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Induced Ovulation, Spawning, Egg Incubation, and Hatching of the Cyprinid Fish *Labeo victorianus* in Captivity

JUSTUS RUTAISIRE

Department of Wildlife and Animal Resources Management, Makerere University, P.O. Box 7062, Kampala, Uganda

ANTHONY J. BOOTH

Department of Ichthyology and Fisheries Science, Rhodes University, P.O. Box 94, Grahamstown, 6140 South Africa

Abstract

Two spawning inducing agents—Aquaspawn (a rapidly metabolized synthetic decapeptide gonadotropin-releasing hormone [GnRH]) and Dagin ([D-Arg^6, Pro^9-NEt]-sGnRH) combined with 20 mg/kg of the water-soluble dopamine receptor antagonist metoclopramide (GnRH + MET)—were tested for their efficacy in stimulating ovulation in *Labeo victorianus* held in its natural environment and under captive conditions. Successful ovulation, when migratory vesicle oocytes became completely transparent, was obtained with GnRH + MET, while GnRH only caused oocyte clearance up to the highly translucent phase. *L. victorianus* eggs were non-adhesive, semi-buoyant, and transparent at ovulation. First hatching occurred after 26 h and lasted for 8 h at 24 C. Water temperature was shown to significantly affect spawning latency and incubation time. Thus, *L. victorianus* could be successfully induced to spawn using a synthetic gonadotropin-releasing hormone coupled with a dopamine antagonist followed by natural fertilization in floating net cages at temperatures between 24 and 27 C.

Ningu *Labeo victorianus* is the only labeine fish within Lake Victoria and its catchment (Greenwood 1966; Reid 1985). This species, once widely distributed in the Lake Victoria basin and supporting a commercial fishery until the late 1950s, has declined due to overfishing (Cadwalladr 1965; Ogutu-Ohwayo 1990; Seehausen 1996). The *L. victorianus* fishery has not only collapsed but the species has also disappeared from some of its former habitats. Recent surveys in Uganda have only found two distant populations—one in the Sio River on the Uganda–Kenya border (0°13′53″N, 34°00′30″E), and the second in the Kagera River on the Uganda–Tanzania border (0°56′28.1″S, 31°46′18″E) (Rutaisire 2003) (Fig. 1). Currently, there is growing interest to breed the fish for wild stock enhancement and culture as a food fish.

Previous attempts to induce spawning in *L. victorianus* were unsuccessful (Fryer and Whitehead 1959; Kajjansi Aquaculture Research Station, unpublished data), possibly due to inadequate knowledge concerning aspects of its reproductive biology. Unpublished records at the Kajjansi Aquaculture Research Station, Uganda, have, however, noted that *L. victorianus* reared in earthen ponds and fed on locally formulated feeds based on maize bran and cotton seed cake reached an average weight of 500 g after 7 mo. With this growth performance, coupled with its popularity as a table fish, this species has the potential to replace the presently farmed exotic common carp *Cyprinus carpio* as an aquaculture fish.

Additional information regarding its reproductive biology including maturity patterns, environmental conditions associated with natural spawning, juvenile intersexuality, sperm ultrastructure, oogenesis (Rutaisire 2003), and spermatogenesis (Rutaisire et al. 2003) are available. Unfortunately no data are available on the role of various reproductive hormones.

It is well known that reproductive processes in fishes are controlled by endoge-

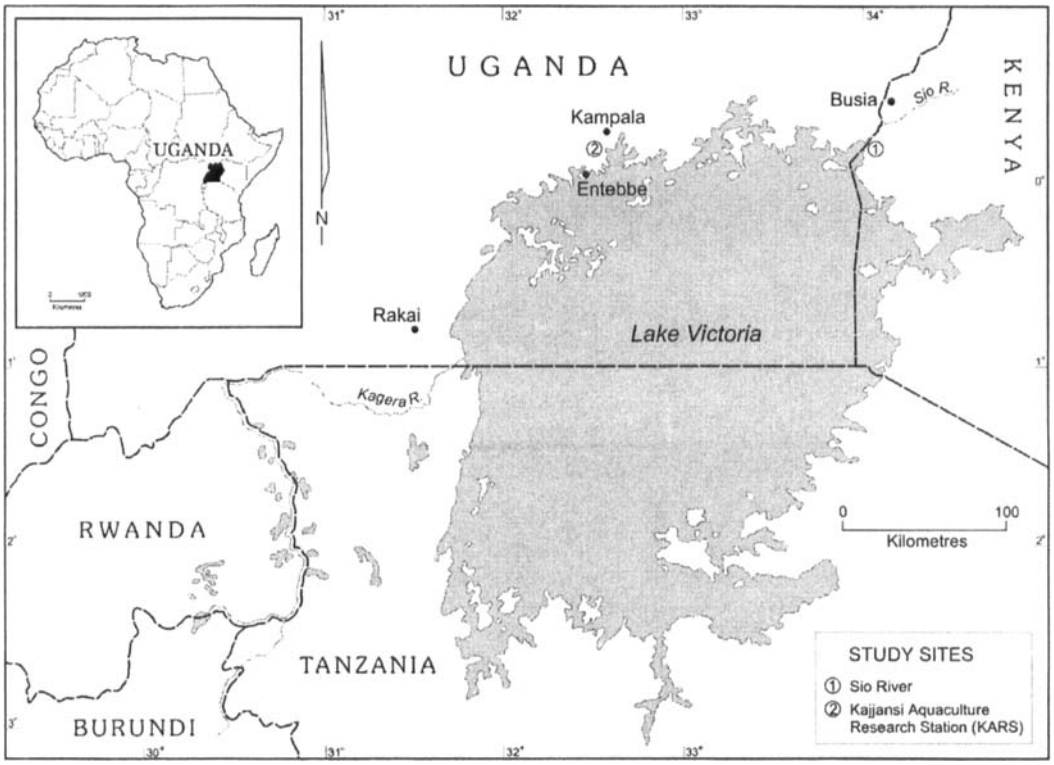

FIGURE 1. *Location of the Sio River and Kajjansi Aquaculture Research Station, Uganda, on Lake Victoria.*

nous biological rhythms as well as by environmental cues (Munro 1990). Endogenous control is mediated through actions of various hormones along the brain-hypothalamus-pituitary-gonad axis. Under natural conditions environmental stimuli are detected and relayed to the brain, resulting in a release of hormones and neurotransmitters that regulate ovulation (Yaron 1995; Peter and Yu 1997). The most important reproductive hormone is gonadotropin-releasing hormone (GnRH) that regulates gonadotropic hormone, GtH (Peter and Yu 1997). Gonadotropin release in teleost fishes is also influenced by a gonadotropin-inhibiting factor (GRIF) from the hypothalamus. This factor has been identified as dopamine and demonstrated to have inhibitory activity on the release of GtH (Peter et al. 1986, 1988). Combinations of GnRH and dopamine antagonists have been used to induce ovulation in several cyprinids including *Carassius auratus* (Sokolowska et al. 1984), *L. rohita, Cirrhinus mrigala* (Halder et al. 1991), and *C. carpio* (Driori et al. 1994).

This study investigates a synthetic GnRH, with and without a dopamine antagonist, as a stimulant for the induction of ovulation and the initiation of spawning *L. victorianus,* and elucidates feasible methods that are available locally that could be used to spawn the fish outside its natural environment.

Materials and Methods

General Methods

L. victorianus broodfish were caught using gill nets (27–75-mm stretched mesh) at the mouth of the Sio River (Fig. 1). The time for collection of ripe fish was predicted based on cyclic variations of gonadosomatic index and the prevalence of oocytes

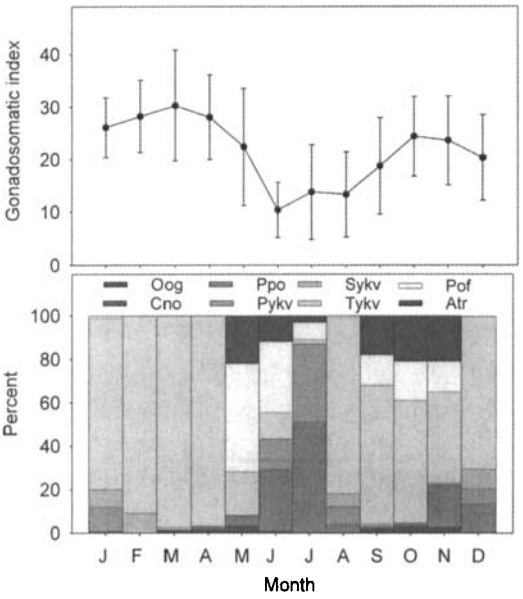

FIGURE 2. *Cyclic changes in the ovary of* Labeo victorianus *from the Sio River, Uganda. The top panel illustrates the mean gonadosomatic index (± SD) with two spawning peaks. The lower panel illustrates the proportion of oocytes in various stages of development. Spawning was indicated by presence of high percentage of tertiary yolk vesicle stages and fresh post-ovulatory follicles. Atr = atretic oocytes, Cno = chromatin nucleolar oocytes, Oog = oogonia, Pof = post-ovulatory follicles, Pykv = Primary yolk vesicle oocytes, Ppo = pre-perinucloelus oocytes, Sykv = secondary yolk vesicle oocytes, Tykv = tertiary yolk vesicle oocytes.*

in advanced stages of development (Rutaisire 2003). Ripe fish were obtained over two time periods of the year that were characterized by high gonadosomatic indices, and a dominance of tertiary yolk vesicle oocytes and fresh post-ovulatory follicles (Fig. 2).

To determine the system under which the fish would successfully spawn, induction was conducted under three different experimental conditions. Experiment 1 was conducted in tanks and involved stripping ripe fish. Experiment 2 was conducted in floating cages set within the Sio River and involved both stripping and natural fertilization. Experiment 3 was conducted as in Experiment 2 but the floating cages were set in ponds instead of the river. Both spawning-inducing agents were tested for their efficacy in stimulating ovulation in *L. victorianus* under the three experimental conditions.

Detection of Oocyte Maturity and Male Ripeness

Prior to hormonal administration, fish were tested for readiness to spawn by siphoning a sample of oocytes from the ovary using a cannula tubing of 1.9 mm in diameter. The samples were cleared in SERA fluid (ethanol : formaldehyde : acetic acid 6: 3:1 v/v) and observed under a light microscope. Females were selected for induction when 80% of the sampled oocyte sample had eccentric nuclei.

Male fish were ready to spawn without hormonal induction. Males were selected from the wild population, and milt was released after application of gentle pressure on the abdomen.

Hormonal Administration

Fish were weighed and anesthetized with 2-phenoxyethanol (Fluka-Chemie-Sigma Aldrich) prior to intramascular injection of the inducing agent between the dorsal fin and the lateral line. Dagin ([D-Arg^6, Pro^9-NEt]-sGnRH) combined with 20 mg/kg of the water-soluble dopamine-receptor antagonist metoclopramide (GnRH + MET) (purchased from Kibbutz Gan Shmuel Fish Breeding Centre, Israel) was administered at a dose of 10 μg/kg. Aquaspawn, a simple, rapidly metabolized synthetic decapeptide GnRH (purchased from Exico, South Africa) was administered at 10 μg/kg as recommended by the manufacturer. Fish from a control group were injected with volumes of 0.6% saline solution (SS) equal to the volume of the hormone solutions used. Water temperature was constantly monitored during the incubation period. Progression towards ovulation involved change of the oocyte from opaque yellowish-cream color to becoming transparent. This process was divided into five phases that were used to assess ovulatory response

to treatments. Oocytes were considered to be in Phase I if they were opaque, II if starting to clear, III if they showed low translucency, IV if highly translucent, and V, the final oocyte maturation stage, if completely transparent.

Experiment 1

Twenty females and 30 males were collected from Sio River (Fig. 1) in October corresponding to the second spawning peak (Fig. 2) and transported in an oxygenated 500-L plastic container to Sunfish Farm near Kajjansi Aquaculture Research Station. Water temperature was maintained at approximately 18 C using ice blocks during transportation. On arrival at the farm, the fish were placed in rectangular concrete tanks supplied with gravity fed natural spring water. The tanks were covered with nets to prevent jumping out of the fish and to reduce possible injury. After 6 h of acclimatization in the tanks, the fish were grouped into six sets of two females and four males. Two sets were injected with either GnRH, GnRH + MET, or SS. After injection, the fish were put in tanks according to theirs sets, and females were kept separate from males. The fish were monitored for ovulation using gentle pressure applied to the abdomen of the females. Those fish whose oocytes had become transparent were stripped. Prior to stripping, a plastic container was cleaned, dried, and weighed. Eggs from the ovulated females were stripped into the container and its weight recorded. The weight of the eggs was obtained as the difference between the weight of the container plus the eggs and the empty container. To determine the number of spawned eggs, a sample of eggs was taken out before addition of milt, weighed, and counted. Eggs were fertilized using the dry method described by Rothbard (1981).

The fertilized eggs were non-adhesive and were therefore spread onto plastic mesh trays (mesh size of 0.6 mm) held in wooden frames. After 2 h, fertilized eggs were discernable by a dark spot of the developing embryo in the transparent vitelline membrane. One-hundred eggs were counted using a tally counter in five sections of the tray and the fertilization rate determined. Time at the onset and end of hatching was recorded. Hatching success was given by the percentage difference between unhatched and the total eggs at the start of incubation.

Experiment 2

This experiment was conducted in April corresponding to the first spawning peak (Fig. 2). Broodfish were caught in reed enclosures as they migrated upstream in the Sio River at Sitengo. Fish were transported in a plastic bucket downstream to the Maddwa landing site and tested for ripeness. Ripe fish were anesthetized and hormonal injections administered. In this experiment concrete tanks were replaced by floating net cages held in a reed enclosure that had been erected in tributaries of the river close to the main lake.

Cage I measured 1 × 1 × 1.5 m and contained two females used for study of oocyte clearance. Cage II measured 2 × 4 × 1.5 m and was subdivided into eight compartments each measuring 1 × 1 × 1 × 1.5 m with 0.5-mm mesh netting. Each compartment contained one female and two males. This cage was set to allow natural fertilization without stripping. Cage III measured 2 × 4 × 1.5 m with two compartments containing fish for stripping. One compartment contained six females and the other twelve males.

Oocyte biopsies were obtained from the two females in Cage I of each treatment. The fish in Cage II and III were closely monitored for ovulation by lifting the cages out of the water and visually inspecting the fish. When ovulation was detected, fish in Cage III were stripped, while those in Cage II were left to fertilize in the cages.

Two h after fertilization, spawners were removed from the cages. The number of eggs spawned was estimated by lifting the net out of the water to spread the eggs on

the net. The total area occupied by the eggs was measured. Eggs in a quarter of the area were counted with a tally counter and used to estimate the total number of eggs spawned. Stripping was conducted as in Experiment 1 but the eggs were irrigated by river water from an overhead tank at 2 L/min. Three h post-spawning a total of 500 fertilized eggs from each compartment in Cage II were collected and incubated in a plastic basin supplied by the same tank as the stripped eggs and at the same flow rate.

Experiment 3

This experiment was conducted at Kajjansi Aquaculture Research Station (KARS) using broodfish that were collected from the Sio River and rested in ponds for 8 d. The procedure for Experiment 2 was followed except that the cages were suspended in the ponds.

Statistical Analysis

Comparison of mean number of eggs was tested using ANOVA. Time taken to induce ovulation, incubation period, and duration of hatching were $\log_{10}$ transformed, while fertilization and hatching rates were arcsine transformed and tested for homogeneity of variances using a Bartlett's test. Comparison of means was by a Student's *t*-test or a Mann-Whitney test if the data did not fulfill parametric assumptions.

Results

Experiment 1 was unsuccessful as all the eggs and larvae died after approximately 10 h. This experiment did, however, provide baseline information for Experiments 2 and 3. Comparisons between the induction times, fertilization rates, and incubation and hatching durations could be drawn between Experiments 2 and 3. Results from the experiments are summarized in Table 1. At night water temperature rose in the Sio River whereas in the ponds and tanks it decreased. A reversed trend was observed during the day (Fig. 3).

Induction of Spawning

The GnRH + MET treatment induced all fish to spawn in all replicates for all three experiments. In fish injected with GnRH and SS, spawning did not occur. The time taken to induce spawning was significantly shorter in the river than in ponds ($P < 0.05$). Eggs ovulated per unit body weight in the two environments were not significantly different ($P > 0.23$). Ovulated eggs were semi-buoyant and non-adhesive. On contact with water, the vitelline membrane of the ovulated eggs swelled and increased from 1.2 ± 0.1 mm to 5.8 ± 0.3 mm in diameter. Swelling occurred whether the eggs were fertilized or not. GnRH only caused oocyte clearing up to Phase IV (Fig. 4).

Fertilization

Percent fertilization was greater than 69% in all experiments with no significant difference in fertilization rates between eggs spawned in the river cages (Experiment 2) and those spawned in pond cages (Experiment 3) ($P > 0.05$). Fertilization rates in the cages were significantly lower in the stripped eggs than those fertilized naturally ($P < 0.01$).

Incubation and Hatching

Incubation took a significantly shorter time to occur in the river than in the pond cages ($P < 0.01$). There was low hatchability in Experiment 1 (Table 1). In this experiment, all the larvae died on the day of hatching. Hatching was found to be significantly higher in the river fish than those in ponds ($P < 0.01$), but was greater than 77% in both experimental conditions. Attempts to hatch stripped eggs in Experiments 2 and 3 were unsuccessful. Highest embryo mortality occurred between 4 and 6 h post-fertilization.

A hatching rate of 69.2% was obtained with the eggs collected 6 h after fertilization in the cages and incubated in plastic containers using the same water as the one in which the cages were floating. The hatching

TABLE 1. *Summary of the effect of natural spawning/artificial stripping, and riverine/pond cages on ovulation, fertilization and hatching of* Labeo victorianus *for three hormonal treatments. Columns with different superscripts are significantly different from each other at the 5% level.*

	Site	Spawning	Eggs (1,000/kg)	Time to ovulate (min)
Saline solution	River	Natural	0	—
	River	Stripping	0	—
	Pond	Natural	0	—
	Pond	Stripping	0	—
GnRH	River	Natural	0	—
	River	Stripping	0	—
	Pond	Natural	0	—
	Pond	Stripping	0	—
GnRH+Met	River	Natural	104.53 ± 10.73[a]	541.24 ± 85.94[a]
	River	Stripping	100.18 ± 65.01[a]	—
	Pond	Natural	101.31 ± 9.39[a]	896.31 ± 17.03[b]
	Pond	Stripping	97.63 ± 6.23[a]	—

process involved multidirectional larval movements at a rate of 78 ± 5.1 movements/min, with embryos repeatedly hitting the vitelline membrane. The larvae measured 5.9 ± 0.2 mm total length at hatching. Immediately after hatching, the newly hatched larvae lay in lateral recumbence on the bottom of the container. The larvae made vibrations as if to gain momentum followed by a thrust of vertical movements to the water surface and back to the bottom at a rate of 7 ± 1.5 movements/min. The movements ceased after 30 min. Hatching took significantly more time in the ponds than in the river cages ($P < 0.01$).

Discussion

Due to the scarcity of *L. victorianus* broodstock, oocyte maturation and ovulation was attained in this study through the hypothalamic approach (Yaron 1995). Unlike the hypophyseal approach, this technique does not involve killing donor fish. The 100% success achieved with GnRH + MET could be attributed to being based on the linpe technique (Peter et al. 1988), which consists of a combination of gonadotropin-releasing hormone analogue (GnRHa) plus a dopamine antagonist. Success of the linpe technique has been reported in several other cyprinids (Peter et al. 1988). Driori et al. (1994) reported high spawning ratios when the same technique was used in *C. carpio* and attributed the good spawning response to rapid absorption of metoclopramide from the injection site. The failure of GnRH alone to cause oocyte clearance beyond Phase IV in this study could be explained by the lack of an effective dopamine antagonist and the fact that in many cyprinids, the endogenous inhibitory impact of dopamine is so strong that it severely compromises the effectiveness of externally applied GnRH to increase GtH release that would lead to ovulation and subsequent spawning (Peter et al. 1991; Driori et al. 1994).

The swelling of the egg vitelline membrane in *L. victorianus* has also been reported to occur in *L. mesops* and *L. cylindricus* (Msiska 1990; Hiroyuki et al. 1999). Fryer and Whitehead (1959) suggested that the swelling is an adaptation to ephemeral flood conditions by providing a standard microenvironment to the developing embryo, effectively safeguarding it from a wide variety of environmental conditions. Production of semi-buoyant eggs also seems to be a characteristic of African labeine fishes whose spawning has been studied, except *L. umbratus* where no precise information is available.

TABLE 1. *Extended.*

Ovulation (%)	Fertilization (%)	Time to start of hatching (min)	Hatching rate (%)	Time to end of hatching (min)
—	—	—	—	—
—	—	—	—	—
—	—	—	—	—
—	—	—	—	—
—	—	—	—	—
—	—	—	—	—
—	—	—	—	—
—	—	—	—	—
100.00	0.94 ± 0.03[a]	1554.18 ± 23.27[a]	0.90 ± 0.04[a]	710.12 ± 162.44[a]
—	—	—	—	—
100.00	0.92 ± 0.06[a]	1628.88 ± 20.09[b]	0.77 ± 0.02[b]	1595.81 ± 159.35[b]
—	—	—	—	—

The significant differences in GnRH + MET's latency, incubation time, hatching rate, and duration of hatching between river and pond experiments were attributed to temperature differences in the two environments (Fig. 4). The same factor is thought to have caused prolonged hatching and low hatchability in the pond cages. Ambient water temperatures are known to affect latency of response to induced spawning treatments in *C. carpio* (Driori et al. 1994). The low hatchability of stripped and artificially fertilized eggs in Experiment 1 was originally attributed to the water conditions in tanks.

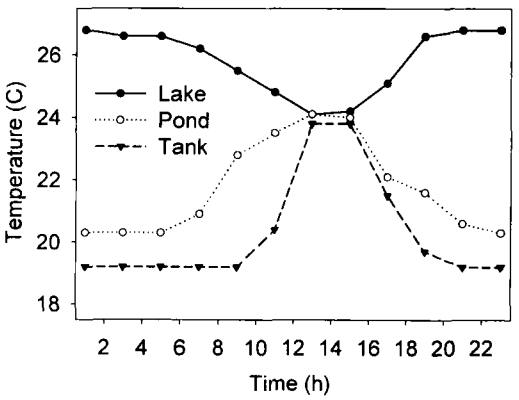

FIGURE 3. *Diurnal temperature variations in the experiments during spawning latency, incubation, and hatching of GnRh and [GnRH + MET]-treated* Labeo victorianus.

This was later disproved by incubation of stripped and artificially fertilized eggs using water from the river and ponds in which naturally fertilized eggs were simultaneously being incubated. All the stripped and artificially fertilized eggs died before hatching, whereas those in the cages had a hatching rate of greater than 89%. Similar results were obtained during Experiment 3 where the water quality was the same as in tanks used in Experiment 1. It can, therefore, be concluded that induced spawning followed by natural fertilization was the most successful method for obtaining viable *L. victorianus* eggs.

The process of breaking through the vitelline membrane during hatching seemed to be energy intensive. It took 8–12 h for some larvae to break through the membrane, while others died after apparently becoming exhausted. During this study the longest time to first hatching was in the concrete tanks and occurred after 27 h, 44 min. In the other experiments it took less time for first hatching to occur. This contrasts sharply with Fryer and Whitehead's (1959) findings in which the first hatching was reported to have occurred after 45 h. Results of this study are similar to the incubation time for *L. mesops* eggs, which were reported to hatch after 28 h (Anon 1964).

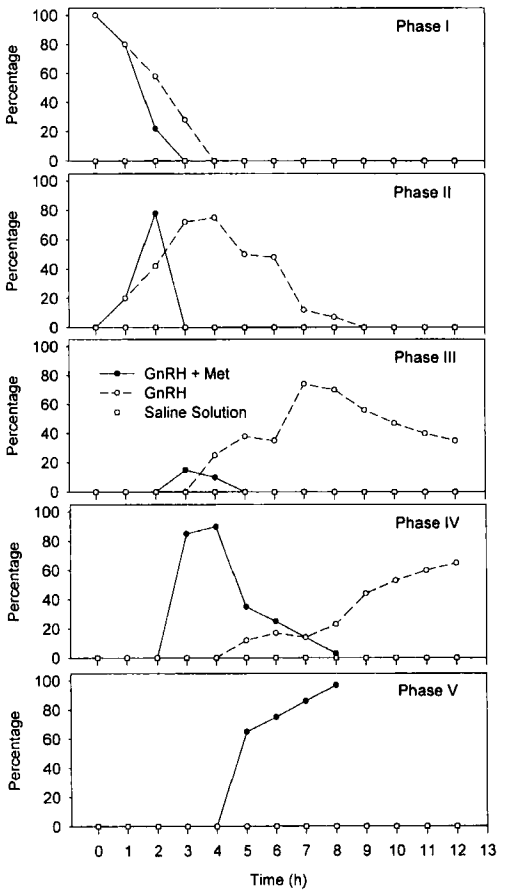

FIGURE 4. *Temporal progression of oocyte develop through five phases for* Labeo victorianus *injected with gonadotropin-releasing hormone (GnRH), gonadotropin-releasing hormone and a dopamine receptor antogonist (GnRH+Met) and a saline solution control.*

The inability to obtain an adequate seed supply is a major constraint to initiation or expansion of aquaculture (Reay 1984). Culture of some fish species depends on collection of wild fry, a method considered inefficient and unreliable (Kuo and Nash 1975). For *L. victorianus* it would be hard to collect wild fry because of difficulty in identifying spawning grounds and harvesting of the widely dispersed semi-buoyant eggs. Propagation of *L. victorianus* will, therefore, be dependent on successful induced spawning.

This study has established that *L. victorianus* can be successfully induced to spawn in captivity. The method used in this study does not require sophisticated equipment of modern hatcheries and is, therefore, affordable by potential breeders in the Lake Victoria basin. The study has also indicated that once fertilization has occurred in cages, eggs could be removed and successfully incubated in locally available receptacles. Incubation of eggs in a controlled environment would reduce predation on the developing embryos and larvae. The temperatures under which *L. victorianus* spawned in this study are within the normal range for most regions around the Lake basin. Induction of spawning should be planned such that fertilization, early embryonic development, and hatching occur when diurnal water temperatures are high. Under controlled conditions, temperature at approximately 26 C would be optimal for induced spawning and hatching. The high fecundity, high fertilization, and hatching rates would enhance the potential of *L. victorianus* as an aquaculture species.

Acknowledgments

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