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Effect of broodfish exchange strategy on the spawning performance and sex steroid hormone levels of *Oreochromis niloticus* broodfish in hapas

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Abstract

The productivity of Nile tilapia (*Oreochromis niloticus* L.) broodfish, spawned in large hapas (120 m²) in ponds under different exchange and management strategies, was monitored over a 70-day period. Female fish were checked for eggs twice weekly and returned to the same spawning hapa throughout the experiment (0 day) or exchanged for females maintained in separate single sex groups at high density for a period of 7 days at each (3.5 days) or alternate (7 days) harvests. Exchanged females were conditioned in separate hapas (20 m²) in the same pond. Production of eggs per unit area were significantly higher ($P < 0.05$) in females exchanged weekly (7 days, 64 eggs m⁻² day⁻¹) or at each harvest (3.5 days; 55 eggs m⁻² day⁻¹) than for fish remaining in the spawning hapas throughout the trial (0 day, 33 eggs m⁻² day⁻¹). A greater proportion of conditioned females (3.5 days and 7 days) was spawned than in the control group (18% females compared to 11% per harvest). Conditioning of female broodfish had no effect on the egg clutch size ($P > 0.05$). Female productivity (eggs kg⁻¹ day⁻¹) was significantly lower for fish exchanged after every harvest (3.5 days) than those remaining in spawning hapas for alternate harvest (7 days) or throughout the experiment (0 day). Gonadal steroid (testosterone, T and oestradiol, 17 β -E₂) and calcium levels sampled at the end of the experiment reflected spawning performance; serum T and E₂ were highest in exchanged females. Non-spawned fish had elevated steroid levels compared to females incubating seed at harvest (e.g., 19.5–91.4 and 7.2–21.5 ng ml⁻¹ T for non-spawned and spawned fish, respectively). Stocking of the Indian Major carp

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Labeo rohita in spawning hapas to control fouling did not improve water quality and led to a significant depression of seed production ($P < 0.05$). Regular exchange of female broodfish from spawning hapas with conditioned fish can improve productivity of hapa-in-pond systems. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Nile tilapia; Broodstock; Broodfish; Hatchery; Seed; Sex steroids; Testosterone; 17β -oestradiol

1. Introduction

The rapid growth in production of the major cultured species of tilapia, *Oreochromis niloticus* (L.), in recent years (Laureti, 1996), has been associated with increasing efforts to intensify hatchery production. Productivity of broodfish in current hatchery systems is heavily constrained by inherent low fecundity and the asynchronous nature of tilapia spawning cycles. This has tended to result in the use of methods yielding low outputs of fry per unit area of hatchery, often resulting in poor quality and unpredictable harvests (Little, 1998).

A particularly promising approach is the mass production of seed (eggs and yolk-sac fry) removed from mouthbrooding females after being spawned in large nylon hapas (fine net cages) suspended in earthen ponds (Hughes and Behrends, 1983; Bautista et al., 1988; Little, 1989). Previous studies have shown that both broodfish condition and spawning synchrony can be significantly enhanced if cohorts of females are conditioned separately at high density prior to stocking for spawning at a lower density (Little, 1989; Lovshin and Ibrahim, 1989). This builds on the understanding that reproduction in tilapiine species is inhibited at high density (Coche, 1982; Paessum and Allinson, 1984; Coward et al., 1998) owing to a breakdown in hierarchy (Little, 1989) and a shift towards schooling behaviour (Falter and Debacker, 1988). The physiological basis of the density-dependent shift in behaviour that leads to the cessation of spawning in crowded or densely stocked tilapia has been investigated recently in laboratory stocks of the substrate spawning *Tilapia zillii* (Gervais) (Coward et al., 1998), but remains unresearched in the commercially more important *O. niloticus*. Coward et al. (1998) noted that levels of important gonadal steroids, such as 17β -oestradiol (E_2) and testosterone (T), were suppressed in densely-stocked female *T. zillii* exhibiting total cessation of spawning activity (Coward et al., 1998). Hormone levels increased dramatically (concomitant with marked spawning activity) upon transfer to aquaria in which females were held individually. This suggests that serum levels of these steroids may be important during the high density conditioning of *O. niloticus*. This study, therefore, aimed to investigate the influence of conditioning and broodfish exchange upon sex steroid levels and reproductive performance in *O. niloticus* broodfish. Importantly, the study was performed on a commercial scale and under realistic commercial conditions.

The optimal duration and density for conditioning female fish has already been investigated (Ambali and Little, 1996; Little, 1996), but these factors have not been related to the hormonal status of the fish. Although more frequent exchange of female fish could result in more intensive spawning, it might also inhibit mature females from spawning all ripe eggs; disturbance of spawning females is known to result in 'residual',

unspawned eggs (Peters, 1983). Frequent exchange of females from spawning hapas and replacement with females conditioned for an optimal period also increase the number of females required as well as the associated costs.

A major constraint to prolonged use of hapas for spawning relatively low densities of tilapias is the fouling of the inside panels of spawning hapas that leads to progressive deterioration in water quality. Growth of periphyton or build up of inorganic silt can reduce water exchange in fine-mesh cage materials. Manual exchange of hapas is effective but time consuming, and other methods have been researched. Some species, such as the suckermouth catfish (*Plecostomus* sp.), are effective controllers of fouling but they reduce seed production (Kingnate, 1995). The impact of stocking a periphyton-grazing species such as the Indian major carp *Labeo rohita* (Hamilton), which is often stocked together with the Nile tilapia in ponds to grow food-size fish, on the productivity of hapa-based spawning systems was assessed.

2. Materials and methods

The experiment was carried out in hapas suspended in ponds at the Asian Institute of Technology (AIT), near Bangkok, Thailand. Blood serum samples from broodfish were analyzed at the Institute of Aquaculture, University of Stirling, Scotland UK.

2.1. Fish

Individual fish of the Thai *Chitralada* strain of *O. niloticus* were obtained from a mature stock raised as a single group from yolk-sac absorption in a hapa-in-pond (day 1–30) and fertilized earthen pond (day 31–130) before final conditioning in hapas (day 131–150). Mean (\pm SE) initial weights of female and male fish at stocking were 38.3 ± 0.4 and 48.5 ± 0.5 g, respectively. The strain has been raised and spawned in hapa-in-pond systems at the AIT for more than 10 generations. Indian major carp, rohu (*L. rohita*), were obtained as fry from a local hatchery and nursed to an average size of 25 g before use.

2.2. Experimental system

A factorial design was used to compare the effect of female broodfish exchange frequency and the use of rohu to control hapa fouling on reproductive performance and sex steroid levels. A 70-day experiment was conducted using six spawning hapas ($24 \times 5 \times 0.9$ m) and 12 conditioning hapas ($8 \times 2.5 \times 0.9$ m) suspended in each of the two replicate fertilized earthen ponds (P1 and P2; area = $1750 \text{ m}^2 \text{ pond}^{-1}$). Treatment hapas were positioned randomly in each pond, with conditioning hapas located close to the same spawning replicate to facilitate fish transfers. Water was added from an open irrigation system when necessary to maintain a water depth of 0.6 m in the hapas. A sex ratio of one was maintained in each spawning hapa throughout the experiment (360 males and 360 females), at an initial total density of 6 fish m^{-2} ; fish lost from the hapas during the experiment were not replaced. Females were held at an initial density of 20

Table 1

Number of spawning hapas (SH), conditioning hapas (CH) and total number of female Nile tilapia (NF) used in each replicate treatment with no female exchange and treatments with females exchanged every 7 and 3.5 days. Rohu were stocked into half of the spawning hapas

Rohu stocked	Female exchange								
	No exchange (control)			7 days			3.5 days		
	SH	CH	NF	SH	CH	NF	SH	CH	NF
+	2	0	360	2	4	720	2	8	1080
–	2	0	360	2	4	720	2	8	1080

fish m⁻² in conditioning hapas. A total of 20 harvests were carried out during the experiment; all females were checked every 3.5 days for the presence of eggs in the mouth. Eggs were shaken from the female mouth into a double-hand net. Egg batches were sorted by arbitrary development stage and counted (Little et al., 1993) before being combined and weighed using an electronic balance. Alternate harvests occurred in the morning or late afternoon to ensure that a period of 3.5 days was maintained between harvests.

Females were exchanged in spawning hapas at every harvest (3.5 days) or alternate harvest (7 days), with females conditioned for 7 days in separate, single sex groups at high density in hapas (Little et al., 1996). The numbers of broodfish and hapas used is indicated in Table 1. Conditioning hapas were allowed to sun-dry in situ to control fouling of the inside walls after fish were removed for exchange. A control treatment in which female broodfish remained in the spawning hapa throughout the experiment was also set up. In spawning hapas stocked with rohu, 30 rohu were maintained in each spawning hapa throughout the experiment. Nine hundred sex-reversed male *O. niloticus* and 16 *Notopterus notopterus* (Pallas) were stocked in each pond to control external hapa fouling and recruitment from wild-spawned tilapia, respectively. Fish in all hapas were fed with a floating starter catfish pellet (Chareon Pokphand; 30% crude protein) twice daily to satiation.

Water quality parameters were monitored throughout the experimental period from inside each hapa and at two points outside the hapas in each pond. Temperature, dissolved oxygen, and pH were measured weekly at 6 and 16 h. Alkalinity, ammonia-N, soluble reactive phosphorous (SRP), total suspended solids (TSS), total volatile solids (TVS), chlorophyll-*a*, and pheophytin of water were analyzed bi-weekly (APHA, 1989).

2.3. Blood sampling and analysis

At final harvest, blood was removed from female tilapia; 2–4 fish were sampled per replicate treatment from both spawned and non-spawned fish in spawning hapas and from fish randomly sampled from conditioning hapas. Blood was allowed to clot at 4°C and centrifuged at 5000–6000 rpm for 15 min. The serum was stored at –25°C and transported to the Stirling laboratory with dry ice in vacuum flasks until radioimmunoassay.

Levels of serum E_2 and T were measured by established radioimmunoassays (Duston and Bromage, 1987). Serum levels of calcium were measured using an atomic absorption spectrophotometer (Perkin-Elmer 2280, UK). Calcium ions are incorporated into the post-translational modification of the vitellogenin molecule prior to its release from the liver (Tata, 1978). As a result, serum calcium levels rise during vitellogenesis; an increase that is directly correlated to blood vitellogenin concentration (Elliot et al., 1982). Total serum calcium is therefore considered an accurate indicator of serum vitellogenin concentration. Intra- and inter-assay coefficients of variation were 7.3% and 10.1%, 4.6% and 7.4%, and 6.2% and 6.9% for calcium analysis, E_2 assay, and T assay, respectively.

2.4. Egg sampling and counting

The mean number of eggs per female was calculated for eggs harvested of different stages (Little et al, 1993) using the following formula:

$$\text{Mean number of eggs female}^{-1} = \text{Total weight of eggs} / (F \times E)$$

where F is the total number of females from the same hapa and E is the mean individual weight of eggs.

Mean individual egg weights were based on counts of 200 individual stage 1 eggs from each hapa at each harvest; eggs of stages 2 and 3 were assumed to be of the same size based on previous AIT studies.

2.5. Statistical analysis

Reproductive performance data (egg output, number of egg clutches, growth and survival of broodfish) were analyzed by a 2 (presence/absence of rohu) \times 3 (female exchange) factorial analysis in which ponds were treated as blocks (Table 1). Percentage data were arcsine-transformed before the analysis of variance (ANOVA) and Student's t -test. Correlation analysis was performed for levels of serum T, E_2 , and total calcium (SPSS for MS Windows). Serum samples obtained from spawned and unspawned fish were compared using multifactor ANOVA and Student's t -test (Statgraphics ver. 7.0).

3. Results

3.1. Reproductive performance

Reproductive performance, as indicated by seed output per unit area or as a percentage of females spawned at each harvest, was enhanced in females regularly exchanged for fish conditioned in single sex groups for 7 days ($P < 0.05$). Mean egg production (eggs $m^{-2} \text{ day}^{-1}$) for females conditioned for 7 days was almost double the output from hapas in which females were maintained in spawning hapas continuously ($P < 0.05$; Fig. 1). A similar, but less pronounced trend, was found for intensity of female spawning (percentage of females spawned harvest^{-1} ; $P < 0.05$). Relative seed

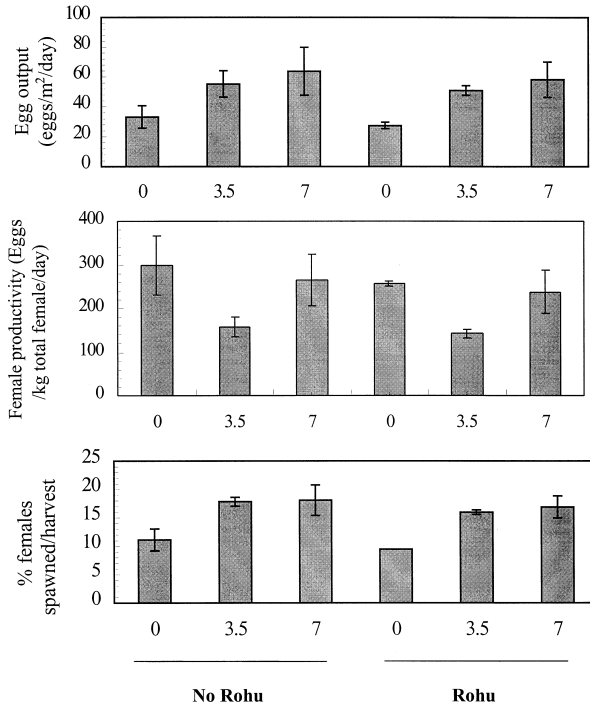


Fig. 1. Effects of exchange (no exchange (0 day), exchanged with conditioned fish every harvest (3.5 days) or alternate harvests (7 days)) of female *O. niloticus* breeders in large nylon hapas harvested twice weekly on (a) egg output (eggs m⁻² day⁻¹), (b) female reproductive productivity (eggs kg⁻¹ total females day⁻¹), and (c) spawning frequency (% females spawned harvest⁻¹). Half the hapas were stocked with *L. rohita*.

output, expressed as the number of seed produced per kilogram total of initial females stocked, indicated that frequent exchange (3.5 days) reduced productivity of female broodfish significantly ($P < 0.001$). Exchange strategy did not influence the fecundity of females, or numbers of eggs clutch⁻¹ ($P > 0.05$), mean values of which ranged between 370–415 eggs clutch⁻¹.

The pond had a significant impact on productivity, with one pond producing nearly one third more reproductive output than the other. Water quality was consistently higher in the more productive pond, that is, both mean early morning dissolved oxygen and chlorophyll levels were higher (> 1.0 compared to 0.7 mg l^{-1} and 103.5 compared to 61.2 g l^{-1} , respectively).

The presence of rohu had a negative impact on egg production, reducing the percentage of females spawning at each seed harvest and resulting in a 12% decline in seed output ($P < 0.05$).

3.2. Growth and survival of broodfish

The mean total duration spent in the spawning hapa clearly affected fish growth (Table 2) and reproductive performance. Females exchanged every 3.5 days, and thus

Table 2

Growth and survival of Nile tilapia broodfish

Notations a, b, and c allow comparison of conditioning periods within the no Rohu or Rohu groups, i.e., to observe the effects of conditioning period for each Rohu and no Rohu group. Values in columns with the same notation are not significantly different ($P > 0.05$).

Rohu	Conditioning period (day)	Broodfish survival (%)		Daily weight gain (mg fish ⁻¹)	
		Female	Male	Female	Male
No	0	98 ± 5 ^a	87 ± 4 ^a	-37 ± 34 ^a	17 ± 5 ^a
	3.5	73 ± 3 ^a	94 ± 3 ^a	350 ± 6 ^b	31 ± 32 ^a
	7	85 ± 3 ^a	89 ± 1 ^a	171 ± 96 ^{ab}	25 ± 56 ^a
	Mean ± SE	86 ± 7	90 ± 2	161 ± 112	24 ± 4
Yes	0	105 ± 14 ^a	86 ± 4 ^a	7 ± 8 ^a	-25 ± 25 ^a
	3.5	83 ± 16 ^a	95 ± 5 ^a	287 ± 83 ^{ab}	39 ± 14 ^a
	7	76 ± 4 ^a	74 ± 8 ^a	208 ± 19 ^b	137 ± 152 ^a
	Mean ± SE	88 ± 9	85 ± 6	167 ± 84	51 ± 47

spending less time in the spawning hapa overall, gained more weight during the experiment than non-exchanged fish. Females kept in spawning hapas throughout the trial lost weight (0 day). Exchange of females resulted in a significantly poorer ($P < 0.05$) survival to final harvest. Males grew more slowly than females in all treatments and actually lost weight in four replicates over the 70-day period. A replicate in which males gained weight was related to poorer survival of male fish (66%) in this hapa; male survival was more than 80% in all other replicates. The pond had no effect on broodfish growth ($P > 0.05$). There were no significant ($P > 0.05$) effects of broodfish exchange and the presence of rohu or pond on the survival of broodfish.

Table 3

Effects of Nile tilapia female exchange on serum levels of sex steroids and total calcium in spawned (S) and non-spawned (NS) female fish removed from spawning (SH) and conditioning hapas (CH) after an experimental period of 70 days (mean values ± SE)

N.B. mean values with the same superscripts are not significantly ($P > 0.05$) different within the same column and row. Notations a, b, and c, are for comparisons within a vertical column and x, y, and z are for comparisons within a horizontal row.

Broodfish exchange strategy	Testosterone (ng ml ⁻¹)			Oestradiol (ng ml ⁻¹)			Total calcium (mg/%)		
	SH	SH	CH	SH	SH	CH	SH	SH	CH
	S	NS	NS	S	NS	NS	S	NS	NS
Control	7.2 ^{a,x} ± 0.9	19.5 ^{a,x} ± 6.7	-	1.7 ^{a,x} ± 0.3	4.8 ^{a,x} ± 1.5	-	14.6 ^{a,x} ± 0.7	13.3 ^{a,x} ± 0.6	-
Females exchanged every 3.5 days	18.7 ^{b,x} ± 2.7	76.5 ^{b,y} ± 15.1	51.5 ^{a,y} ± 10.7	3.5 ^{b,x} ± 0.7	16.6 ^{b,y} ± 2.6	14.5 ^{a,y} ± 2.3	23.6 ^{b,xy} ± 1.4	28.8 ^{c,y} ± 2.2	23.5 ^{a,x} ± 1.3
Females exchanged every 7 days	21.5 ^{b,x} ± 2.4	91.4 ^{b,y} ± 14.1	55.7 ^{a,y} ± 10.6	8.4 ^{c,x} ± 1.5	15.3 ^{b,y} ± 1.6	16.7 ^{a,y} ± 2.7	21.1 ^{b,x} ± 1.3	22.7 ^{b,x} ± 1.2	21.2 ^{a,x} ± 0.6

3.3. Serum testosterone, 17β -oestradiol, and total calcium levels

In females without conditioning, levels of serum T, E_2 , and total calcium, were similar in spawned and non-spawned females (Table 3). In conditioned females, spawned fish had lower levels of both E_2 and T than in unspawned fish ($P < 0.05$). Levels of both E_2 and T were also higher in fish conditioned for 3.5 and 7 days than those that had remained in the spawning hapa continuously ($P < 0.05$). Levels of both steroids were similar in conditioned fish maintained for both 3.5 and 7 days in the spawning hapa, and there was no effect of rohu nor of pond. Levels of E_2 and T in females removed from conditioning hapas were significantly higher ($P < 0.001$) than those of spawned females removed from spawning hapas. Serum levels of both hormones in fish removed from conditioning hapas were similar to unspawned fish from conditioned treatments harvested from spawning hapas (Table 3). Calcium levels were similar in spawned and unspawned females, but levels in conditioned females were significantly higher than control females ($P < 0.001$). In spawned females, there was a highly significant ($P < 0.001$) correlation between T and E_2 . Total calcium exhibited a significant ($P < 0.05$) correlation with T, but not with E_2 . In non-spawned females, T, E_2 and total calcium, were highly correlated ($P < 0.01$).

4. Discussion

This study aimed to describe the serum steroid levels in female Nile tilapia held under a variety of management conditions, and to relate them to reproductive performance. The decline in reproductive performance of Nile tilapia broodfish over time has been observed by several workers in ponds (e.g., Mires, 1982), tanks (Guerrero and Guerrero, 1984), and hapas (Bautista et al., 1988). The use of short spawning cycles and frequent exchange with 'conditioned' females has proved a practical means to maintain system output, at a cost of using more broodfish (Little et al., 1993; Macintosh and Little, 1995). The physiological basis of the conditioning/spawning management has not been described until now although the results of Coward et al. (1998) suggest that circulating gonadal steroids play vital regulatory roles.

This study demonstrates how steroid levels in female broodfish, managed in different ways, reflect spawning readiness and reproductive performance. Higher levels of T and E_2 in fish that were pre-conditioned at high density were related to improved reproductive performance compared to fish that were not exchanged. These steroids are suppressed in both *Tilapia tholloni* (Sauvage) and *T. zillii* held under crowded conditions (Coward and Bromage, 1995; Coward et al., 1998); these reduced levels being associated with lack of final oocyte maturation. In the present study, non-spawned fish (except in control groups) had more than double the levels of T and E_2 than spawned fish, which were similar to spawned and conditioned females. This suggests that 'readiness to spawn' remained high post-spawning in conditioned fish and concurs with observations of multiple spawning (Lee, 1979; Fishelsen, 1983) and Peters' explanation of polymodal ripening of eggs (Peters, 1983). The highly asynchronous nature of ovarian recrudescence is part of the flexible reproductive strategy found in *Oreochromis* species, which

can be exploited by appropriate conditioning and exchange. The high reproductive performance of fish remaining in the spawning hapa for 7 days (7 days) suggests that a higher proportion of ripe fish had the opportunity to spawn than when fish were changed more frequently (3.5 days). Possible explanations for this include a shortage of males or the presence of hierarchies that may have restricted spawning activity to the most dominant fish over the shorter, more intense, exchange interval. In a previous study, selected exchange of spawned fish with conditioned fish after 5 days spawning opportunity enhanced seed production compared to exchange of all females; this suggested that 5 days is an adequate time for ripe fish to spawn if hierarchy formation is inhibited or weakened (Little et al., 1996). The close correlation of spawning performance and serum E_2 in spawned females ($r = 0.76$, $P < 0.01$) suggests weekly exchange optimized active vitellogenesis in the current study. Total calcium levels remained higher in all exchanged females indicating elevated vitellogenin production.

Mean levels of T in non-spawned, exchanged fish taken from spawning hapas were in the range identified for spawning *T. zillii* after transfer from crowded, confined conditions to individual isolated conditions (Coward et al. 1998), suggesting a similar hormonal basis for reproduction. In the present study, E_2 levels of spawned fish from exchanged or non-exchanged females were similar to those found by Coward et al. (1998; 3.5–8.4 ng ml⁻¹), but non-spawned fish taken from either spawning or conditioning hapas exhibited elevated levels (14–17 ng ml⁻¹) suggesting readiness to spawn. The lower levels of E_2 and T in the control fish might reflect the higher levels of stress in females maintained constantly in spawning hapas. Stress has previously been related to poor reproductive performance and low levels of serum sex steroids in tilapia (see Foo and Lam, 1993a,b; Coward et al., 1998). As exchanged fish were handled more often than controls, results of the present study suggest that handling stress in such hapa-based systems may be less important than stress through interaction with conspecifics in low density spawning hapas. The presence of rohu in the spawning hapa, while depressing seed production, had no effects upon female steroid levels. However, stocking rohu had no effect on clutch size, suggesting that courtship, rather than the fecundity or brooding efficiency was affected.

Verdegem and McGinty (1987) found that seed removal every 2 days optimized productivity of females maintained without exchange, but that weekly harvests were more economic because of reduced labour costs. Our results indicate that exchange of females at each twice-weekly harvest did not improve productivity over once weekly exchange, and costs are increased by the more frequent collections. A subsequent trial has confirmed that a period of 10 days conditioning and 5 days spawning opportunity optimize seed production but may be economically less attractive than a weekly cycle of conditioning and spawning (Little et al., unpubl. data).

More frequent female exchange (3.5 days) depressed the number of eggs spawned (eggs kg⁻¹ female day⁻¹ and m⁻² day⁻¹) rather than the frequency and intensity of spawning (% spawning harvest⁻¹) compared to weekly exchange (7 days). The number of females with eggs was similar, but females remaining in spawning hapas longer produced larger clutches and had significantly higher levels of E_2 than females exchanged more frequently (8.4 and 3.5 ng ml⁻¹ for 7 and 3.5 days, respectively). Interruption of spawning may have been more pronounced among fish in which females

were exchanged more frequently. This phenomenon is also believed to inhibit overall productivity at sub-optimal broodfish densities (Little et al., 1993). The poorer survival of female fish at harvest in exchange treatments also demonstrates a cost of using such methods. Handling losses during transfer of females from spawning to conditioning hapas were probably the major cause.

The use of rohu to moderate water quality within hapas was clearly inconsequential. The depression of seed production observed in hapas stocked with rohu was most likely related to disturbance or interruption of *Oreochromis* spawning rather than interspecific competition for food. Kingnate (1995) found the suckermouth catfish effective at removing fouling from hapas, but it also resulted in reductions in seed yield. Essential water quality parameters remained within the range typical of commercial hapa-in-pond systems throughout the relatively short experimental period described.

The use of exchange techniques in hapa-based systems can be an important method for hatcheries to increase and maintain seed productivity. This study suggests that high levels of circulating steroids (and total calcium as an indirect measure of hepatic vitellogenin production) are indicative of females maintaining good condition and readiness to spawn. Steroid levels explain how broodfish exchange strategies can intensify seed production. Weekly exchange (7 days) of females was not only the most productive method but also reduced handling and broodfish costs compared to more frequent exchange (3.5 days). Broodfish costs include both the costs of broodfish purchase or production and maintenance (labour, feed, etc.) during their production life. The stocking of a periphyton-grazing fish in spawning hapas had no clear impacts on water quality but rather depressed seed yields possibly through negative interactions on broodfish during courtship.

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