

Introduction

Although *Clarias gariepinus* is alien to most hydrographic basins of Cameroon, its culture has been widely adopted by local smallholders. Cognizant of their inability to avoid escapes into surrounding ecosystems and cognizant of the risks associated with the culture and/or introduction of alien species (Nairobi Declaration, 2002) Yong-Sulem et al (unpublished) assessed the distribution and dominance of catfish species in Cameroon and found that *Clarias jaensis* was the dominant clariid of the Sanaga (Cameroon's most important river system) as well as of the Wouri, Nyong, Dja and Ntem hydrographic basins. Palatability tests showed that consumers make no difference between the two clariids. As aquaculture expands in Cameroon, the lack of a suitable indigenous species will undoubtedly lead to the widespread introduction of alien species. To prepare for substituting the alien *Clarias gariepinus* with indigenous *Clarias jaensis* and for aquaculture extension into threatened rainforest regions, we undertook to develop protocols for producing the fingerlings of *Clarias jaensis*

Materials and Methods

Through co-operation with fishers of the Mefou, Nyong, So'o, Fala and Angah Rivers (Nyong River basin) in the Central Province of Cameroon, 500 live catfish adults were collected and characterized. Four hundred of them, identified as *Clarias jaensis*, were acclimated in ponds prior to being used for reproductive studies. Studies were carried out at the facilities of local catfish hatcheries in the vicinity of Yaoundé, which participate regularly in WorldFish on-farm demonstrations and research trials.

Initial methods were as used locally for *C. gariepinus*: Determination of gravidity was through testing whether caressing of abdomens could provoke expression of any eggs. Hormonal treatment was through injection into the dorsal muscles (PG doses were in terms of ratios of the weights of donor fish to those of gravid females), fish were hand-stripped, fertilization was by the dry method and incubation was through egg spreading to form a single layer on incubation trays.

Experiment 1

A range of doses of conspecific pituitary glands (PG), Human Chorionic Gonadotropins (HCG) and Luteinizing Hormone Releasing Hormone (LHRHa) were used to induce final maturation and ovulation of eggs. Pituitary glands were extracted as described by Viveen and crushed to obtain a paste which was suspended in a few drops of water to facilitate injection with a 1-ml syringe (Table 1). HCG [format 5000 International Units (IU)] was dissolved to obtain 1 ml and the volumes to be injected were determined through simple proportion (Table 2). One mg of LHRHa was dissolved in 25 ml of primperant (metochloropramide) and simple proportion was also used to calculate the volume that was required to inject a fish of a given weight (Table 3). Injected brooders underwent

their latency period under indoor temperature conditions which usually varied from 24 to 27°C and ovulated eggs were manually expressed and incubated as described for *Clarias gariepinus* by de Graaf and Janssen (1996).

Table 1: Hypophysation dose [(Weight of donor fish - D) / Weight of receptor brooder - R)] and final maturation as well as ovulation of *Clarias jaensis* eggs under a variable indoor temperature of 24 – 26 °C.

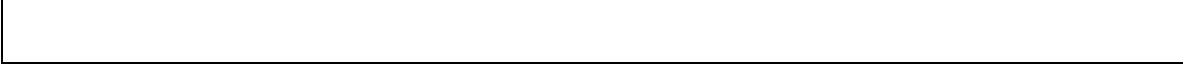
Desired Dose (D/R) (g)	Weight of Donor (g)	Weightt of ♀ (g)	Actual Dose (D/R) (g)	Weight of eggs (g)	Wt of eggs / Wt of ♀
1.0	320	316	1.01	20	0.06
	293	286	1.02	8	0.03
	213	210	1.01	13	0.01
1.5	509	350	1.45	19	0.05
	612	403	1.52	22	0.06
	360	240	1.50	15	0.06
2.0	623	310	2.01	13	0.04
	465	230	2.02	12	0.05
	410	204	2.01	10	0.05

Table 2: Administration of HCG at 3 experimental doses and weight of eggs obtained.

Dose (IU/kg)	Wt. of ♀ (g)	No of IU	HCG solution (ml)	Wt of eggs (g)
4000	180	720	0.15	7
	356	1424	0.29	11
	270	1080	0.22	12
4500	618	2472	0.50	29
	512	2048	0.41	30
	430	1720	0.15	16
5000	428	1712	0.34	25
	168	672	0.14	10
	297	1188	0.24	19

Table 3: Administration of LHRHa at 3 experimental doses and weight of eggs obtained.

Dose (mg/kg)	Weight of ♀	Volume of LHRHa (ml)	Weight of eggs
0.02	414	0.21	0
	360	0.18	0
	207	0.11	0
0.05	190	0.24	5
	518	0.65	16
	488	0.61	20
0.07	526	0.92	18
	277	0.49	10
	349	0.61	19



Experiment 2

Because all eggs failed to hatch, regardless of kind of hormone and dose at which gravid females were injected, a number of hypotheses on possible causes were tested. They were to do with single or double injections (SI or DI) as well as with water quality and duration of latency (Table 4).

Because the pH (8) of incubation water in Experiment 1 was suspected for having been too high for successful egg incubation, subsequent trials were done at a pH of 6 (as is common in the Nyong River from where broodfish were obtained) using HCG at a dose of 4,000 IU/kg which had been confirmed (experiment 1) as effective for inducing final maturation and ovulation. Modal weight (150-300g) broodfish and a 1500 I.U. HCG format were preferred. The effects of water temperature and salinity were surveyed based on fish spawning ground conditions in their native Nyong river and on indications from the literature. Thermostatically controlled heaters were used to maintain triplicate constant incubation temperatures of 26 and 30° C which average rainy and dry-season temperatures in their source River .

Since Phleps and Wasler (1993) obtained higher hatchability of *Ictalurus punctatus* eggs in saline (0.5–2.5 g of salt/liter) than in fresh water, the effect of incubation water salinity (1.5 g of uniodised salt/liter) was also tested. Based on Cacot et al's (2002) recommendation that ovulated eggs of *Pangasius bocourti* should be fertilized immediately after ovulation to avoid their degeneration, stripping of broodfish after temperature-dependent latency durations determined for *Clarias gariepinus* by de Graaf and Janssen (1996) and trial stripping per hour with effect from 5 hours post latency onset were also undertaken. For double injections, first ones comprised a quarter of the determined dose and three quarters were administered 2 hours after the first.

Finally, *Clarias gariepinus* and *Clarias jaensis* were concurrently subjected to identical conditions of hypophysation, latency (time required for final egg maturation and ovulation), gamete collection, fertilization and incubation, followed by microscopic (40X) examination of eggs once every 5 hours (4 times between 06H00 and 21H00) for 2 days. All eggs developed normally, right to closure of blastopheres (delimiting their central yolk sacs). Yet, while those of *C. gariepinus* continued right to hatching (> 60 %), those of *C. jaensis* died, notwithstanding their larger size (which leads us to believe that if we can get them to hatch, the resulting larvae should be more robust, resistant to predation and easier to feed than the much smaller *C. gariepinus*).

Table 4: Effect of single or double injections (SI and DI) of broodfish with HCG and of incubation water temperature (T) and salinity (S) on final egg maturation, ovulation and hatchability. TS = Trial stripping of brooders as necessary to minimize the time lapse between ovulation and fertilization of eggs.

Treatments			Wt. of ♀ (g)	No of IU		Volume (ml)		Wt. of eggs (g)
SI/DI	T (°C)	S (g/l)		1 st I	2 nd I	1 st I	2 nd I	
SI	26	0	164	656	-	0.66	-	5
			217	868	-	0.87	-	8
			289	1156	-	1.16	-	7
SI	30	0	313	1252	-	1.25	-	9
			150	600	-	0.60	-	4
			222	888	-	0.89	-	8
SI	30	1.5	248	992	-	0.99	-	7
			193	772	-	0.77	-	5
			304	1216	-	1.22	-	7
DI	30	0	260	260	780	0.26	0.78	4
			241	241	723	0.24	0.73	4
			145	145	435	0.15	0.44	3
DI+TS	30	0	179	179	537	0.18	0.54	Brood
			208	208	624	0.21	0.63	fish
			242	242	726	0.24	0.73	died

Results and Discussion

All but one of the treatments to induce final egg maturation and ovulation were effective, eggs flowing out very easily after a latency duration equivalent to that determined for *C. gariepinus*. Only the LHRHa dose of 0.02 mg/kg produced unviable eggs which did not seem to have completed their final maturation phase. This dose could have been insufficient for *C. jaensis*, albeit enough for *C. gariepinus*. The optimum LHRH dose should lie between 0.2 and 0.5 mg/kg. Ovulated eggs were generally less adhesive, yellow in color and larger (about 1.5 mm in diameter) than those of *Clarias gariepinus*. Yet, none developed right to hatching as would have been expected from their apparent high quality. Hourly trial stripping to facilitate fertilization of eggs shortly after ovulation resulted in the death of brooders, an indication that *C. jaensis* could be more vulnerable to handling stress.

References

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I. Plans for follow-up (maximum length: 200 words)

Despite problems with egg hatchability, the large egg size, the wide natural distribution (implying a general ability to be cultured under a range of conditions) and indigenous nature of *Clarias jaensis* means that this species could have wide applicability for environmentally sound Cameroonian aquaculture. The urgency of finding a good aquaculture candidate is growing along with interest in aquaculture. New projects in the rainforest zone are beginning nearly every day. We therefore plan to continue this work as a high priority.

The natural breeding season for *C. jaensis* now appears to be over for the year, however we shall continue to monitor the brooders for gravidity (for the better-studied *C. gariepinus*, there is another, smaller reproductive peak in March-April) whereupon we shall test the hypothesis that the quality of the eggs may have been affected by the late application of hormone and instead apply progressive hormonal treatments consisting of low daily doses over 10 days prior to the decisive treatment (Cacot et al, 2002) or even intra-muscular implantation of hormonal pellets (Lee et al, 1986) which mimic the gradual natural slow release of hormones into the bloodstream until attainment of the gonadotropic surge.

Instead of stripping, we shall also try to fertilize eggs naturally by allowing males and females to mate at will in holding tanks (Bruton, 1979). In case of further failure, we shall test the hypothesis that some unknown water quality parameter may be to blame by performing the trails in camp on the banks of the Nyong in order to enable artificial reproduction in their natural spawning grounds.

In the meantime, further literature review will be complemented by field studies to learn more about the natural reproductive cycle and attempt to identify key environmental parameters that might affect hatching.

**III. Report budget utilization including whether budget was spent as planned
(maximum length: 100 words)**

The funds arrived pretty late but thanks to advance payments by Worldfish Cameroon, we suffered no delays. Timely disbursements are of paramount importance to the success of a project with seasonal exigencies like fish reproduction. The budget was effectively used as planned, with substantial matching contributions from cooperating farmers and WorldFish.

**IV. Assessment of the fellowship experience and general comments.
(maximum length: 300 words)**

I noticed that my research skills were significantly growing. My capacity to interact with fishers, fish farmers and retailers has been improved – I am becoming more able to harness contradictions, gather consensus and proceed towards defined goals.

My collaboration with WorldFish Cameroon was strengthened, with her representative (Dr. Randy Brummett) finding more time to criticize my designs, prescribe references and suggest alternatives. I experienced for the first time that such promptings can enkindle and maintain a real passion for research and only now can I understand why and how some researchers turned workaholics.

Dr. Brummett did not always share my disappointment with repeated incubation failures but rather read information from each, developed implied hypotheses and encouraged further experiments to test them. Nor did he limit his interventions to my level alone. He also talked IITA and IRAD technicians / officers into more collaboration (repair of computers and involvement of grantee in relevant workshops). I learned tremendously from his simplicity and from that of his CGIAR colleagues. I can now boast of not only being more able to conduct, but also to manage research works.