally it can be said that the water quality in the culture tanks was suitable for growth and survival of fish larvae.

Growth of Larvae

The growth data are presented in Fig. 1 and Table 3. In week 1, the total length and weight of fish receiving Diets I, II, III and IV were 5.7 mm (1.9 mg), 5.9 mm (1.9 mg), 8.4 mm (7.0 mg) and 6.2 mm (3.6 mg) respectively. The fish

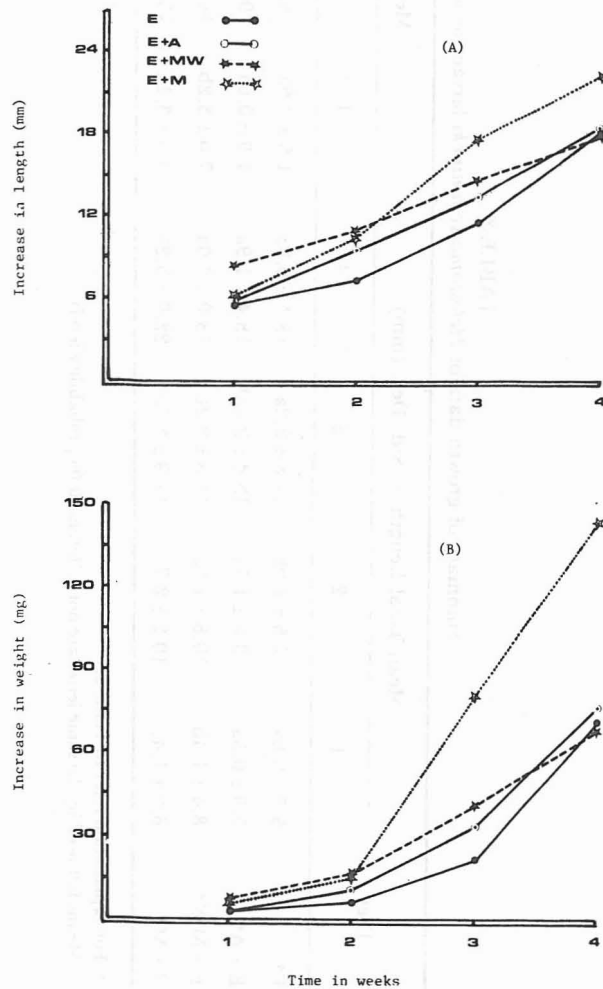


Fig. 1. Effect of diet on the increase in length (A) and weight (B) of *Helostoma temminckii*. (See Table 1)

TABLE 3
Summary of growth data of *Helostoma temmincki* larvae for week 1 to 4

Diet	Mean Total Length \pm Std. Dev. (mm)				Mean Weight \pm Std. Dev. (mg)			
	1	2	3	4	1	2	3	4
E*	5.7 \pm 0.6a	7.5 \pm 1.9b	11.8 \pm 2.2a	18.1 \pm 0.3a	1.9 \pm 0.6a	6.5 \pm 5.9a	20.4 \pm 13.1a	71.6 \pm 33.4a
E + A*	5.9 \pm 0.5a	9.6 \pm 1.7a	13.4 \pm 2.5ab	18.4 \pm 1.9a	1.9 \pm 0.6a	9.8 \pm 4.9a	30.3 \pm 18.5a	77.9 \pm 24.4a
E + MW*	8.4 \pm 1.4b	10.8 \pm 1.9a	14.8 \pm 2.2b	18.0 \pm 2.0a	7.0 \pm 3.2b	16.0 \pm 11.0b	41.0 \pm 18.3a	67.7 \pm 22.2a
E + M*	6.2 \pm 1.4a	10.2 \pm 2.7a	17.9 \pm 3.1c	22.0 \pm 3.2b	3.6 \pm 3.1a	15.7 \pm 11.3b	81.4 \pm 40.3b	145.8 \pm 59.3b

* For explanation refer to Table 2.

Means followed by the same letter are not different (0.05 probability level)

TABLE 4
Chemical composition of egg yolk, *Artemia* nauplii, Microworms and *Moina*

Food/Organism	Moisture %	Dry matter %	Protein	Lipid % of Dry Weight	Carbo- hydrate	Ash	Source
Egg yolk	51.1	48.9	33.2	56.6	2.5	3.2	Cook and Briggs (1973)
<i>Artemia</i> nauplii	—	—	50.6	23.2	6.0	14.7	Dutrieu (1960) in Ivleva (1973)
Microworms <i>Turbatrix</i> / <i>Rhabditis</i>	76	24	40.0	19.5	—	—	Ivleva (1973)
<i>Moina</i> <i>rectirostris</i>	90.6	9.4	70.5	16.1	—	11.0	Ivleva (1973)

that were fed Diet III (E + MW) were larger ($P < 0.05$) than those that were fed with the other diets. The fish fed Diet I, II and IV had similar sizes ($P > 0.05$). The superiority of Diet III is consistent with the findings of Jocher (1973) and Masters (1975) that microworms are suitable for feeding larvae that had outgrown the use of infusoria.

In week 2, the total length and weight of the larvae fed Diet I, II, III and IV were 7.5 mm (6.5 mg), 9.6 mm (9.8 mg), 10.8 mm (16.0 mg) and 10.2 mm (15.7 mg) respectively. Even though the larvae that were fed Diet III (E + MW) recorded the largest size, the sizes of fish that were fed egg yolk and live food were similar ($P > 0.05$) but were significantly larger than fish that were fed solely egg yolk ($P < 0.05$). Genkel (1979) reported that *Coregonus fera* fry fed homogenized yolk registered no growth in the first three weeks. Beck (1979) concluded that live food was superior to non living or artificial diets.

On the third week, the total length and weights of the larvae fed Diet I, II, III and IV were 11.8 mm (20.4 mg), 13.4 mm (30.3 mg), 14.8 mm (41.0 mg) and 17.9 mm (81.4 mg) respectively. The larvae that were fed Diet IV (E + M) recorded the largest size over the sizes of fish fed the other diets ($P < 0.05$). Common carp fry with a total length of 12 to 13 mm require zooplankton of larger size such as *Moina* and *Daphnia* (Tamas, 1979). In this experiment fish fed Diet IV (E + M) must have attained this size between the sampling days of week 2 and 3 so that by week 3, fish fed this diet were significantly larger than fish fed the other diets. The fish that were fed Diet III were similar in size to fish fed Diet II ($P > 0.05$) but larger than fish fed Diet I ($P < 0.05$). The superiority of the diet combination of egg yolk and microworms over egg yolk alone was thus maintained.

At the end of the experiments, the total length and weight of fish in Diets I, II, III and IV were 18.1 mm (71.6 mg), 18.4 mm (77.9 mg), 18.0 mm (67.7 mg) and 22.0 mm (145.8 mg) respectively. Fish that were fed egg yolk and *Moina* were very much larger than fish fed the other diets ($P < 0.05$). Ivleva (1973) reported that the

protein content of *Moina rectirostris* was 70.5% (Table 4) and this was reflected in better growth of larvae. *Daphnia* which possesses similar characteristics as *Moina* has a high concentration of haemoglobin, a high protein pigment, and is therefore rich in food value (Masters, 1975). The high nutritive value of *Moina* may thus explain the good growth exhibited by fish fed Diet IV from week 3 onwards till the end of this experiment.

Survival Rate

The mean survival rates for fish fed Diets I, II, III and IV were 25.7% \pm 21.1%, 50.6% \pm 11.1%, 55.8% \pm 14.8%, 21.6% \pm 7.3% respectively and these values were similar ($P > 0.05$). Even though fish which were fed Diet IV recorded the highest growth, low survival rates were encountered. However, fish which were given egg yolk that was enriched with *Artemia* nauplii and microworms recorded higher survival rates. The low survival rates of fish fed egg yolk alone suggest that its use in larval rearing needs to be reevaluated. The low survival rate of fish fed Diet IV is probably due to the lack of suitable size *Moina* at the beginning of the trials.

CONCLUSION

Taking into account the growth and survival data collected as well as the cost of the diets used, one would conclude that *Helostoma temmincki* larvae can be reared in tanks by providing egg yolk and microworms as starter feed for the first two weeks followed by a switch to egg yolk and *Moina* after the initial two-week period.

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