COMPARISONS OF REPRODUCTIVE PARAMETERS AMONG IMPROVED STRAINS OF NILE TILAPIA *Oreochromis niloticus* L.

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Abstract

The Nile tilapia *O. niloticus* is presently the dominant species in freshwater tilapia culture. Recent research in tilapia genetics has resulted in the development of superior genotypes for aquaculture with fast growth and high survival. Most of this work has been centered in Asia where nearly 80% of global tilapia production originates. Our study compared the growth, survival, sexual maturation and various reproductive parameters in four tilapia strains, three of which have been improved through various selective breeding approaches (GIFT, IDRC and Fishgen-selected) and a local stock (Chitralada) was included as a non-improved control. This presentation focuses on maturation and reproductive parameters including total and relative fecundity, inter-spawn intervals (ISI) and spawning frequency (SF). The four strains were originally on-grown in extensive culture systems with fertilisation only. Growth (weight and length) and reproductive parameters (gonadosomatic index, hepatosomatic index, and stages of sexual maturation) were measured on fish sampled every 21 days. Based on staging of gonad development, GIFT were found to become sexually mature marginally later than the other two strains. At 9 months of age, broodstock from each strain were stocked in 5m² breeding hapas with 5 males and 15 females per hapa and four replicate hapas per strain. Broodstock were sampled for eggs every week and data on fecundity and inter-spawning interval for the four strains over the 17 months of reproduction are reported. Seasonal and environmental variances appear to be the major determinants of egg/fry production with the only strain difference observed being a lower relative fecundity in GIFT. Across all strains, fecundity per female increased over time whilst fecundity per unit weight of female remained constant. SF and ISIs fluctuated widely between individual fish and ISIs were even highly variably within individual females making it very difficult to identify trends. Many females spawned very infrequently and means of identifying fecund females could have huge impacts upon hatchery efficiency. The results will be discussed in the context of the respective uptake of these strains by growers and hatchery operators.

Introduction

Genetic improvement of cultured fish remains in its infancy relative to other agricultural sectors. Significant progress has been made in the last decade including the implementation of breeding programmes in species used extensively in aquaculture in the developing world such as the tilapias and carps. There have in particular been a number of breeding programmes in tilapia, the best known of which is probably the Genetically Improved Farmed Tilapia (GIFT) project initiated by ICLARM (Eknath and Acosta, 1998) but there have also been other programmes (see below). One of the particular problems in tilapia aquaculture is that the fish is very easy to breed and sexual maturation and unwanted reproduction in mixed sex fish within the culture systems can have considerable negative impacts upon productivity. This has promoted the widespread use of monosex fish in the industry but also raises a consciousness about sexual maturation and breeding not considered in the culture of most other species. Late maturation and to a lesser extent, reduced fecundity, are thus considered desirable traits by growers. These are however undesirable traits for hatchery producers. Given that tilapia is a low value species, profit margins on seed production can be relatively small. The extra cost of maintaining broodstock for a longer time prior to initiating seed production and thus maintaining larger fish as broodstock with higher resource requirements (feed and space) and greater difficulty in handling would dissuade many hatcheries from using a late maturing strain unless the growth benefits and thus selling price of the seed warrants it. This appears to be a reality in the case of application of the GIFT tilapia in hatcheries in Thailand where anecdotal information from hatchery managers have indicated that this strain is indeed later maturing than alternative unimproved strains available in Thailand (Turner, pers. comm.). This has resulted in some hatcheries using only GIFT males as broodstock, crossing those to earlier maturing females from a local strain, thus losing some of the potential benefit of the breeding programme which has produced the GIFT. The GIFT available in Thailand has not, to the author's knowledge, been deliberately selected for later maturation and if the later maturation and lower fecundity of this strain was confirmed, these might be considered correlated traits on the growth trait that is selected, which would be considered positively by the target clientele, the growers but negatively by the hatcheries who carry the responsibility of supplying quality seed to the growers.

Uraiwan (1988) confirmed that a genetic relationship exists between growth rate, age and size at maturity in tilapia. This observation led to a suggestion that selection can be made more efficient by combining selection for body weight at a particular age with selection for increasing fish growth. It was further suggested that environment and genotype x environment interactions were important factors that must be considered in selection for growth and maturation. Similar to growth variation, most of the variation in age and size at maturation was found to be due to environment and genotype x environment interaction. High environmental effects were found to be responsible for low estimated heritability for these traits and inconsistent genetic gains.

Falconer (1990) and Lynch and Walsh (1988) indicated that most phenotypic characters are correlated and that an improvement in one may result in positive or negative impact on other traits. However, Hörstgen-Schwark and Langhölz (1998) argued that the question of whether the selection response for growth rate indicates a beginning of an antagonistic relationship between maturation and body weight still remains unanswered. Longalong *et al.* (1999) threw some light on this topic in their study on realised heritability for selection for later maturation in the GIFT strain. They observed a highly significant response to selection for early maturation indicating this as a trait with moderate to high heritability. They also observed, however, a correlated response in growth rate with males and females in the early maturing line growing faster than those in the later maturing line, significantly so in the case of the males. This indicates that the inclusion of late sexual maturation in a selection index within a tilapia breeding programme, as suggested by Uraiwan (1988), might reduce the response to selection for growth traits. If this correlation applies in the opposite direction it might also be the case that selection for faster growth may result in a correlated response for earlier sexual maturation. This would be contrary to the anecdotal findings of the Thai hatcheries and counter to the established hypothesis in tilapia that energy expenditure on gamete production and spawning has a negative impact upon growth rate. Clearly this is an issue that warrants further research.

Materials and methods

The overall objective of this study was to compare the initial growth, maturation and reproductive performance of three selectively improved Nile tilapia strains (GIFT, Fishgen-selected and Philippine-Selected) and one locally adapted strain (Chitralada) in low input pond systems in Thailand. The specific objectives were:

1. To compare the growth rates of strains during grow out in a fertilized pond systems (with separate and communal stocking)
2. To evaluate traits of sexual maturation during initial grow-out
3. To evaluate initial reproductive parameters of broodstock following maturation
4. To carry out long term evaluation of comparative reproductive performance of the four strains

Source of broodstock

GIFT: This stock originates from the Genetically Improved Farmed Tilapia project in the Philippines and was obtained from the Thai National Aquaculture Genetics Research Institute in 2002. This strain is the result of five generations of combined selection for growth on a genetically variable synthetic base population derived from eight separate strain accessions. The GIFT selection programme is reported to have resulted in an accumulated genetic gain of 85% in relation to the base population (Eknath and Acosta, 1998). The GIFT tilapia is established as a good strain for aquaculture and has been widely distributed throughout Asia.

Fishgen-selected: This stock was obtained directly from the Philippines in 2000 and is a strain developed as a female line for crossing with YY males to produce superior genetically male tilapia Mair *et al.*, 1997, Abucay and Mair, this issue). This strain was the high line in a breeding programme incorporating three generations of intensive within-family divergent selection for growth with rotational mating. This breeding programme combined five distinct accessions of the pure species which had already been established to have superior growth rates relative to other strains of the species (Capili, 1995). Estimates of divergence in growth performance between the high line and low line ranged from 32% to 102% with an average of 60%, equivalent to an estimated response to selection of 30% assuming equal response in high and low lines (Abucay and Mair, in press). This strain is not commercially cultured in its own right but is used in several Asian countries as the female line in the production of GMT.

Philippine-selected: This stock was also obtained from the Philippines in 2000 and represents the product of 12 generations of within-family selection for 16-week growth (Bolivar and Newkirk, 2000). The stock, known locally as the IDRC strain after the funding agency that supported the initial research, is derived from a base population of locally adapted strains of tilapia available in the Philippines in the late 1980s. Performance evaluations showed that the strain had consistently higher final body weight in tanks, hapas and ponds than two variants of control lines (random-bred control and mean selected control) and also than unrelated controls including the GIFT strain and a local 'Israel' strain. The reported response to selection was about 3.6 % per generation. There were also no significant differences ($P<0.05$) reported between the final body weight of IDRC and GIFT strains and more recent unpublished data (various sources) has also indicated that the strain has culture performance broadly comparable with GIFT. Subsequent generations of this strain has been distributed for aquaculture, mainly within the Philippines, where it is now known as the FaST strain.

Chitralada: This strain was included to represent a non-selected, but locally adapted control. The Thai-Chitralada strain is derived from a stock of 50 fish which was introduced to the Royal Chitralada Palace in Thailand in 1965 and originates from Egypt via Japan. This introduction formed the base population for the large majority of tilapias cultured in Thailand up until the early 2000s. This strain has been reproduced intensively at the Asian Institute of Technology (AIT) for at least 10 generations in hapa based seed production systems (Tuan *et al.,* 1999). The strain, though not deliberately selected, has been found to have culture performance at AIT comparable with that of selected strains. For example, in studies conducted in the late 1990s, no significant differences were found in grown and reproductive traits between Chitralada and GIFT traits (Bhujel, 2000; Yakupitiyage, 1998).

The grow-out phase

Broodstock of Chitralada, Fishgen-selected and Philippine-selected (IDRC) were bred at AIT using standard methods of pooled spawning of broodstock in fine-mesh hapas (Bhujel, 2000) with a minimum of 12 spawning females providing the 4000 fry representative of each strain. The GIFT fry of the same age (maximum age difference across all fish was two weeks) were obtained from NAGRI. Fry from the different strains were nursed through three stages of nursing at standardised densities, in hapas suspended either in concrete tanks or an earthen pond, with densities reducing through each phase.

After a total of four or six of nursing, fry were stocked, at average weights of 5-6 g, into two growth comparison trials under extensive conditions in fertilization only ponds. Emphasis in this trial was not on optimum growth given that the fish were intended for use as broodstock.

In a separate stocking trial, fish were stocked at 3 per m² in 3 replicate half ponds (100 m²) making 75 fish per strain per replicate (totaling six ponds, each divided into two with a fine mesh screen). Fish were grown out for a period of 91 days with 30 fish being sampled for weight every 21 days. In the later samplings 20 fish per half pond were removed for determination of GSI and stage of sexual maturation, thereby reducing the overall stocking density of the ponds. All fish were weighed, measured and sexed at harvest.

In the second trial in a communally stocked pond, randomly selected 6 week old fish at mean weights of 15-22 g, representative of each strain were marked with combinations of fin clipped and coded wire tags (Mair, 2002) and stocked together in a single 200 m² earthen pond (600 fish with 150 per strain). Sampling was as for the separately stocked trials and all fish were harvested after 85 days of grow-out.

In both trials fish were not fed throughout but ponds were fertilized with urea at a rate of 28 kg N/ha/week and triple super phosphate at a rate of 7 kg P/ha/week to stimulate primary productivity.

Assessment of sexual maturation

In fish sacrificed for assessment of sexual maturation, gonads were weighed for estimation of gonadosomal indices and the stage of sexual development of the gonads was recorded based on criteria developed by Hörstgen-Schwark and Langhölz (1998) as shown in Table 1.

Table 1. Criteria used in determining stages of sexual maturity in tilapia by examination under a dissecting microscope.

Female Maturity Stage	**Appearance of ovaries**
1. Immature/inactive	No egg visible
2. Inactive-active	< 20 eggs visible, size < 0.2 mm
3. Active	> 20 eggs visible, size < 0.2 mm
4. Active - ripe	Eggs yellow, size 0.2 – 1.1 mm
5. Ripe-ripe running	Eggs yellow, size > 1.1 mm
6. Spent	Absorption of yolk material, egg white
Male Maturity Stage	**Appearance of testis**
1. Immature	Thread-like, colorless
2. Inactive	Translucent, wider than above
3. Inactive	Flesh color, still thin
4. Inactive - active	White/yellowish, thickened, no milt apparent when cut
5. Active - ripe	Cream colored, thick and enlarged
6. Ripe	Distended fully over visceral cavity, milt evident if testis cut
7. Ripe - running	White/silvery, milt runs freely under pressure

Comparative evaluation of breeding

Fish from the grow out phases were pooled and maintained in hapas on a maintenance diet of commercial catfish grower pellets up to the age of 180 days at which time they were stocked into breeding hapas for evaluation of breeding. Fish were stocked in 5 m² hapas with 20 fish (5 males: 15 females) per hapa with four replicate hapas per strain. All 16 hapas were placed in the same 200 m² earthen pond.

Eggs were collected from each hapa every seven days according to standard protocols developed at AIT (reviewed by Bhujel, 2000). Each female was tagged with a PIT tag at first spawning and all tag numbers of spawned females were recorded in subsequent collections along with the post-spawning weight, providing full breeding profiles for all spawning females in each hapa. Eggs collected were staged according to their development, weighed and counted using volumetric estimation. Evaluation of reproduction in the strains is a continuous process and is on-going at the time of writing. The data reported here is a preliminary presentation of data collected over 70 weeks representing the production from fish in the age range of 6 to 22 months. A problem arose with collection of the data that whilst most females were tagged (only those that never spawned did not receive a tag) the total number of males and females in a hapa was not counted during the early part of the experiment. Also some mortality occurred in broodstock over time which affected the number of breeders remaining in the breeding hapa. These broodstock were replaced after 9 months of production, using spare stock that had been retained after harvest at the end of the growth phase and maintained in similar condition to the breeding fish. As a result of the omission of total counts of broodfish it was difficult compare overall productivity of the strains other than by preparing total counts factoring in both fecundity and survival parameters. Variables that could be calculated or estimated from the data included:

- Absolute fecundity – the number of eggs per spawning per female
- Relative fecundity – the number of eggs per unit weight of female
- Absolute spawning frequency – number of spawnings of the 70 week period
- Spawning frequency – number of spawning per unit time
- Inter-spawn interval (ISI) – the average interval between spawning (either over fixed periods e.g. 10 weeks, or from the total period from the first to the last spawning of any individual fish)

Data analysis

Growth data from the separately stocked trials was affected by different pond means and sampling bias, for which correction factors were applied (Jere, 2002). Raw and corrected data were compared using ANOVA. Trends in breeding data were subject to preliminary analysis through basic linear correlations and ANOVA for comparisons of parameters between strains.

Results

The grow out phase

There have been a number of studies in recent years on the relative culture performance of various strains of tilapia with varying degrees of scientific rigour and varying results. The growth comparison component of this study is thus not emphasised in this paper and will be dealt with in more detail in another publication. In summary, the analysis of data from the growth trial which were analysed in theirs raw form and also corrected for pond means and sample bias (from seining) indicated no significant differences between growth of strains in the separate stocking trials despite the fact that the three selected strains were all ranked with higher mean harvest weights than the unimproved Chitralada. Growth curves for the strains in separate stocking are illustrated in Figure 1. There were also no significant differences in survival or sex ratio between strains.

Similar rankings and growth trends were seen in the comparison of strains under communal stocking but this time growth rates were highly significant in analyses with individual fish as replicates. Fishgen-selected had significantly higher ($P<0.05$) harvest weight than IDRC and Chitralada whilst GIFT had significantly higher harvest weight than Chitralada only. There were no significant differences in sex ratio or survival between the strains.

Assessment of sexual maturation

The results of the staging of sexual maturation of males and females of the four strains at harvest in the separate and communally stocked trials are shown in Figures 2 and 3, respectively.

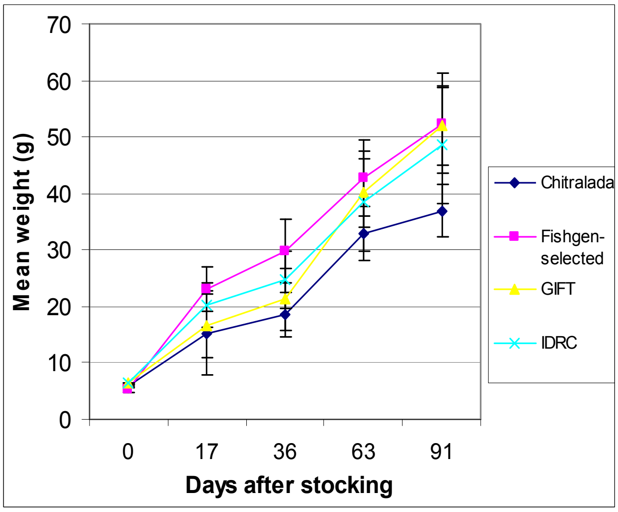

Figure 1. Growth curves of the four tilapia strains in separate stocking after 91 days for grow out.

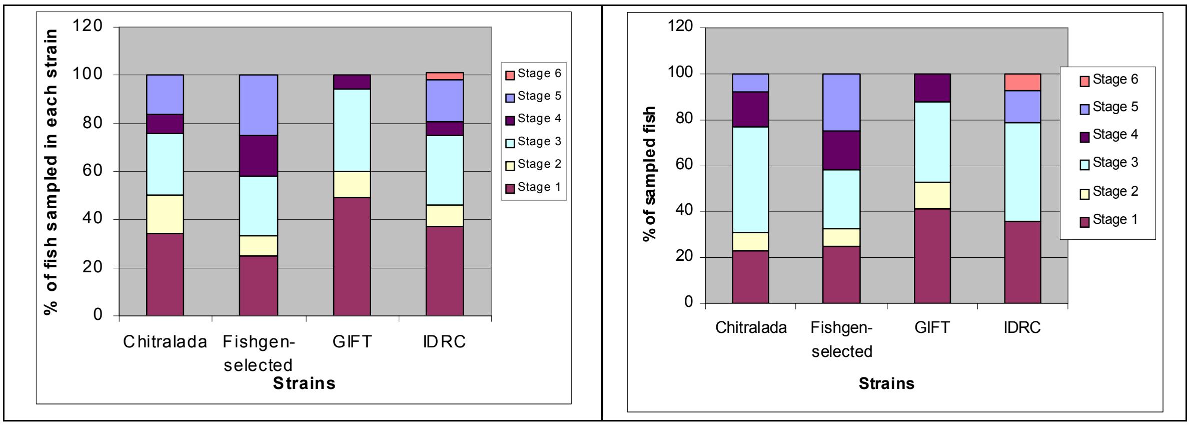

Figure 2. Stages of sexual maturation of males at harvest of fish from separately stocked (left – aged 119 days) and communally stocked (right – aged 127 days) growth comparisons.

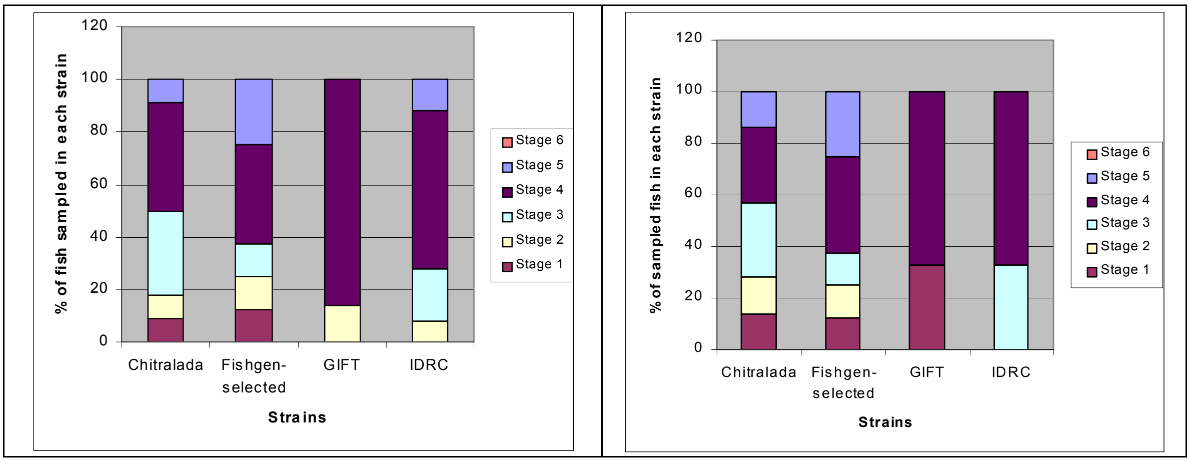

Figure 3. Stages of sexual maturation of females at harvest of fish from separately stocked (left – aged 119 days) and communally stocked (right – aged 127 days) growth comparisons.

There were no significant differences in the frequency of the different stages of sexual maturation between the strains although an absence of any Stage 5 males or females was noted in GIFT. In the fish reared in separate stocking there were significant differences in GSI in males (Table 2) with GIFT having lower GSI than Fishgen-selected and IDRC. There were no significant differences in GSI among the females in separate stocking. The GSI data from communally stocked fish did not contain sufficient numbers of individuals to warrant analysis.

Table 2. GSI (%) for the four strain reared in separate stocking for 91 days (results from ANOVA were based on square root transformed data) with fish aged 119 days (n=30 per strain).

Strain	Male GSI (%)	Female GSI (%)
Chitralada	0.2618[a,b] ± 0.1462	1.2711 ± 0.1685
Fishgen-selected	0.5801[a] ± 0.0043	0.9079± 0.0207
GIFT	0.1334[b]± 0.0688	1.5736± 0.8478
IDRC	0.4358[a]± 0.1530	1.8965± 0.0497

Comparative evaluation of breeding

Due to the failure to count the total number of fish in the breeding systems during the early part of the monitoring it was not possible to compare the overall productivity of the different strains. Comparisons between strains were made of total production (across all replicate hapas) in the eight week periods immediately after initial stocking (at 180 days old) and after restocking (at 450 days old) which were valid as it was known that insignificant mortality occurred during these periods and thus the number of broodstock were known. In the first of these periods the IDRC strain had a significantly ($P<0.05$) higher mean weekly egg production (7,903) compared to Fishgen-selected (1,399) and Chitralada (1,440) with the production from GIFT being intermediate at 4,821. A similar trend was observed in the second period, although production was much higher as the fish were older and larger. During this period IDRC had significantly ($P<0.05$) higher average total production (18,830) than all the other strains and GIFT (11,615) also had higher production than Fishgen-selected (4,069) and Chitralada (3,145).

Looking at fry production throughout the 70 week period it was evident that there was a very high degree of variability in production across the season but that the temporal variation in production seemed to be broadly common to all the strains (as illustrated by the trends in relative fecundity over the first 12 month period of production shown in Figure 4).

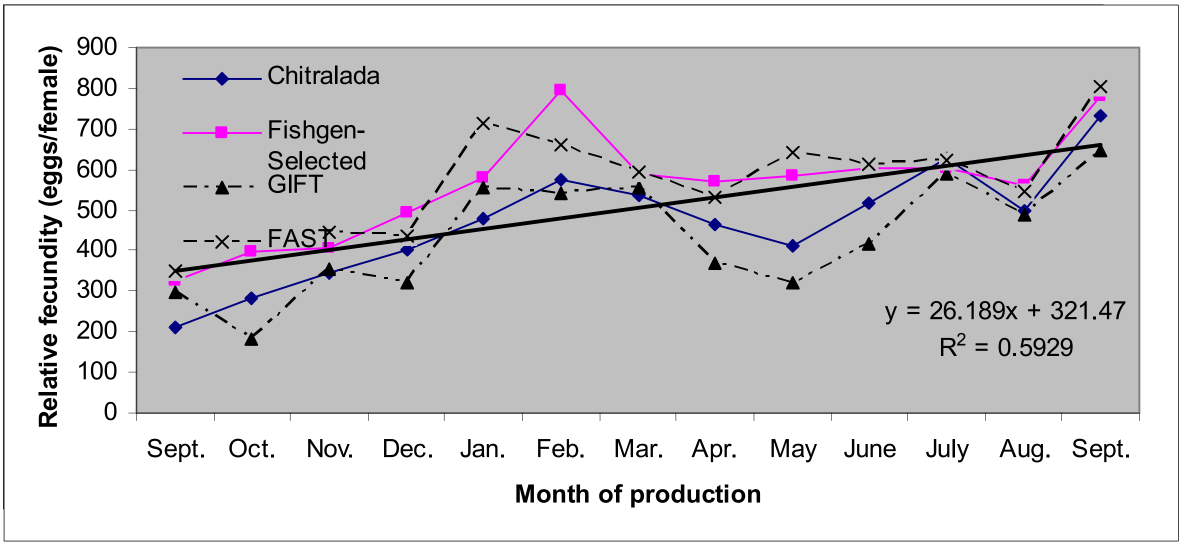

Figure 4. A summary of the relative fecundity of spawning females across the four strains during the first year of the trial.

Examination of a summary of the fecundity data over the entire 70 week period does reveal some differences between the strains (Table 3). The average number of spawnings per month and the mean egg/fry production per month are both variables which depend on the fecundity of individual females and the number of surviving females and thus no meaningful comparison can be made without more detailed information on mortality of broodstock. The data on absolute and relative fecundity, generated from spawning females, can however be directly compared. These data revealed that the GIFT strain had significantly lower absolute and relative fecundity than the other strains. IDRC had the highest ranked absolute fecundity with the locally adapted Chitralada strain having the highest ranked relative fecundity.

Table 3. Comparison of overall production of eggs, by the four strains, over the 70 week evaluation period.

	No of spawnings per month	Mean egg/fry no per month	Absolute fecundity (eggs/fry per female)	Relative fecundity (eggs/fry per g female)
Chitralada	24.38	5014	468[a]	5.957[a]
Fishgen-selected	19.23	6830	560[a]	5.424[b,c]
GIFT	20.77	6898	433[b]	5.225[c]
IDRC	18.46	6865	579[a]	5.927[a,b]

Investigation of spawning frequency over the 70 week period revealed no significant differences between the strains with average spawning frequencies ranging between 5 and 7 (Figure 5).

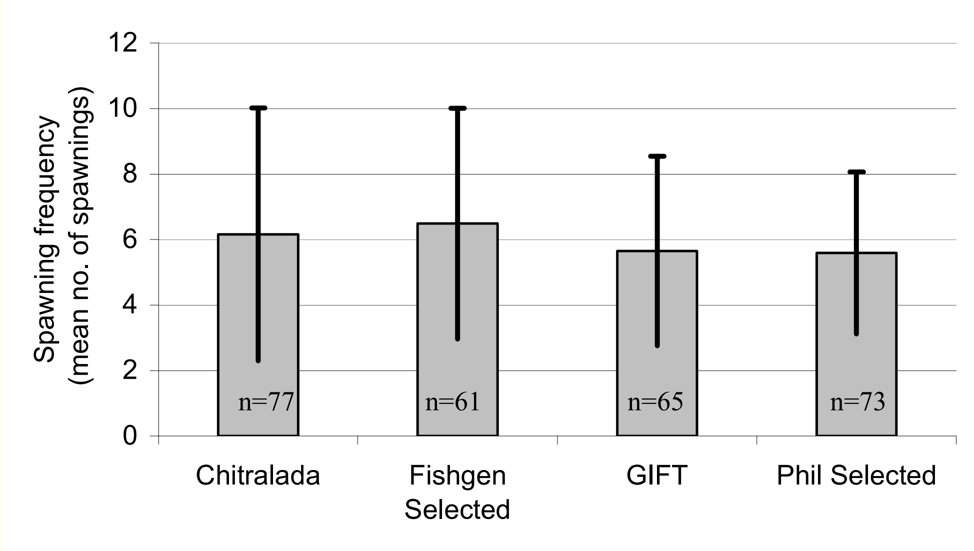

Figure 5. Comparison of mean spawning frequency of spawning females in the four strains over the 70 week evaluation period.

Investigation of temporal trends in fecundity across all strain combined, revealed that absolute fecundity is increasing with age of the fish whilst relative fecundity appears to remain fairly constant although neither correlation is significant (Figure 6).

A similar lack of correlation was observed between relative fecundity and the post-spawn weight of females (Figure 7).

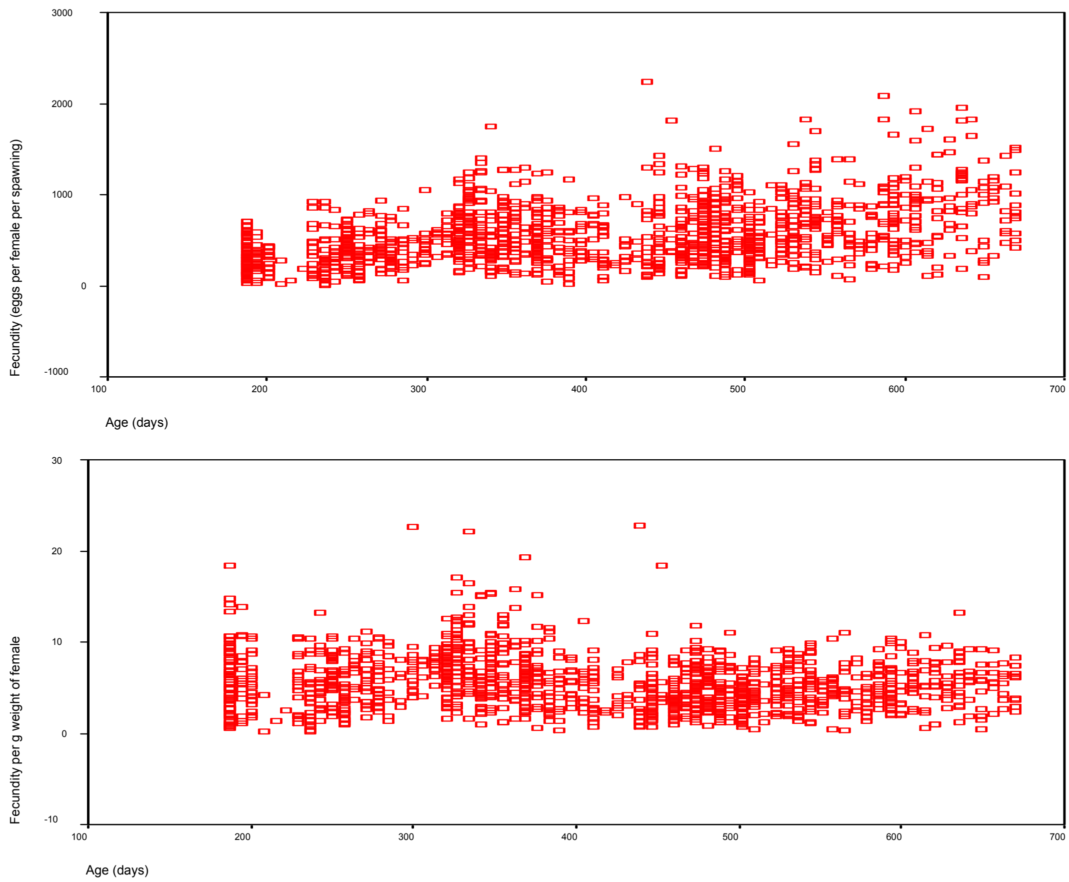

Figure 6. Relationship of absolute (upper) and relative (lower) fecundity with age (in days from 180 to 675) in all strains combined.

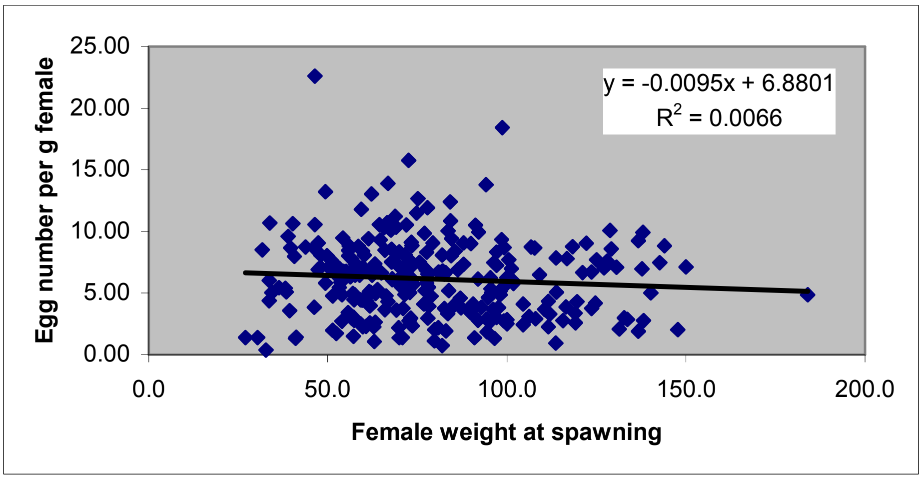

Figure 7. Plot of post-spawn weight of females and relative fecundity indicating the lack of any correlation.

Discussion and conclusions

These results throw some light on the debate over the relationship between growth and fecundity in tilapia and the impact of selection for growth on this relationship, although some of the findings appear ambiguous. The growth trials, although not a major element of this study, appear to confirm the superior growth rates of the growth selected strain over the non-selected Chitralada although these differences were not significant in the replicated trial. The development of gonads was characterized in the strains at the end of the grow-out phase when the fish were at an age when around 50% of the fish might be expected to be sexually mature (Kronert *et al.,* 1987) despite their relatively small size. The absence of the most mature males and females in GIFT provided some indication that this strain is later maturing than the other strains. The differences were not extreme and were not in accord with the GSI data from females in which it might have been expected (in none spawning fish) that a later maturing strain would have had an overall lower GSI. The timing of sexual maturation and the verification of anecdotal information from hatcheries regarding the late maturation of GIFT females, clearly requires more substantive examination.

The data set developing from the long term comparative evaluation of breeding is perhaps unique in that it is tracing the spawning patterns of individual fish and may enable us trace the fecundity parameters of tilapia broodstock (focusing on females) throughout their productive life and help determine the optimum ages for using broodstock. It is clear that there is huge variability in the spawning patterns between individual females and over time. It appears probable that environmental factors and variation between individual fish are more important factors in hatchery productivity than is strain. However, there were some significant differences in fecundity between strains with GIFT having overall lower fecundity that the other strains supporting the anecdotal evidence supplied by hatcheries. However, the data from total egg/fry production when the fish were first stocked at 180 days and again at 450 days old showed higher total fecundity from the IDRC and GIFT strains, appears to contradict this observation. Initial spawning patterns after stocking were atypical to the continuous production as broodstock females would respond to being placed in a 'breeding environment' (previously they would have been maintained with sexes separate) with a large number of females spawning in the first week or two followed by a large drop off in spawning activity. It is thus probable that the long term fecundity data provides a more reliable assessment of comparative fecundity of the strains.

The age fecundity relationships are in line with expectations with regard to absolute fecundity although, based on findings with other species, admittedly over larger age differences, relative fecundity might have been expected to decline with age (Siraj *et al.,* 1983, Ridha and Cruz, 1989). The lack of any relationship between female size (weight) and relative fecundity is also perhaps surprising given the findings of other authors (Srisakultiew, 1993).

Overall, the results from this study do not fully resolve the ambiguity concerning the relationship between size, age and growth rate in tilapia and the presence or absence of a correlated response in sexual maturation in growth selection programmes. The IDRC strain, selected for growth in a within family selection programme would appear, if anything, to have a positively correlated response in fecundity parameters whereas the response in GIFT would appear to be more of a negative correlation. It is thus difficult to advise hatcheries with regard to their expectations of fecundity in growth-selected strains.

One point of far greater importance to hatcheries perhaps, is the huge variability in the frequency of spawning of individual females (within or between strains). Many individual females had not spawned at all or had spawned only once in 70 weeks whilst others have spawned up to 17 times during this period. Clearly some method for identifying and separating out the high and low spawning frequency fish could have a dramatic impact upon hatchery efficiency and profitability far in excess of any fecundity differences that might exist between different strains.

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