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Full Length Research Paper

Comparison of broodfemales selection methods used at early maturity to improve seed production in Nile tilapia, *Oreochromis niloticus* (*L*.)

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Abstract

The effects of three broodfemales selection methods: i) Broodfemales selection by size at maturity as small and large, ii) Broodfemales selection by age at maturity as early and late maturing and iii) Broodfemales selection by 90 d post-maturity spawning performance were evaluated on Nile tilapia, *O. niloticus* reared in a hapa-in-pond system over 12 month experimental period. The method broodfemales selection by size at maturity didn't improve total seed production while broodfemales selection by age as early and late maturing groups failed to effectively separate the broodfemales into productive and unproductive groups. On the other hand, the method brood females selection by 90 d post-maturity spawning performance resulted in similar age at first spawning (d), initial and final body weight (g), eggs spawn^{-1}, eggs kg female $^{-1}$ d^{-1} and ISI (d) group to the method broodfemales selection by age at first spawning as early and late maturing. However, it had advantages in effectively separating the broodfemales into productive and unproductive groups and also in increased eggs female^{-1} d^{-1} and spawn female^{-1} y^{-1}. Therefore, selecting broodfemales by 90 d post-maturity spawning performance allowed improved synchronization of broodfemeles through maintaining 83 ± 2.02% active broodfemales in the population, higher by 42, 20 and 50% than the methods broodfemales selection by size, by age at first spawning and the control, respectively.

Keyword: Nile tilapia, Oreochromis niloticus, seed production, selection method, maturation, spawning activity

INTRODUCTION

Large variations within and between strains of tilapia have been reported for fecundity, frequency of spawning and size of broodfemales at first spawning that resulted in wide variations in reproductive potential and seed production rate (Behrends and Smitherman 1983; Rana1990; Macintosh and Little 1995; Getinet and Bart 2007).One reason for huge differences in seed production between strains and species could be the proportion of spawning females in a population. For example, proportion of spawning females was 22.5-39.5% for Philippine red tilapia, 25.5% for Florida red tilapia and 42.6 % for Thai Chitaralada (Smith, Watanabe, Chang, Ernst, Wicklund and Olla 1991; Eguia 1996; Getinet and Bart 2006). Variations in age and size at maturity within population could also influence the rate and synchronization of seed production (De Silva 1986; Duponchelle, Pouyaud and Legendre 1998). In general, it is presumably accepted that tilapias mature either early at a small size, or late at large size (Duponchelle *et al.,* 1998) but size at maturity has no effect on the fecundity and the spawning potential of individual females when cultured (Getinet and Bart 2007). The huge variation in broodfemale size within even same age broodfemales' population is also assumed to be a factor that affects the reproductive performances and seed production. However, size of broodfemale is not correlated with reproductive performances (Getinet and Bart 2007). This is evidenced by Ens *et al.,* (1995) who reported highly fecund females but small in size while Mair and Little (1991) and Macintosh and Little (1995) reported highly fecund but large in size. Moreover, smaller females were found to delay maturation was noted by Eyeson (1983). The existence of low fecund-large size females in a population was also reported by

Getinet and Bart (2007). This leads to one point of far greater importance particularly to hatcheries perhaps, is the huge variability in the frequency of spawning of individual females (within or between strains). Clearly some method for identifying and separating out the high and low spawning frequency broodfemales could have a dramatic impact upon hatchery efficiency and profitability far in excess of any fecundity differences that might exist between different strains.

Generally, using large size fish to improve productivity are routine in broodfish management. However, female selection by size in Nile tilapia may result in selecting either productive and/or unproductive females (Getinet and Bart, 2007). In an effort to synchronize broodfemales in a population distribution of females within a population by age and size at first maturity as early (90 - 190 d) and late maturing (Bolivar *et al.,* 1993; Longalong and Eknath 1995) is widely used by many authors to study growth and reproduction and also to improve rate of seed production too. The author however, realized broodfemales selection method called 'selection of broodfemales by 90 d post-maturation spawning performance'. Therefore, the intention of this study was to comparatively investigate the effects of the conventional broodfemales selection methods known as broodfemales selection by size at maturity as large and small size, broodfemales selection by age at maturity as early and late maturing and broodfemales selection by 90 d post-maturation spawning performance as frequent and less frequent spawn with the aim to improve tilapia hatchery seed production.

MATERIALS AND METHODS

Descriptions of the three broodfemales selection methods

1) *Broodfemales selection by size at maturity as large and small in size*: This method is commonly used by hatchery managers presumably to improve seed production. In this study population mean weight at first spawning 48g was considered to separate the experimental fish into small (< 48 g) and large (≥ 48 g) size.

2) *Broodfemales selection by age at first maturity as early and late maturing:* This is a common method used in tilapia breeding programs. In this study broodfemales were grouped into early maturing those matured ≤ 190 d post-hatch and late maturing those matured > 190 d post-hatch (Bolivar et al., 1993; Longalong and Eknath 1995).

3) *Broodfemales selection by 90 d post-maturation spawning performance*. This is a new method developed by Getinet and Bart (2007). It assumes in Chitralada strain of Nile tilapia, *O.niloticus* frequently spawning females have successive spawning cycles greater than two within 90 d post-maturity under optimal management and environment. Therefore, in this study individual female spawning frequency ≥ 2 within 90 d post maturation was used to group females as sexually active females and spawning frequency < 2 within 90 d post-maturation as sexually less active females.

Experiment culture facility preparation and management

Five earthen ponds (200 m² each) were prepared to run the experiment. The ponds were fertilized every month with urea (17 kg hectare $^{-1}$) and triple super phosphate (1.3 kg hectare $^{-1}$) and 20% of the water was exchanged every 2 weeks over 12 month experimental period.

Experimental fish

The experimental fish used in this study were same age group (full-sibs) fingerlings of Thai-Chitralada strain of Nile tilapia, *O. niloticus* nursed using supplemental feeding, 25% protein commercial catfish pellet (30% protein, 4% fat, 12% moisture and 8% ash; Charoen Pokphand Group - CP, Thailand) at 1% body weight (BW) day^{-1} for 125 days in communal hapas in earthen ponds at the Asian Institute of Technology (AIT), Thailand. Fish were hand sexed and the females were checked for their maturity by visually examining the morphological characteristics. All experimental fish were tagged with Passive Integrated Transponder (PIT).

Experimental design and sampling procedure

Phase I: The broodfemales were first randomly grouped into four equal populations (n=300 each) and were kept separately in four hapas-in-pond of 200m^2 for 90 days. The groups were designated to serve as a source population to apply the three broodfemales selection methods: i) broodfemales selection by size at maturity as large and small size ii) broodfemales selection by age at first spawning as early and late maturing iii) broodfemales selection by 90d post-maturity spawning activity and iv) a control set. Feeding commercial catfish pellets continued at 1% BW. Weight (g) and fecundity (eggs spawn^{-1}) were monitored over 90d. Eggs were collected directly from the mouth of incubating females every five days using standard protocol described by Little *et al.,* (1993). Fish were individually weighed and all eggs removed from the mouth were counted to determine fecundity (egg spawn $^{-1}$). Weights of all experimental fish were also monitored and recorded every month. This provided breeding and growth profiles for all broodfemales over the 90d experimental period.

Phase II: Using the breeding and growth profile records over Phase I representative broodfemales (n=68) from each group were selected by applying the

Table 1. Treatments formed as the result of applying broodfemales selection methods on respective populations (n=300) of *Oreochromis niloticus*, Nile tilapia over 90d post-maturation

Broodfemales selection methods	Treatment group (n=68 each)	Selection criteria over 90d post maturity
Size at maturity	Large Size	≥ 48g
	Small Size	< 48g
Age at maturity	Early maturing	Matured ≤ 190d post-hatch
	Late maturing	Matured > 190 d post-hatch
Spawning activity 90d post-maturity	Sexually active	SF ≥ 2 within 90d post maturity
	Sexually less active	SF < 2 within 90d post maturity
Control	Population	Randomly mixed

SF= Spawning frequency

perceived methods and the control.

Table 1 Treatments formed as the result of applying broodfemales selection methods on respective populations (n=300) of *Oreochromis niloticus*, Nile tilapia over 90d post-maturation

The selected broodfemales groups were stocked in seven hapas-per-pond in four replicate ponds (200m^2 each). Each replica hapa-in-pond (2.0 x 2.5 x 1.0m) was stocked with 5 males and 17 females. Same age group males (75.1 ± 2.2 g) and same batch with the females were used. Monitoring of growth and reproduction continued following the procedure mentioned above.

Descriptions of terminologies used

Total fecundity (eggs $female^{-1}y^{-1}$), the number of eggs per female per year; relative fecundity (eggs kg $female^{-1}$), the number of eggs per kilogram female weight; spawning frequency (spawn $female^{-1}$ $year^{-1}$), number of spawns per female per year and inter-spawn-interval (ISI; d $spawn^{-1}$), the number of days elapsed from one spawn to the next were determined from the regular weekly sampling for entire 12-month experimental period.

Statistics used to analyze variations

The data on body weight, size and age at first spawning, inter-spawn-interval, fecundity and spawning frequency were analyzed by One way analysis of variance (ANOVA) and Least significant difference (LSD) test was used to determine mean differences between groups and across treatments. Correlation coefficient using "standard" correlation (Pearson's product-moment correlation) test was used to test relationships among spawning frequency, fecundity, total fecundity, relative fecundity, ISI , age and size at first spawning, growth rate and final body weight. Total fecundity was regressed with spawning frequency. All analysis were carried out using statistical package SPSS (20 version) and all values were considered significant when $P < 0.05$ and expressed as the mean ± S.E.M.

RESULTS

Broodfemales variations within a population

Analyses of the population using growth and spawning frequency parameters four distinct broodfemales phenotypic groups were observed: high fecund-large size (HL), high fecund-small size (HS), low fecund-large size (LL) and low fecund-small size (LS) with a proportion of 37, 6, 39, and 18%, respectively. Non-spawning female was not observed. Overall there was no difference in mean fecundity (416 ± 21.1 eggs $female^{-1}$) among the four distinct broodfemale groups, the three treatments and the population mean (the control) as well. There was no correlation between the eggs $spawn^{-1}$ and body weight gain (g d^{-1}; $r = - 0.107$) or/and final weight (g; $r = - 0.061$) of broodfemales. Eggs $female^{-1}$ d^{-1} was not correlated with growth rate (g d^{-1}; $r = - 0.089$, d.f. = 59, $P < 0.05$) but correlated with spawn $female^{-1}$ ($r = 0.926$, d.f. = 59, $P < 0.05$). Eggs $female^{-1}$ d^{-1} correlated negatively with age at first spawning (d; $r = - 0.304$, d.f. = 59, $P < 0.05$) and it was not also correlated with weight at first spawning (g; $r = - 0.041$, d.f. = 59, $P < 0.05$). Spawning activity of individual female observed at early maturity remained unchanged over the experimental period (Figure 1).

Broodfemales selection by size at maturity

The method broodfemales selection by size at maturity resulted in grouping the broodfemales into two considerably small (34 ± 0.84 g) and large (65 ± 3.11 g) sized females as compared to other methods. But the weight difference observed at early maturation was significantly reversed at the end of the experimental period while the initial and final weight trends within

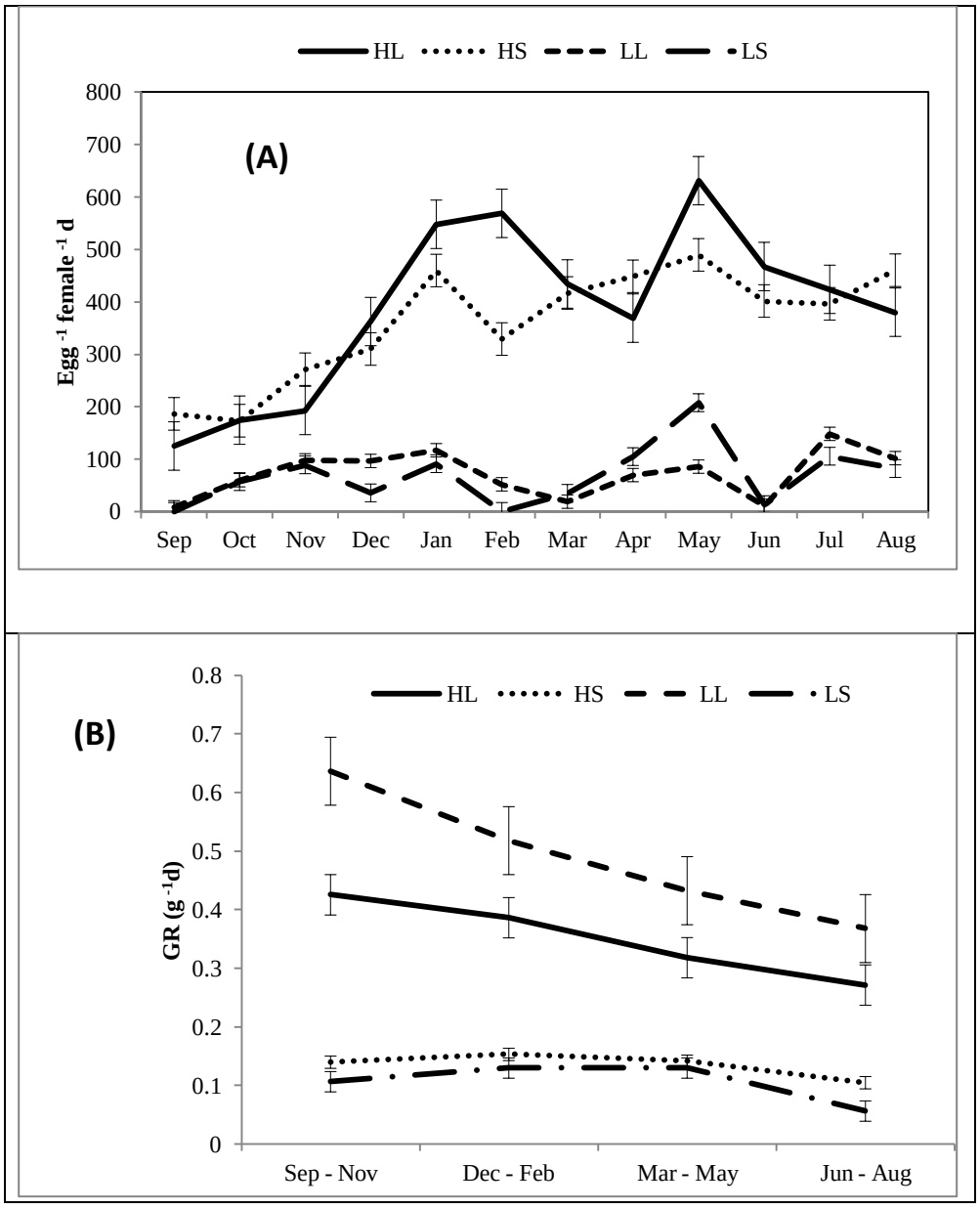

Figure 1. Trends of (A) reproduction and (B) growth of broodfemales separated at maturation (90 d age) by size and spawning activity into four groups: HL, HS, LL and LS groups in a population of Nile tilapia *O. niloticus* (n=68). Fecundity of individual female remained unchanged over 12 month experimental period while growth difference observed between high spawning frequency groups at maturation disappeared over time. Data are means ± SEM of four replications (ANOVA, LSD, $P < 0.05$).
Abbreviations: HL= High spawning frequency Large size; HS= High spawning frequency
Small size; LL= Low spawning frequency Large size and; LS= Low spawning frequency Small size.

treatment groups under the other selection methods remained unaffected (Table 2).

There was no difference in eggs spawn^{-1}, spawn female^{-1}y^{-1}, inter-spawn-interval (d) and proportion of active broodfemales between the size groups and the control but the large size broodfemales had higher eggs kg female^{-1} d^{-1} as compared to the small size (Table 2).

Selecting large size broodfemales at maturity resulted in high relative fecundity similar to the frequent spawn groups selected by 90 d post-maturity spawning performance method. The small size broodfemales group at maturity had higher growth rate per day and final body weight than the large size broodfemales at

Table 2. Effects of selecting broodfemales of Nile tilapia, *Oreochromis niloticus* at maturity by size at first spawning (g), age at first spawning (d) and 90d post-maturity spawning activity on growth, reproduction and distribution of active females reared in hapa in pond over 12 months. Data are means ± SEM of four replications (ANOVA, LSD, $P < 0.05$).

Parameters	Population Mean	By size at first maturity		By age at first spawning		By 90 d post-maturity spawning activity	
		Small size	Large size	Early mature	Late mature	Active	Less active
Age at first spawning (d)	180 ± 10.00^{bc}	168 ± 14.19^{c}	192 ± 16.12^{bc}	127 ± 3.06^{d}	235 ± 15.11^{a}	128 ± 5.60^{d}	204 ± 13.09^{ab}
Weight at first spawning (g)	49 ± 2.30^{b}	34 ± 0.84^{c}	65 ± 3.11^{a}	48 ± 3.46^{b}	50 ± 3.29^{b}	50 ± 3.66^{b}	49 ± 3.06^{b}
Weight final (g)	172 ± 12.30^{ab}	204 ± 22.95^{a}	138 ± 7.36^{b}	181 ± 18.03^{ab}	163 ± 17.22^{ab}	165 ± 18.58^{ab}	175 ± 16.14^{ab}
Eggs spawn^{-1}	416 ± 21.20	370 ± 29.69	463 ± 33.08	421 ± 27.17	410 ± 33.62	438 ± 31.82	405 ± 27.79
Eggs female^{-1} d^{-1}	6 ± 0.80^{b}	6 ± 1.11^{bc}	6 ± 1.26^{bc}	9 ± 1.23^{ab}	4 ± 0.74^{c}	12 ± 1.70^{a}	4 ± 0.55^{c}
Eggs kg female $^{-1}$ d^{-1}	62 ± 10.30^{bd}	57 ± 13.80^{cd}	100 ± 19.58^{ab}	92 ± 14.45^{abc}	63 ± 17.18^{bd}	136 ± 25.96^{a}	51 ± 9.00^{d}
Spawn female^{-1}y^{-1}	5 ± 0.50^{c}	5 ± 0.73^{bcd}	5 ± 0.76^{bcd}	7 ± 0.71^{b}	3 ± 0.55^{de}	9 ± 0.93^{a}	3 ± 0.35^{e}
ISI (d)	78 ± 7.20^{b}	75 ± 12.41^{bc}	81 ± 9.06^{ab}	54 ± 5.80^{cd}	104 ± 12.11^{a}	43 ± 6.10^{d}	95 ± 9.40^{ab}
Active females proportion (%)	42 ± 1.94^{c}	45 ± 2.53^{c}	40 ± 1.96^{c}	67 ± 1.55^{b}	19 ± 1.75^{e}	83 ± 2.02^{a}	27 ± 1.49^{d}

Population (control) mean weight at first spawning 48g was considered to group ≥ 48g females at maturity as large size and <48g as small size broodfemale.

Broodfemales were grouped into early maturing those matured ≤ 190 d post-hatch and late maturing those matured > 190 d post-hatch.

Spawning activity at early maturity was considered based on the number of spawns per individual within 90 d post-maturity, categorized as active spawn ≥ 2 spawns and less active spawn < 2 spawn;

Population (control) mean spawn 5 spawns female^{-1} year^{-1} was considered to group the broodfemales, active females those ≥ 5 spawns female^{-1}year $^{-1}$ and less active females those spawn < 5 spawn female^{-1} year^{-1}.

maturity, the other treatment groups and the control (Figure 2).

Broodfemales selection by age at first maturity as early and late maturing

The method broodfemales selection by age at first maturity as early and late maturing showed a highly variable age at first spawning that ranged from 109 to 417 d and the method separated the broodfemales into early (127 ± 3.06 day-old) and late (235 ± 15.11 day-old) maturing. This is similar to the effect of separating the broodfemales by 90 d post-maturity spawning performance (Table 2). This method also resulted in grouping the broodfemales into early maturing 39% (of which 76.9% high fecund and 26.2% low fecund) and late maturing 61% (of which 21.31% high fecund and 78.69% low fecund) broodfemales. There was no difference either in initial or final body weight (g) between the early and late maturing broodfemales. The early maturing broodfemales produced higher seeds than the others, except the frequently spawning broodfemales group separated by 90 d post maturity spawning performance.

Broodfemales selection by 90 d post-maturation spawning performance

The method broodfemales selection by 90 d post-maturation spawning performance grouped the broodfemales population into two distinctly different productive groups, frequent spawning (9±0.93 spawns female $^{-1}$ y^{-1}) and less frequent spawning (3±0.35 spawns female $^{-1}$ y^{-1}). The two groups had no difference either in initial or final body weight. This method also efficiently separated the broodfemales into early maturing (128±5.60 d) as frequent spawns and late maturing (204± 13.09 d) as less frequent spawns similar to selection of broodfemales by age at first maturity while the method broodfemales selection by size at first

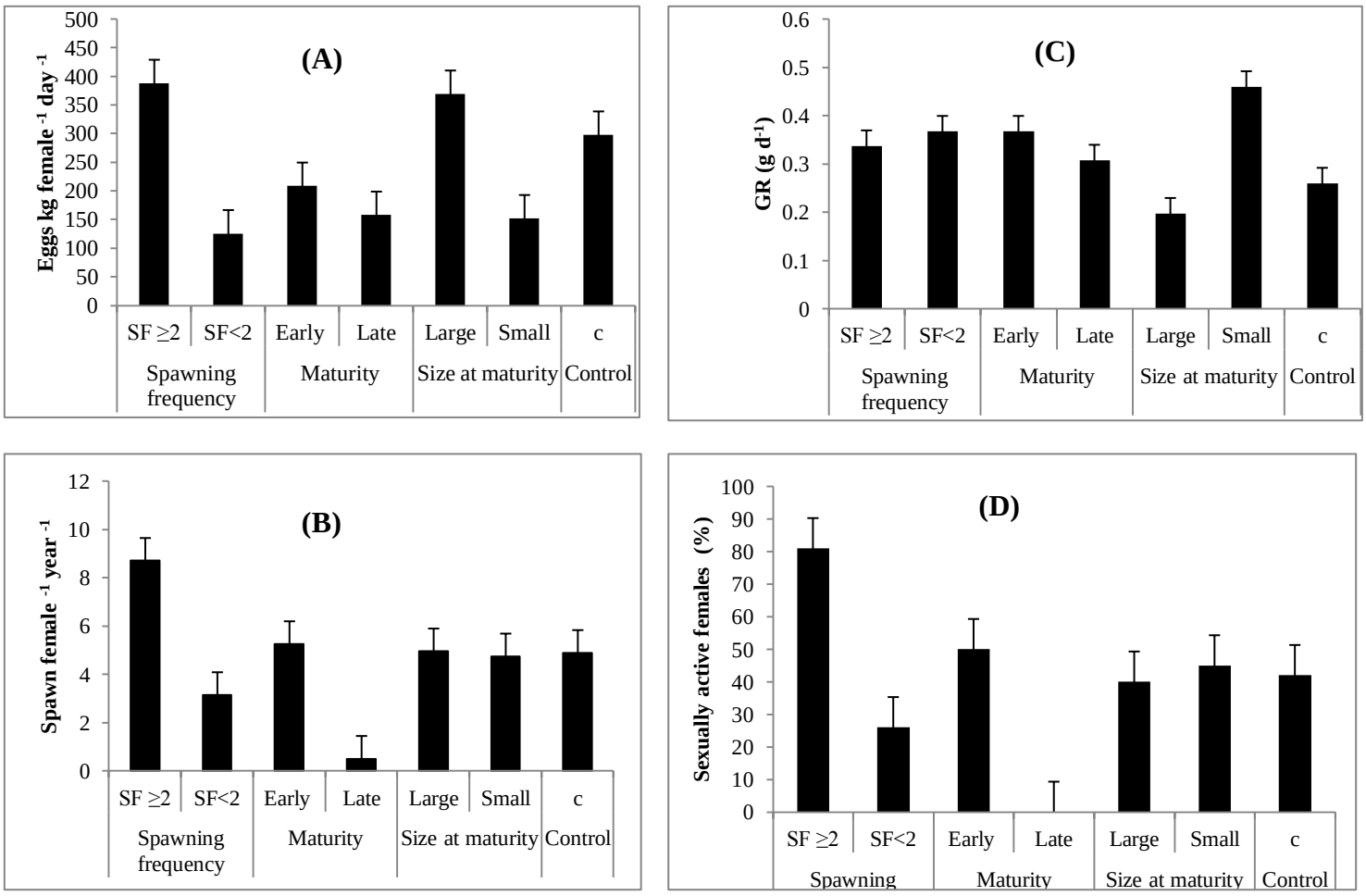

Figure 2. Effects of grouping broodfemales by spawning frequency (SF≥2 and SF<2) within 90days post-maturation, by age at maturation (early and late) and by size at first spawning (large and small) on growth and reproduction of Nile tilapia *O. niloticus* (n = 68) reared in hapa in pond system for 12 months. Effects of grouping on: (A) relative fecundity (eggs kg female^{-1} d^{-1}), (B) spawning frequency (spawn female^{-1} year^{-1}, (C) growth rate (g d^{-1}) and (D) proportion of sexually active females within group (%) after 12 months experimental period. Data are means ± SEM of four replications (ANOVA, LSD, *P < 0.05*).

maturity failed to separate the population into two age at maturity groups (Table 2). But broodfemales selection by 90 d post-maturity spawning performance improved seed yield (12.02 eggs female^{-1} d^{-1}) by 52 % over the population mean. Frequent spawn broodfemales had more eggs female^{-1} (67%), spawns female^{-1} y^{-1}(67%) and a reduced ISI (55%) than less frequent spawn broodfemales. Twenty four percent of the less frequent spawn broodfemales were active broodfemales while 17 % of the frequent spawn broodfemales were less frequent spawns. The method broodfemales selection by 90 d post-maturity spawning performance resulted in similar age (d), initial and final body weight (g), eggs female^{-1}d^{-1} and ISI (d) to broodfemales selection method by age at first spawning as early and late maturing. However, spawns female^{-1} y^{-1}and proportion of active females in the broodfemales group selected by 90 d post-maturity spawning performance were higher by 22 and 20% than those grouped by size at maturity and age at first spawning, respectively. Moreover, the method broodfemales selection by 90 d post-maturity spawning performance increased the proportion of active broodfemales to 83 ± 2.02%, higher by 42, 20 and 50% than the broodfemales selection methods by size, age at maturity and the control, respectively.

DISCUSSION

Broodfemales variations within a population

Using factor of spawning frequency combined with weight gain as indicator of reproduction performance

four distinct broodfemales stocks were realized within a population of same age broodfemales of Nile tilapia, *O. niloticus*: high fecund-large size, high fecund-small size, low fecund-large size and low fecund-small size. The observed classes of broodfemales within a population of *O. niloticus* could be justified by previous reports that reported distinct broodfemales within tilapia population. Accordingly highly fecund broodfemales but small in size (Ens *et al.,* 1995) and highly fecund broodfemales but large in size were reported (Mair and Little 1991; Macintosh and Little 1995). Smaller females were found to delay maturation (Eyeson 1983) and the existence of low fecund-large size females was also reported (Getinet and Bart 2006). Non-fecund broodfemale was not observed in this study over 12 moth experimental period, however, Bolivar *et al* (1993) reported 57 % non-fecund females in a long term (210 d) experiment.

The proportion of spawning females in a population varies between species and starains. For example, proportion of spawning females was 22.5-39.5% for Philippine red tilapia and 25.5% for Florida red tilapia (Eguia 1996; Smith *et al.,* 1991). However, this study noted 100% spawning females of which 42% frequent spawn (≥ 6 spawn year^{-1}) in the population of Nile tilapia, *O. niloticus* (Chitralada strain) suggesting high productivity. This study suggested that broodfemale could be either frequent spawn, less frequent spawn or non-spawning regardless of its size. However, this finding contradicted with earlier findings that reported tilapia mature either early and small, or late and large; and different sizes at maturity are associated with differences in fecundity and brood frequency, with small maturing individuals being relatively more fecund and breeding more frequently than large maturing ones (Iles 1973; Duponchelle et al., 1998).

Broodfemales selection by size at early maturation

Overall the method broodfemales selection by size at early maturation didn't improve age at first spawning, eggs female^{-1} d^{-1}, spawn female^{-1}y^{-1}, ISI (d) and proportion of active females when compared with the population mean and the other methods. This observation agrees with collimation method, i.e. removal of larger fry and smaller fry at 21 d to generate a population that has an increased ratio of genetic to phenotypic variation (Doyle and Talbot 1986). Non-genetic or environmentally induced size variation is a particular problem for pond produced tilapia fry (generally as a result of asynchronous spawning) making selection based on large phenotypic size often a poor indicator of breeding value. Huang and Liao (1990) also failed to obtain a significant response to mass selection for weight in an*O. niloticus* strain imported from Japan in 1966. This study found no difference in eggs spawn^{-1} and spawn female^{-1} between small and large sized broodfemale groups, Cisse (1988) also found no significant correlations between the weight and the number of eggs per spawn for *Sarotheradon melanotheron* (Ruppel). On the other hand, many studies reported larger females produce more eggs spawn^{-1} than smaller females (Peters 1983; Macintosh et al.1985). In this study the observed significant difference between the small and large size groups at maturity was found reversed by the end of over 12 month experimental period. This is in agreement with initial size didn't seem to affect later growth performance (Eknath, Reyes, Boliver, Palada-de Vera, Danting, Dionisio and Longalong 1995).

Broodfemales selection by age at first maturity as early and late maturing

This method failed to effectively separate the broodfemales into two distinctly productive and unproductive groups. As the result outputs of this broodfemales selection method could be biased due to huge variations in the distribution of broodfemales by age and size within a population of different species and strains of tilapia. For example it was reported 28% of broodfemales matured within 60 d and a further 15% between 61 and 210 d, while 57% had not matured by the end of the experiment at 210 d (Bolivar *et al.,* 1993). There is evidence of considerable within-population variation in age and size at maturity. This is obvious from maturity-at-age or at-length curves derived for natural populations (e.g. De Silva, 1986; Duponchelle et al., 1998). The basis of this variation has not been evaluated: possibilities include genetic, as well as non-genetic factors such as behavioral interactions (Lorenzen, 2000).

Broodfemales selection by 90 d post-maturity spawning performance

The period, 90 d post-maturation spawning performance recommended in this method is specific to Chitralada strain of Nile tilapia, *O. niloticus*. Therefore, the post-maturation period could be elongated or shortened depending on the productivity of a strain or species considered for the purpose and it is also subject to culture management and environmental conditions (Eguia 1996; Smith *et al.,* 1991; Getinet and Bart 2006). The method broodfemales selection by 90 d post-maturity spawning performance resulted in similar age at first spawning (d), initial and final body weight (g), eggs spawn $^{-1}$, eggs kg female $^{-1}$ d^{-1} and ISI (d) to the method broodfemales selection by age at first spawning as early and late maturing. However, the method broodfemales selection by 90 d post-maturity spawning performance had advantage over the others by effectively separating the broodfemales into productive and unproductive groups and also by increased eggs female^{-1}d^{-1} and

spawn female^{-1} y^{-1}. Therefore, selecting broodfemales by 90 d post-maturity spawning performance allowed maintaining 83 ± 2.02% active broodfemales in the population, higher by 42, 20 and 50% than selecting broodfemales by size, age at first spawning and the control, respectively.

CONCLUSION

Early maturation and frequent spawning are management challenges when working to grow tilapia; however it is a huge opportunity in tilapia seed production to exploit the situation to supply enough fry to the rapidly growing tilapia industry. In this regard the method, broodfemales selection by 90 d post-maturity spawning performance improved by 97.6 % the proportion of active broodfemales in breeder's population. Commercial Nile tilapia, *O. niloticus* seed production industries would benefit directly from this method while application for selection program need progeny evaluation of the broodfemale groups.

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