Technical note

Comparison of humidity chamber, MariSource hatching-tray and "Zuger" glass funnel incubation systems for breeding of *Cyprinus carpio* (L.) and *Clarias gariepinus* (Burchell)

JF Prinsloo*, LC Hoffman and J Theron
Aquaculture Research Unit, University of the North, Private Bag X1106, Sovenga 0727, South Africa

Abstract

Humidity chamber, MariSource hatching-tray and "Zuger" glass funnel incubation systems were compared for hatching successes of both *Cyprinus carpio* and *Clarias gariepinus* fertilised eggs. The humidity chamber incubation system proved superior for *C. carpio*, whilst the MariSource hatching-tray system was better for *C. gariepinus*. Fungal infections greatly influenced hatching results.

Introduction

The economic viability of a freshwater fish hatchery depends on optimal production of larvae and juveniles. Breeding methods and systems employed are largely determined by the specific hatching requirements of the particular species. Likewise, the sophistication of the breeding system(s) used is usually determined by the degree of technological expertise available, especially in developing regions.

The European common carp *Cyprinus carpio* L. is one of the most popular freshwater aquaculture species, with an annual global production of 900 000 metric tonne (Nash and Kensler, 1990). In southern Africa, warm-water fish production is rapidly expanding, with the sharptooth catfish, *Clarias gariepinus* (Burchell) being the major species cultured (Hecht and Britz, 1990).

In-depth studies have already been conducted on the environmental requirements and methodology employed for the successful hatching and rearing of *C. carpio* (Woynarovich, 1962; Soin, 1977; Woynarovich and Horváth, 1980; Rothbard, 1981; Schoonbee and Brand, 1982; Schoonbee and Prinsloo, 1984, 1986; Prinsloo et al., 1987) and *C. gariepinus* (Schoonbee et al., 1980; Hecht et al., 1982; Viveen et al., 1985; Hecht et al., 1988).

In South Africa, "Zuger" glass funnels and MariSource (Heath Techna) hatching-trays, or adaptations thereof, have proved to be relatively successful in the large-scale incubation of *C. carpio* and *C. gariepinus* eggs (Schoonbee et al., 1980; Hecht et al., 1982; Schoonbee and Prinsloo, 1984; Prinsloo et al., 1987). The removal of egg adhesiveness when using glass hatching funnels is, however, time-consuming and labour-intensive, and may lead to mechanical and chemical damage of eggs (Prinsloo et al., 1987), resulting in variable hatching success, especially for *C. gariepinus*. Furthermore, the prolonged time required for the hatching of carp eggs (approximately 50+ h - Woynarovich and Horváth, 1980) increases the probability of severe fungal infections (e.g. *Saprolegnia* spp.) thus negatively affecting optimal production of larvae (Theron et al., 1991).

The above-mentioned constraints necessitated the search for a less labour-intensive, more efficient incubation system for the large-scale propagation of carp and catfish larvae. In the present investigation, the hatching results of a humidity chamber developed at the University of the North, Sovenga, South Africa, were evaluated for *C. carpio* and *C. gariepinus* against existing incubation systems.

Materials and methods

Hatching systems

Three incubation systems for the mass production of *C. carpio* and *C. gariepinus* larvae were used. These systems included "Zuger" glass breeding funnels (Woynarovich, 1962), MariSource (Heath Techna) hatching-trays (Prinsloo et al., 1987) and a recently developed humidity chamber incubation system.

The humidity chamber incubation system is designed to ensure that adhesive eggs are kept moist during embryonic development in air containing a high relative humidity. The humidity chamber (Fig. 1) consists of a Perspex unit 1 000 x 500 x 250 mm in size, mounted on top of a 1 000 ℓ PVC water tank. The unit is subdivided and each subdivision fitted with 5 vertical sponges (465 x 205 x 35 mm) attached to Perspex hangers. Netting material (460 x 170 mm, 250 to 300 µm size) for the attachment of the adhesive eggs is mounted on both sides of each sponge. Total surface area of netting material for the humidity chamber therefore amounts to 1,564 m^2, providing potential attachment for 0,8 million *C. carpio* and 3,1 million *C. gariepinus* eggs respectively. Two perforated Perspex trays fit tightly on top of the unit, with these perforations (4 mm diam. 40 mm apart) being sited above each vertical hanging sponge. Water is pumped into the trays and allowed to run through the perforations onto the centre line of each hanging sponge. Two water outlets (20 x 90 mm) are situated 20 mm above the bottom of each unit, ensuring entrapment of egg shells and debris.

The different incubation systems were connected to a common

*To whom all correspondence should be addressed.
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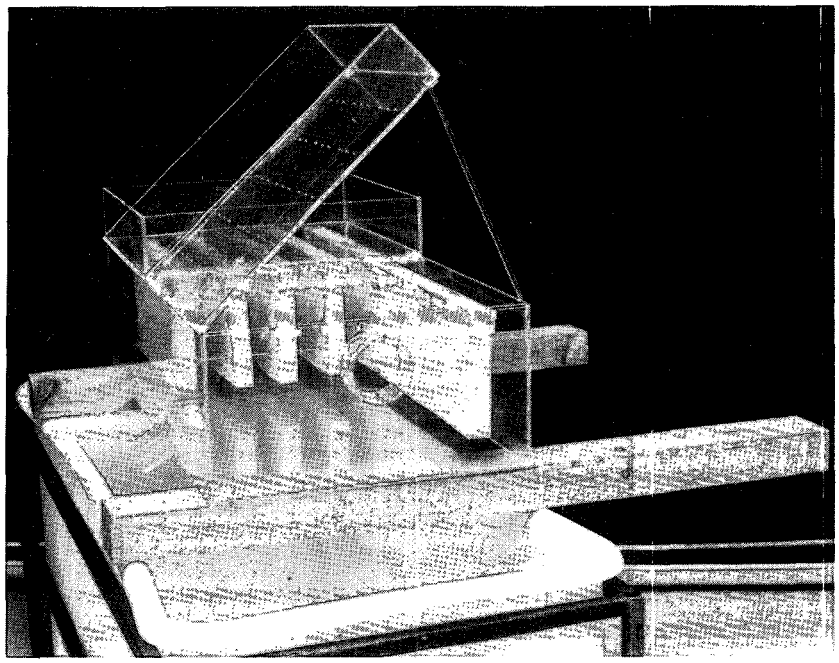

Figure 1
A photo of the humidity chamber incubation system

12 000 ℓ recirculation unit provided with a rotating biodrum, gravel filter and ultraviolet sterilisers.

Water temperature was maintained at 23 ± 1°C for *C. carpio* and 27 ± 1°C for *C. gariepinus*. Relative humidity in the humidity chamber was 94% and 96% at the above-mentioned temperatures, respectively. Water was well aerated, maintaining oxygen levels above 6 mg·ℓ$^{-1}$.

Hatchery procedures

Pond-reared *C. carpio* and *C. gariepinus* spawners were used. Selection of the spawners and procedures followed for the induction, stripping and fertilisation of the eggs were done according to Schoonbee et al. (1980) and Schoonbee and Prinsloo (1984; 1986).

For both *C. carpio* and *C. gariepinus*, the pooled eggs of 3 females were fertilised with semen obtained from 2 males. A 100 g sub-sample of fertilised eggs was used in each incubation system.

In the MariSource hatching-tray system, the sub-sample was divided and spread in a monolayer in 5 trays. In the humidity chamber, the egg sub-sample was evenly divided and spread over 10 sheets of netting material. These nets were attached to both sides of the vertically hanging sponges. In both these systems, spreading of eggs was done in water by means of a soft brush to facilitate even distribution with minimum damage.

The removal of egg adhesiveness of the egg sub-sample for use in the "Zuger" glass funnel system, was based on procedures developed by Woynarovich and Horváth (1980), Woynarovich and Woynarovich (1980), as adapted by Hecht et al. (1982) and Schoonbee and Brand (1982). Thereafter, the swollen fertilised eggs were evenly divided between 3 funnels.

Total water flow through the MariSource hatching-tray and humidity chamber systems was maintained between 8 to 10 ℓ·min^{-1}. In the breeding funnels, the water flow was adjusted according to the procedures described by Woynarovich and Horváth (1980).

Water samples for physical and chemical analysis (*Standard Methods*, 1980) were collected from the outlets of the individual breeding systems: at 6 h and 4 h for *C. carpio* and *C. gariepinus* respectively. Sampling commenced 2 h after fertilisation, and continued until hatching. A final water sample from each incubation system was analysed 24 h after hatching.

As hatching commenced, the sponges in the humidity chamber were lowered into the holding tank. This procedure is necessary to expedite the hatching process, and to prevent trapping of larvae amongst the egg shells. The breeding trays and sponges containing the attached egg shells and unhatched eggs were removed and discarded 4 h after the onset of hatching. Simultaneously, the larvae and egg shells from the funnels were transferred to the water tanks. This procedure was necessitated by the inability of newly hatched *C. gariepinus* larvae to escape from the funnels.

Four days after hatching, all holding tanks were cleaned and the number of larvae in each tank estimated as follows: the water level in each tank was lowered to 300 mm. A sieve (120 mm diam., 5 mm deep, 118 µm mesh size) was used to collect samples for counting. This sieve was placed on the bottom of the tank. An equal distribution of larvae through-out the water column was achieved by gentle stirring of the total water volume. As soon as an equal distribution of larvae was observed, the ring was raised quickly, but smoothly, vertically out of the water. This procedure was repeated 3 times per holding tank. The calculated total number of larvae per tank was derived by dividing the surface area of the holding tank by the surface area of the ring and multiplying by the mean of the sub-samples.

Results

Water chemistry

Results of selected physical and chemical parameters of water in the different incubation systems used for *C. carpio* and *C. gariepinus* are summarised in Table 1. Temperature and oxygen concentrations of the water in the different systems remained

TABLE 1
MEAN VALUES FOR SELECTED PHYSICAL AND CHEMICAL PARAMETERS OF WATER FROM THE DIFFERENT INCUBATION SYSTEMS FOR *CYPRINUS CARPIO* AND *CLARIAS GARIEPINUS*

Parameter		Humidity chamber	MariSource hatching-trays	Glass funnels
C. carpio				
Temperature °C		23,50	23,18	22,99
± S.D.		0,402	0,503	0,672
Oxygen mg·ℓ$^{-1}$		7,11	7,41	7,30
± S.D.		0,385	0,298	0,305
Conductivity (µS·cm^{-1})		92,88	93,66	93,44
± S.D.		2,233	2,054	2,165
pH	max	7,09	7,80	7,12
	min	6,60	6,60	6,32
C. gariepinus				
Temperature °C		27,30	27,20	26,70
± S.D.		0,394	0,503	0,261
Oxygen mg·ℓ$^{-1}$		7,90	6,40	7,70
± S.D.		0,110	0,115	0,170
Conductivity (µS·cm^{-1})		111,90	111,00	111,90
± S.D.		0,734	0,778	1,217
pH	max	8,04	8,00	7,99
	min	7,72	7,69	7,55

TABLE 2
WATER AMMONIA LEVELS (NH_3 mg·ℓ$^{-1}$) AS MEASURED IN THE DIFFERENT INCUBATION SYSTEMS 2 h AFTER FERTILISATION, AT HATCHING AND 24 h POST-HATCHING FOR *CYPRINUS CARPIO* AND *CLARIAS GARIEPINUS*

Time (h)	Humidity chamber	MariSource hatching-trays	Glass funnels
C. carpio			
2	0,061	0,012	0,073
48	0,000	0,000	0,000
72	0,085	0,207	0,158
C. gariepinus			
2	0,000	0,000	0,000
22	0,000	0,010	0,000
46	0,000	0,010	0,110

TABLE 3
ESTIMATED NUMBER OF 4-d OLD *CYPRINUS CARPIO* AND *CLARIAS GARIEPINUS* LARVAE HATCHED FROM MARISOURCE (MS), HUMIDITY CHAMBER (HC) AND GLASS FUNNEL (GF) INCUBATION SYSTEMS

Species	Estimated number of larvae per incubation system		
	HC	MS	GF
Cyprinus carpio	33 269	21 861	27 534
Clarias gariepinus	12 327	22 019	10 563

relatively constant, with the exception of the MariSource hatching system used for *C. gariepinus*, where the oxygen concentration was 6,4 mg·ℓ$^{-1}$. For both *C. carpio* and *C. gariepinus*, no differences in conductivity between the different systems were observed. The differences in conductivity and pH values for the 2 species are attributed to different water sources being used.

For both *C. carpio* and *C. gariepinus*, the ammonia concentrations in the holding tanks remained insignificantly low (<0,10 mg NH_3·ℓ$^{-1}$), even 24 h after hatching (Table 2).

Hatching results

Of the 3 incubation systems used for *C. carpio*, the humidity chamber (33 269 larvae) and "Zuger" glass funnels (27 534 larvae) produced 52% and 26% more larvae than the MariSource hatching-trays (21 861), respectively (Table 3).

For *C. gariepinus* (Table 3), the MariSource hatching-tray (22 019 larvae) system proved superior, producing 44% and 52% more 4-d old larvae than the humidity chamber (12 327) or the "Zuger" glass funnel (10 563) systems, respectively.

Fungal infections

Heavy *Saprolegnia* spp. fungal infections were noted on the *C. carpio* eggs in the MariSource and "Zuger" glass funnel incubation systems, whilst the humidity chamber showed no infections. No fungal infections were observed on the *C. gariepinus* eggs.

Discussion

The low water ammonia concentrations noted were due to effective nitrification by the biofilters. The other physical and chemical parameters (Table 1) were all within the accepted limits for larvae of these 2 fish species (Woynarovich and Horváth, 1980; Viveen et al., 1985). Differences in numbers of 4-d old larvae obtained from the incubation systems are, therefore, inherent to the incubation system used and not the water environment.

Determining the number of larvae 4 d after hatching, when the yolk sac has already been absorbed, the air bladder filled with air, and when the larvae have developed a fish-like swimming motion and commenced exogenous feeding (Woynarovich and Horváth, 1980), gives a better estimation of larval viability as most of the weak and deformed larvae have already died off.

The humidity chamber incubation system proved to be superior to the "Zuger" glass funnel system, producing 21% more viable *C. carpio* larvae. This result is mainly due to the avoidance of mechanical and chemical damage caused to the eggs during the removal of egg adhesiveness (Prinsloo et al., 1987) and the absence of fungal development on the eggs. Although mechanical and chemical damage to eggs do not play a role in the MariSource hatching-tray system, the resulting number of viable larvae produced is disappointingly low (only 65% compared to that of the humidity chamber). The primary cause of this poor performance could mainly be attributed to the heavy *Saprolegnia* spp. fungal infection of the eggs observed.

No fungal infections were observed on *C. gariepinus* eggs in all the incubation systems used, as *C. gariepinus* eggs already started hatching 18 h after fertilisation at 27°C. Of the 3 incubation systems, the MariSource hatching-tray system proved

to be superior, producing respectively 79 and 108% more viable larvae than the humidity chamber and the glass funnel systems (Table 3). The extremely low number of larvae produced in the "Zuger" glass funnel system can possibly be ascribed to the chemical and physical damage caused to the eggs during removal of the egg adhesiveness. The more fragile nature of *C. gariepinus* eggs, as compared to those of *C. carpio*, make them more susceptible to physical damage during the stirring process. It was also noted that *C. gariepinus* eggs in the humidity chamber system, showed signs of dehydration and degradation prior to hatching, resulting in lower numbers of larvae. This phenomena can be eliminated by submerging the eggs into the water at least 1 h before the expected commencement of hatching.

From the results of this investigation, the humidity chamber system proved to be most suitable for the production of *C. carpio* but not *C. gariepinus* larvae. Although the MariSource hatching-tray system, or adaptations thereof, are also suitable for *C. carpio*, problems with respect to fungal infections are still encountered. This system was, however, still the most efficient for the production of *C. gariepinus* larvae. The problems encountered with the egg adhesiveness in the case of the glass funnel system, makes this method of incubation less suitable for production of larvae of both species.

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