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Mixed-sex Nile tilapia (*Oreochromis niloticus*) can perform competitively with mono-sex stocks in cage production

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Abstract

All-male tilapia stocks are widely used by farmers to supply both domestic and international markets with homogenous, large sized fish (500g+). While a number of strategies are possible, hormonal treatment of fry with 17α-methyltestosterone (MT) is the most common method used by commercial farmers due to its low cost and ease of application. However, contrasting to its current widespread use the implications of MT in tilapia farming have raised concerns especially from public and environmental perspectives. Therefore, in this study we tested the impact of stocking a mixed-sex fast growing strain of Nile tilapia (*Oreochromis niloticus*) fry at high density and then grading out females at 4 or 8 weeks intervals during grow-out and compared final production with a standard MT-treated mono-sex system. From a production perspective, the strategies to remove females at 4 or 8 weeks were successful as no differences in harvest weight, survival and feed conversion rate were observed when compared to the MT-treated group. Similarly, no differences at harvest were obtained in terms of external appearance, Fulton's condition factor, gonadosomatic index, fillet yield, fat-somatic index and visceral-somatic index (%) between MT-monosex group and the groups where females were removed (4 or 8 weeks). However, a financial analysis of this approach showed that the additional costs (fry, feed and labour) involved in the mixed-sex strategy resulted in lower profits. This could be mitigated if a proportion of the removed females could be sold at a premium price as potential broodstock. In the model presented, sales of 13% of the removed females for broodfish at current Thai prices, or a premium of at least 8% for non-sex-reversed final product would be sufficient for the mixed-sex system to return a higher profit than the mono-sex system. The latter strategy could also enable further social licence through use of small fish in nutritional and outgrower initiatives.

Key words: *Oreochromis niloticus*, aquaculture systems, hormonal, quality, bioeconomic modelling, Thailand

1. Introduction

Farming of all-male tilapia stocks has been a central strategy for most producers in what has become a highly cost-competitive industry supplying both domestic and international markets (Bentsen et al., 2017). As hormone treated fish are banned in some countries and consumers elsewhere may have concerns based on environmental or welfare factors, we set out to establish whether a mixed-sex production system using a modern fast-growing strain could be developed that would be competitive with mono-sex systems. A potential strategy was tested at a commercial farm in Central Thailand based on stocking mixed-sex fry at high density and then grading out females at 4 or 8 week intervals during grow-out to achieve comparable yields.

Global production of Nile tilapia (*Oreochromis niloticus*) increased significantly from 1.74 to 4.59 million tonnes between 2006-2019 with substantial international trade (imports of 529,096 t of tilapia - fresh, chilled and frozen, whole and fillets, with a value of US$ 1.46 billion in 2018) (FAO, 2021). An increase that has been crucial to ensuring food and nutritional security for poor and vulnerable communities and in contributing to economic development (Macfadyen et al., 2012). Nile tilapia is an excellent species for aquaculture. Being omnivorous, it grows well on diets containing modest protein levels (reviewed by Ng and Romano, 2013) and feeds with a high proportion of plant-based materials can be used (Tacon and Metian, 2008). However, until sex reversal strategies were developed, farming was dependent on mixed-sex populations, which are characterised in semi-intensive systems by uncontrolled breeding resulting in higher numbers of small fish using the available production capacity and stunting individual growth. While in intensive systems, a slower growth rate in females, which is highly strain dependent, inhibited full productivity. The production of all-male stocks via sex-reversal was developed in the 1970s and became widespread during 1980-1990s as an efficient means of maintaining good growth rates to market size (e.g. Guerrero 1975; Shelton et al 1978; Hanson et al 1983; Phelps & Popma 2000).

Several techniques have been devised to produce all male stocks and one strategy, administering 17α-methyltestosterone (MT) in the feed of juvenile fish for 2-3 weeks, has been widely adopted. With appropriate precautions, MT use in the early production stages should not pose adverse human health risks for consumers (Macintosh 2008; Mlalila et al., 2015). Hazards associated with poor practice, however, include occupational risks for hatchery workers and environmental risks from discharging contaminated process water (Megbowon and Mojekwu 2014; Mlalila et al., 2015), which may have wider ecosystem effects (Rivero-Wendt et al., 2020). However, there is considerable pressure on the aquaculture industry to exclude hormones, including 17α-methyltestosterone from supply chains. Although its use is banned in several countries, including China, concern that this chemical was being used meant that tilapia from Hainan, China were assigned a red rating for the criterion 'Evidence or Risk of Chemical Use' by Seafood Watch (2012). This contributed to the entire sector of farmed tilapia production in ponds in China receiving a yellow rating and score of 5.34 on a scale from one to ten. Therefore, despite tilapia being classified as a good protein alternative to other species, the red rating for chemical use may deter potential buyers. UK consumers (for example) are significantly influenced by messages on food safety issues (Balcombe et al., 2020), so adverse ratings in prominent buyer and consumer guides could severely impact an aquaculture sector. For instance, buyers from multiple-retailers in the UK will not purchase cultured fish when hormones have been used in the production process (e.g. Sainsbury's 2017).

With this background and given evidence of 17α-methyltestosterone contamination from tilapia hatcheries in central Thailand with associated risks to environmental health, alternative farming practices need to be considered (Rivero-Wendt et al., 2020; Barbosa et al 2013; Oliveira 2013). Clarification concerning the treatment of hatchery water and recommendations on acceptable use practices to safeguard receiving ecosystems and protect human health were proposed for the Aquaculture Stewardship Council tilapia standard (ASC, 2017). However, only indicator 6.2.3 in the revised standard mentions methyl- or ethyl-testosterone; specifically, that water used to hold fish being dosed must be held for a minimum of 48 hours prior to discharge (ASC, 2019).

Despite these pressures, the economics of tilapia farming encourage continued use of sex-reversal as a means of maximising returns on investment through production efficiency gains. Encouraging the farming of non-sex-reversed tilapia in these contexts will require either a clear price differential in favour of non-hormone treated fish, or a second income stream that at least compensates for additional costs.

Previous comparative trials between mixed- and mono-sex culture systems have generally shown an advantage for mono-sex (e.g. Chakraborty et al 2011). However, factors such as strain, stock density and management are important (Dan & Little 2000). Therefore, this study was designed to re-examine the difference in performance between mono-sex and mixed-sex populations using different stocking and management strategies with a modern fast-growing strain of Nile tilapia (Big Nin strain; Developed from the GIFT strain in the Philippines and imported into Thailand in 2004 with subsequent selection to improve disease resistance) (Moses et al. 2021; Nam Sai Farms, 2022). Trials were carried out in river-based production cages at a commercial farm in Central Thailand. The focus here is on the main grow-out performance between approximately 45g and market size of just under 1 kg. The main strategy to be tested was the use of mixed-sex fingerlings that are stocked to cages at double the usual grow-out density, with females removed (once they can be reliably manually sexed) so as to reduce stock numbers and maintain stock density limits to maximise biological production from the system. Two variations of this strategy were tested, the first starting to remove females after 4 weeks (when sexing became possible) and repeating at 4 week intervals. The second starting to remove females after 8 weeks and then again after a further 8 weeks. These treatments were compared with (a) a stock of conventionally sex reversed tilapia at normal stock density and (b) a stock of mixed-sex tilapia at double density (i.e. no removal of females). The biological performance of each treatment was evaluated and the commercial viability of each strategy was examined using a comparative financial model.

2. Materials and methods

2.1 Experimental design cage-based growth trial

This trial was carried out at Nam Sai Farms Co Ltd. commercial farm in Central Thailand (Bang Sang, Prachinburi, Thailand) using cages suspended in a river. A strategy of over-stocking and then heavy grading of mixed-sex tilapia was compared with mono-sex or mixed-sex populations at conventional stocking densities without subsequent grading.

In preparation for the main trial (see Fig. 1), fifty-six thousand (n=56,000) Nile tilapia fry of the Big Nin strain at an average weight of 1.03 g per 100 individuals approximately were stocked into two 10 m^2 hapas (Hapa 1 and Hapa 2), on 6th January 2017. Each 10m^2 hapa held 28,000 fish. In Hapa 1, fry were fed non-hormone treated feed, and in Hapa 2, fry were fed with 17-alpha methyltestosterone (MT) treated feed (60 mg/Kg) for 21 days. After this nursery period, which lasted 23 days in total, fry from Hapa 1 were transferred into fifteen 20m^2 hapas and fry from Hapa 2 were transferred into five 20m^2 hapas (fingerling nursery period; 1,200 fish/hapa). At this stage, the average weight of the fry was 0.29 ± 0.02 g and survival during the fingerling nursery period was 63%.

Eight weeks later, for the main trial (grow-out period), fish were transferred from the 20m^2 hapas into 12m^2 (14.4m^3) cages positioned on the river (Suppl. Fig. 1) and four treatments were introduced (n=5 replicate cages/treatment); mono-sex (M-300) and mixed-sex (MS-300) both with an initial stocking density of 20.83 fish/m^3 and mixed-sex with removal of identified females every 4 weeks (MS-600-R4) or every 8 weeks (MS-600-R8), both with an initial stocking density 41.66 fish/m^3. Four weeks after stocking was selected as the earliest time for manual sexing based on experience at Nam Sai Farms.

Experimental design and information on treatments is shown in Fig. 1 and Table 1 respectively. Fish were transferred into the cages on 5th May, 2017 and animals were fed "ad libitum" (15 min) 3 times daily (at 08:15, 13:00 and 16:00 with daylength between 12.5 and 12.75 hours and sunrise at approximately 06:00) with floating pellets (30% crude protein) from Krung Thai PLC. The experimental protocol was approved by the Animal Welfare and Ethical Review Board at the University of Stirling (AWERB 16 17/198).

2.2. Data collection and sampling

The amount of feed consumed per cage and fish mortality were recorded daily. During pre-grow out stage, sample fish weight in mono-sex and mixed-sex hapas was recorded weekly for 8 weeks. During the grow-out phase (main trial), sampling was carried out every 4 weeks until harvest (20 weeks later; November 2017). At each of the fish sampling intervals, thirty fish (n=30/cage) were sampled at random from each cage (150 fish per treatment) and their individual weights were recorded using a digital scale accurate to 0.01 g.

At the end of the trial (week 20), fish from each cage (n=30/cage; n=5 cages/treatment) were selected at random and humanely euthanised by physical stunning and pithing. Fish were then gutted, filleted and de-skinned manually. Fillets, gonads (if present), mesenteric adipose tissue, viscera and carcass weights were recorded for each fish.

Proximate composition of fish fillets was also carried out in a random subset of the fish sampled at week 8 of the trial (n=6 cage; n= 5 cages/treatment). In this case, fillets were immediately placed on dry ice after filleting and transported to the Nutrition and Analytical Services, University of Stirling, Scotland, UK for proximate analysis (moisture, ash, protein and lipid). The timing of this sampling was partly determined by resources available (i.e. student project timetable) and also to allow for the sampling of female fish which were removed from the trial at this point.

Water quality measurements were recorded using a multi-parameter portable meter (Hanna HI 9828/10-01) at 08:00 and 15:00 twice a week and found to be typical of this region of Thailand (dissolved oxygen 2.05-5.8 mg/L; temperature 28.5-32.4 °C; 6.0-7.0 pH). Turbidity (15-50 cm) was measured using a Secchi disk and alkalinity (mEq/L), total ammonia nitrogen (mg/L) and nitrite concentrations (mg/L) were recorded and found to be within adequate levels (alkalinity 34-68 mEq/L; ammonia <1 mg/L; nitrite <0.25 mg/L) for the duration of the grow-out period using a sera Koi aqua-test kit (Sera GmbH). Surface water current measurements were between 0.42-0.57 m/s and were taken using a float and measuring rod.

2.3 Calculations

Biometric parameters were calculated using the following formulas. Specific growth rate $(SGR) = 100 \times (LnWf - LnWo) \times D^{-1}$; where Wf is the final weight of fish (g) , Wo is the initial weight (g) and D is the number of days. Feed conversion rate $(FCR) =$

$(((F/N)/D))/(Wg/D)$, where F is the total feed consumed, N is the number of fish and Wg is the weight gain $(Wf - Wo)$. Fulton's condition factor $(k) = 100 \times (Wf/L\hat{}3)$, where Wf is the final weight of fish (g) and L is the total length (cm). Gonadosomatic index $(GSI) = (Wg/Wf) \times 100$, where Wg is the weight of the gonads if present (g) and Wf is the final weight of fish (g). Fat-somatic index $(FSI) = (Wfat/Wf) \times 100$. Visceral-somatic index $(VSI) = (Wv/Wf) \times 100$, where Wv is the weight of the viscera (g) and Wf is the final weight of fish (g). Fillet yield $(FY) = (Wfillet/Wf) \times 100$, where $Wfillet$ is the weight of the fillets (g) and Wf is the final weight of fish (g).

2.4 Proximate composition

Fillets sampled at week 8 of the grow-out stage (n=6/cage; n=5 cages/treatment) were ground, freeze-dried and analysed for proximate composition (AOAC, 2010). Crude protein was determined with the Kjeldalh method using the nitrogen content of the sample $(N \times 6.25)$ ascertained with an automated analyser (Tecator Kjeltec Auto 2030 analyser, Foss, Warrington, UK). Crude lipid content was determined using the method established by Folch et al. (1957). Moisture content was determined by drying the samples in an oven at 110 °C for 24 h (AOAC, 2010) and ash content was determined by incineration in a muffle furnace at 600 °C for 24 h, as per standardised practices.

2.5 Statistical analysis of biometric and proximate composition data

Data is presented as means ± standard error mean (SEM). From a statistical perspective, the cage was considered the experimental unit (n=5/treatment). Data was analysed using SPSS Statistical Package (IBM Statistics 20; SPSS Inc., Chicago, IL, USA). Arcsine transformation was applied to percentage data to ensure binomial distribution. Biometric data collected every 4 weeks (survival, weight, SGR, FCR) and the proximate composition of the fillets at week 8 were analysed by one-way ANOVA with the explanatory variable being ‘treatment’. Biometric data (k, GSI, FY, VSI) collected at the end of the trial (week 20) were analysed by two-way ANOVA with the explanatory variables being ‘treatment’ and ‘sex’. When the overall test indicated a significant difference between treatments a Tukey’s or Games-Howell post hoc test was applied. For pairwise (male/female) comparisons, a 2-tailed T-test was used. Differences were considered significant if $p < 0.05$.

2.6 Bioeconomic modelling

The bioeconomic model used representative fish growth performance data from the cage-based trials in Thailand and additional model parameters from pond-based culture strategies in China (Cai et al., 2018). Cost data was derived from farm accounts and supplementary sources and is presented in US$ equivalents (IRS, 2018). A discounted net cash-flow assessment over a 10-year period was used to evaluate the trial results. A sensitivity analysis using Internal Rate of Return (IRR) was used to examine the contribution of the key variables to economic viability. The production data has been scaled to a more appropriate commercial operation of 1500 m^3 of cage volume for this analysis.

3. Results

3.1 Survival and fish performance during the grow-out period

The mean values of weight at the start of the grow-out trial (after fry nursery-23 days and fingerling nursery-8 weeks) for the MT-fed fish (45.5 ± 0.2 g; mono-sex group) were significantly higher than those not fed with MT (44.3 ± 0.2 g; all mixed-sex groups) (p=0.04; T-test 2-tailed).

At grow-out stage (main trial), the removal of female fish by visual inspection from two of the treatment cages (MS-600-R8, cages B and MS-600-R4, cages C) was an integral part of this research. The removal of females from MS-600-R4 at week 4 involved around 36% of the number stocked (average weight 114 g) and for MS-600-R8 at week 8 it was around 30% of the number stocked (average weight 225g). Subsequent removals (weeks 8, 12 and 16 for MS-600-R4 and week 16 for MS-600-R8) involved a much smaller numbers of females. The proportion of males and females according to the treatment at the end of the trial and final weights are shown in Fig. 2. At the end of week 20, the mean percentage of remaining fish in treatment MS-600-R8 was 52.2% (>96% male) and that of MS-600-R4 was 51.6% (>98% male). The mixed-sex population (MS-300) was 36% female and 64% male at the final sampling.

Survival, weight, SGR and FCR recorded over the sampling period according to the different treatments are shown in Table 2. Over the 20 week grow-out period, the mean fish weight increased from around 45 g to just under 1 kg. Weight from fish treated with MT (M-300) was significantly higher than mixed-sex fish up to week 8. Looking at the different treatments, results indicate that individual weight from groups where females were removed (MS-600-R8 and MS600-R4) improved. From week 16, the only group with a significant lower weight was the mixed group (MS-300) compared to the mono-sex group (M-300). The lower individual weight recorded at the end of the trial in the mixed-sex group (MS-300) was mainly driven by the significantly lower values for females as shown in Fig. 2B. Accordingly, when looking at SGR 4 weeks after females were removed in the groups MS-600-R8 and MS-600-R4 the SGR recorded was the highest (MS-600-R8 at 12 weeks and MS-600-R4 at 8 weeks). On the other hand, both survival and FCR were similar among groups with no clear trends observed (Table 2).

3.2 Fish condition and biometric measurements at the end of the trial (week 20) and proximate composition of fillets at week 8

At the end of the trial, no differences in Fulton's condition factor, FSI or VSI and FY were detected. The GSI was significantly higher in the mixed-sex group (MS-300) an effect driven by the significantly higher GSI (%) in females (Table 3).

Finally, from a fillet proximate composition perspective, no significant differences in the percentage of moisture, ash, protein and lipid were obtained according to the different treatments (Table 4).

3.3 Bioeconomic modelling

To assess the financial viability of the mixed-sex stocking and grading strategies, a hypothetical commercial farm in Central Thailand was modelled with an output in the region of 60-90 tonnes per year. Input data to the bioeconomic model was based on trial results and published sources (Table 5).

The stocking and overall management regimes assumed in the model are based on those of the Thailand trials albeit with larger cage sizes and hence fish numbers (Table 6). Cages are either stocked with a conventional 25 fish/m^3 or at double density (50 fish/m^3) with subsequent grading of females at either 4 or 8 weeks (minor removals at other stages in the trial are ignored) broadly reflecting production conditions of the experimental trial.

The sales value of the fish is normally related to fish size. Typical ex-farm market price quoted at the time of this study is shown in Table 7.

The smaller females graded out at weeks 4 and 8 would therefore normally have a relatively lower value per kg, probably representing a loss in relation to their cost of production to that point. However, if females can be sold live as potential broodstock, a substantial premium might be obtained with prices of up to US$1.41 (50 THB) per fish ($7.05/kg for a 200g fish or $14.10/kg for a 100g fish) estimated (Nam Sai Farms). For the baseline financial model it was assumed that 20% of the removed females per cycle can be sold for broodstock with the remainder available for local food markets.

3.4 Sensitivity analysis

The 10-year internal rate of return (IRR) calculation was used as a basis for the sensitivity analysis (Table 9). The base case suggests that initial overstocking and removal of females after 4 weeks provides the best return on investment whilst also showing why the use of mixed-sex stocking at the same density as all-male culture is not a financially viable option. The most important variable overall is the price received for fish sales, which appears to be lower in Thailand than some other countries. A 6% reduction in price is sufficient to make all scenarios commercially non-viable, whilst a similar increase would raise the 10-year IRR of the all-male production scenario from to 5.8% to 25.7%. The most significant production cost element is feed, followed by fingerlings.

For the mixed-sex tested scenarios, the value of the removed females is of critical importance. The baseline model assumes 20% of these can be sold for potential broodstock rather than for immediate consumption to provide the returns indicated. As this percentage falls, the mixed-sex scenarios become financially unviable (breakeven is at around 10% of removed females sold at high value for potential broodstock). This could be offset however if a higher sales price is achievable for non-sex-reversed fish. A premium of around 8% in final sales price for mixed-sex fish over mono-sex gives the same returns (with no income from potential broodstock). The market price for small fish ($/kg) appears to be lower than the cost of production at this stage, so early sales lead to an immediate loss to the business. A separate model (Fig. 3) illustrates the impact of fingerling price on production costs and potential margins at smaller fish sizes. For female fish sold at 100 g, ten fry must be purchased per kilogram of sales (not considering mortalities) whereas this falls to 5 fry per kilogram for 200 g fish and only 1 fry per kilogram for 1 kg fish. The breakeven sales price for 100 g category fish (including cost elements for feed and additional labour) is estimated to be $1.99/kg and for 200 g category fish $1.43. This is substantially higher than actual reported prices (personal communications) of $0.36 and $0.46 in Thailand and $0.66 and $0.97 respectively in Bangladesh. Sale of these fish for consumption therefore represents a financial loss to the business, the amount depending on the differential between the sales price and the break-even price. In these circumstances therefore, the cost of fry is a critical variable. Both cost and price structures can vary however with ex-farm prices for 200g tilapia exceeding US$2/kg in Ghana for instance (Chamber of Aquaculture Ghana, 2022), suggesting more location-specific modelling could be beneficial.

4. Discussion

4.1. Production performance of mixed-sex populations in cages

In this study, the mixed population had a sex ratio of around 36% female and 64% male, while the MT-treated group led to a resolution of >99% males. Hormone-fed tilapia showed a significant increased body weight after the nursery period, indicating a positive effect of 17α-methyltestosterone on growth at these early stages as reported in other studies (Pandian and Sheela, 1995; Chávez-García et al., 2020). After the grow-out period (20 weeks), tilapia males reached a higher harvest weight than females in the mixed population group (Guerrero, 1975; Hanson et al., 1983) and therefore, final weight in the mixed-sex group was significantly lower than in the mono-sex treatment. Tilapia males are typically preferred due to their fast growth, higher survival and better FCRs. In the present trial, faster growth was recorded in hormone-fed fry. However, after the grow-out period, while a lower harvest weight was obtained in the mixed-sex population, due to the significant proportion of females, cages where females had been graded out at 4 and 8 weeks showed similar harvest weight, survival and FCR to the hormone treated fish. Furthermore, no differences at harvest were observed in Fulton's condition, fillet and visceral yields for any of the groups. In this respect, only a significantly higher GSI was observed in the mixed-sex cages, which again was driven by the substantial presence of females in this group with a higher GSI. Therefore, from a production perspective, the removal of females performed in two of the treatments was a successful strategy and did not detrimentally affect the performance and fillet nutritional quality of the fish at harvest. Small female fish accounted for 6.6% and 10.4% of the biomass harvested from the MS600-R4 and MS600-R8 treatments respectively, and this could be a nutritious source of affordable fish for poorer consumers.

4.2. Financial performance of mixed-sex populations in cages

Despite greater production from mixed-sex cages with higher stock density their profitability is lower than mono-sex cages at lower stock density when all harvested fish are sold for food when using price data from Thailand. This is due to a proportion of the production (removed females) being sold at a loss. If this loss can be reduced through a proportion of the removed fish being sold at a higher price, or the harvested fish achieving a premium, the mixed-sex production system can be more profitable than mono-sex.

To potential to sell female fish at a premium price as potential broodstock is attractive, and hatchery operators could be willing to pay this for females when the strain is known and desirable and the hatchery operator needs an external supply. However, the market for such fish would be limited and depend on the size of the industry, the number of other producers, and the degree to which hatchery operators are anyway self-sufficient. There could also be biosecurity constraints, which would make this model more attractive to vertically integrated or contract farming operations. It is estimated that each potential brood fish would have the capacity to produce 14,600 eggs over a productive life of 2 years. With assumed survival rates of 60% eggs to first feeding fry and 60% to 2g fry, this yields 5,040 fry per brood fish. The model farm used in the calculations has a maximum fry requirement of 75,000 per cycle (150,000 per year or 300,000 over two years). This would require only 60 female broodstock to supply. The assumed 20% of removed fry in the financial model used for broodstock would produce up to 14 million fry per year – sufficient for an industry production in the region of 10,000 tonnes. This is around 4.5% of annual tilapia production in Thailand, so potentially feasible but only for a small number of producers.

4.3. Opportunities for mixed-sex tilapia production

A meta-analysis of willingness to pay (Li and Kallas 2021) suggested a global average premium of 16.6% for sustainably produced seafood compared with seafood without that assurance, and a tendency for this to be higher in Asia and Europe than North America. Sensitivity to food contamination is particularly high in China because of recurrent foods safety scandals (Xu et al 2012; Wang et al, 2020), a trend that is likely to grow in Asia, by far the largest centre for fish consumption (Naylor et a, 2021), as incomes and expectations rise. Mixed-sex tilapia production based around early removal of females may have significance where hormones are unavailable or not used, such as small-scale producers in Zambia (Kaminski et al., 2018), are inconsistent in quality, as reported in Egypt (Eltholth et al., 2015) and/or restricted by Governments such as in some countries in Sub- Saharan Africa where cage production of tilapia is growing fast. These results support the importance of developing faster growing strains; female removal has been ineffective at maintaining cost effective production in many unimproved strains. Cage production allowed for effective removal of females at a small size but such approaches may be less effective in ponds, however Dan and Little (2000) demonstrated removal of recruits in mixed-sex culture by monthly seining limited growth retardation and led to similar performance compared to mono-sex stocks, even with less developed strains of Nile tilapia, suggesting a reappraisal with current strains is warranted.

Consideration could also be given to how smaller fish produced within a mixed-sex strategy might be used to enhance the nutrition and health of local poorer populations and thus serve as an additional factor to avoidance of hormone use in the development of social licence and ethical credentials for producers seeking premium markets. This might involve direct sale of small fish to consumers, or development of smallholder outgrower networks (e.g. Kaminski et. al., 2020).

5. Conclusions

The trials demonstrated the technical feasibility of mixed-sex production of tilapia in cages in based on a strategy of over-stocking with fry and then sorting and removing females after 4 to 8 weeks. Based on the stocking densities selected for the trials, the final production of tilapia was up to 50% greater from the mixed-sex cages, with the final harvest yielding fish of an equivalent mean weight. However, a financial analysis of this approach showed that the additional costs (fry, feed and labour) involved in the mixed-sex strategy resulted in lower profits. This could be mitigated if a proportion of the removed females could be sold at a much higher price as potential broodstock. In the model presented, sales of 13% of the removed females at the higher price quoted in Thailand would be sufficient for the mixed-sex system to return a higher profit than the mono-sex system. A premium of around 8% in final sales price for mixed-sex fish over mono-sex also gives the same returns (with no income from potential broodstock) and would make the smaller females available at low-cost for local enterprise and nutritional improvements.

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Table 1. Treatments used in the cage-based growth trial.

Trial code	Treatment description	Number of replicates
MS-300	300 Mixed Sex fish	5 cages 'A'
MS-600-R8	600 Mixed sex fish; all noticeable females removed at 8-weeks intervals	5 cages 'B'
MS-600-R4	600 Mixed sex fish; all noticeable females removed at 4-weeks intervals	5 cages 'C'
Monosex male	300 Monosex hormone treated males	5 cages 'M'

Table 2. Survival, growth performance and feed utilisation in Nile tilapia during the grow-out stage according to treatment. Results are means ± SEM (n=5 tanks/treatment). Different superscript letters within a row indicate significant differences between treatments (one-way ANOVA with Tukey and Games Howell, $p<0.05$). Furthermore, a superscript asterisk indicates significant differences between all the mixed-sex groups and the mono-sex (T-test, $p<0.05$). SGR, specific growth rate; FCR, food conversion rate.

	Mixed Sex			**Monosex**
	MS-300 (A)	MS-600-R8 (B)	MS-600-R4 (C)	Monosex male (M)
Initial weight (g)	44.1 ± 0.3^{b}	44.5 ± 0.5^{b}	44.3 ± 0.4^{b}	$45.5 \pm 0.4^{a*}$
Sampling 4 weeks				
Survival (%)	97.4 ± 2.1^{a}	98.1 ± 0.5^{a}	99.8 ± 0.6^{a}	99.2 ± 0.4^{a}
Weight (g)	124.3 ± 6.5^{ab}	117.9 ± 4.2^{ab}	113.4 ± 2.6^{b}	$138.2 \pm 6.1^{a*}$
SGR (%)	4.5 ± 0.2^{ab}	4.2 ± 0.1^{ab}	4.1 ± 0.1^{b}	$4.8 \pm 0.2^{a*}$
FCR	1.41 ± 0.05^{a}	1.16 ± 0.06^{b}	1.20 ± 0.02^{ab}	1.30 ± 0.07^{ab}
Sampling 8 weeks				
Survival (%)	99.0 ± 0.6^{a}	97.2 ± 0.3^{ab}	92.5 ± 1.0^{b}	95.2 ± 2.4^{ab}
Weight (g)	274.3 ± 6.0^{ab}	258.4 ± 1.8^{c}	284.3 ± 5.2^{ab}	$292.5 \pm 8.6^{a*}$
SGR (%)	2.8 ± 0.1^{b}	2.8 ± 0.1^{b}	3.3 ± 0.1^{a}	2.7 ± 0.2^{b}
FCR	1.19 ± 0.01^{a}	1.19 ± 0.04^{a}	1.16 ± 0.03^{a}	1.23 ± 0.04^{a}
Sampling 12 weeks				
Survival (%)	92.8 ± 0.8^{a}	89.7 ± 1.2^{a}	94.9 ± 2.3^{a}	90.8 ± 2.6^{a}
Weight (g)	474.1 ± 4.6^{a}	490.0 ± 5.3^{a}	501.0 ± 4.0^{a}	498.9 ± 12.6^{a}
SGR (%)	1.7 ± 0.1^{ab}	2.0 ± 0.05^{a}	1.8 ± 0.04^{ab}	1.7 ± 0.1^{b}
FCR	1.34 ± 0.08^{a}	1.45 ± 0.03^{a}	1.30 ± 0.09^{a}	1.51 ± 0.13^{a}
Sampling 16 weeks				
Survival (%)	97.6 ± 1.0^{a}	95.9 ± 1.1^{a}	96.5 ± 0.8^{a}	97.6 ± 1.0^{a}
Weight (g)	676.3 ± 11.8^{b}	737.0 ± 6.4^{ab}	739.6 ± 12.5^{a}	754.3 ± 25.0^{a}
SGR (%)	1.3 ± 0.05^{a}	1.5 ± 0.05^{a}	1.4 ± 0.05^{a}	1.5 ± 0.1^{a}
FCR	1.66 ± 0.11^{a}	1.41 ± 0.06^{a}	1.48 ± 0.05^{a}	1.52 ± 0.19^{a}
Sampling 20 weeks				
Survival (%)	95.0 ± 1.4^{a}	95.5 ± 1.5^{a}	97.3 ± 0.9^{a}	97.3 ± 1.1^{a}
Weight (g)	888.1 ± 14.9^{b}	966.4 ± 9.7^{a}	958.9 ± 18.4^{ab}	$1002.0 \pm 24.0^{a*}$
SGR (%)	1.0 ± 0.02^{a}	1.0 ± 0.05^{a}	0.9 ± 0.02^{a}	1.0 ± 0.1^{a}
FCR	2.09 ± 0.14^{a}	2.06 ± 0.25^{a}	1.90 ± 1.1^{a}	1.81 ± 0.08^{a}

Table 3. External appearance index, condition factor (k), gonadosomatic index (GSI%), fillet yield (FY%) and visceral yield (VSI%) at the end of the trial according to treatment and sex within each treatment. Results are means ± SEM (n=5 tanks/treatment). Different

superscript letters within a column indicate significant differences between treatments (two-way ANOVA post hoc Tukey, $p<0.05$). Furthermore, a superscript asterisk indicates significant differences according to sex within a treatment (T-test, $p<0.05$).

	k	GSI (%)	FY (%)	FSI (%)	VSI (%)
MS-300 (A)	1.73 ± 0.02	0.43 ± 0.02[a]	34.75 ± 1.08	3.89 ± 0.14	3.79 ± 0.14
MS-300 (A) Males	1.74 ± 0.02	0.21 ± 0.02	35.09 ± 1.10	3.96 ± 0.11	3.74 ± 0.11
MS-300 (A) Females	1.74 ± 0.03	0.86 ± 0.10*	34.43 ± 1.18	3.80 ± 0.19	4.01 ± 0.27
MS-600-R8 (B)	1.79 ± 0.03	0.19 ± 0.00[b]	33.68 ± 2.11	3.72 ± 0.21	4.29 ± 0.18
MS-600 (B) Males	1.79 ± 0.03	0.18 ± 0.01	33.67 ± 2.17	3.74 ± 0.22	4.30 ± 0.18
MS-600 (B) Females	1.81 ± 0.04	0.43 ± 0.16*	34.76 ± 1.20	3.86 ± 0.58	4.32 ± 0.33
MS-600-R4 (C)	1.74 ± 0.02	0.18 ± 0.01[b]	33.26 ± 1.16	3.63 ± 0.09	4.22 ± 0.14
MS-600 (C) Males	1.74 ± 0.02	0.16 ± 0.01	33.27 ± 1.15	3.64 ± 0.09	4.22 ± 0.14
MS-600 (C) Females	1.71 ± 0.05	0.76 ± 0.46*	33.43 ± 2.32	3.29 ± 0.17	4.40 ± 0.47
Monosex male (M)	1.80 ± 0.03	0.18 ± 0.01[b]	33.90 ± 1.22	3.59 ± 0.18	4.28 ± 0.11

Table 4. Proximate analysis of fillets at week 8 according to treatment. Results are means ± SEM (n=6/cage; n=5 cages/treatment). No significant differences were observed for any of the parameters measured according to the different treatments (one-way ANOVA post hoc Tukey, p>0.05).

	Mixed Sex			**Monosex**
	MS-300 (A)	MS-600-R8 (B)	MS-600-R4 (C)	Monosex male (M)
Moisture (%)	77.6 ± 0.6	78.0 ± 0.4	77.3 ± 0.7	77.1 ± 0.4
Ash (%)	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
Protein (%)	18.8 ± 0.9	18.5 ± 0.3	19.2 ± 0.5	19.1 ± 0.5
Lipid (%)	2.5 ± 0.4	2.4 ± 0.1	2.3 ± 0.1	2.4 ± 0.1

Table 5. Data sources for cage-based tilapia culture in rivers in central Thailand

Data source	Cage operation & input/output items	Practices, costs incurred and returns
Trials in Ban Pakong River	Fingerling production and stocking regimes	Based on reported research trial
	Feed and feeding regimes	Commercial grow-out feed is used with 32% protein content; feed is administered by hand, three times daily; the ration is adjusted according to manufacturer guidelines from 4% to 2% of body weight per day over the period between stocking and harvesting; feeding behaviour is, however, monitored and feeding adjusted accordingly; feeding after handling is reduced to allow for a supressed appetite owing to stress.
	Size and value of fish at harvest	Fish are removed at a range of sizes. Approximate market prices for each size grade is given in Table 7. Small females suitable for use as broodstock can command significantly higher prices. The proportion of small females that can be sold for potential broodstock significantly affects overall financial viability
Key informant interviews and scientific literature	Cage size and construction	Commercial producers in northern Thailand typically use 32 m^3 cages constructed from steel tubing and plastic drums and measuring 4m x 4m with depth 2m and mesh of 6-25 mm (Lebel et al., 2013). This model of a larger farm uses 50 m^3 cages measuring 5 x 5 x 2 m.
	Culture practices for cages in rivers	River-based cage culture in northern Thailand involves ≤4 cages for small-scale producers, medium producers have 5-12 cages and large producers ≥13 cages (Lebel et al., 2013).
	Cost implications of scaling-up production	Cost structure was based on Piumsombun et al., 2005 with no adjustment for scale

Note: exchange rate of Baht 35.372 to US$1 for 2017 used for currency conversions (IRS, 2018)

Table 6. Operating parameters for the 4 cage-culture stocking and management scenarios

Operating parameter	Sex-reversed (all males)	Mixed sex (no females removed)	Mixed sex (females removed at 4 weeks)	Mixed sex (females removed at 8 weeks)
Cage volume (m^3)	1500	1500	1500	1500
Stocking rate (no. m^{-3})	25	25	50	50
Total fish stocked (no.)	37,500	37,500	75,000	75,000
Size at stocking (g)	45.5	44.5	44.5	44.5
Culture period (days)	139	139	139	139
Female removal rates: (% total fish stocked) week 4	-	-	36	-
week 8	-	-	-	32
Expected survival rate (%)	94	94	94	94
Expected FCR	1.52	1.53	1.41	1.43
Final harvest weight (g)	1002	888	959	966
Standing stock at harvest (kg m^{-3})	23.5	20.9	28.8	30.9
Table fish biomass per cycle (t)	35.321	31.306	43.270	46.329
Removed female numbers per cycle			27,000	24,000
Total weight of females removed (kg)			3,079	5,369
Mean weight of removed females (g)			114	224

Table 7. Sales price of marketed fish

Size grade	Sales price THB/Kg	Equivalent US$/kg*
100-200g	10	0.28
200-300g	15	0.42
300-400g	20	0.57
400-500g	25	0.71
500-600g	30	0.85
600-700g	37	1.05
700-800g	42	1.20
800-900g	46	1.30
900-1000g	49	1.38
1000g+	51	1.45

*Exchange rate = US$1 = THB 35.372

Table 8. Financial indicators from bioeconomic modelling for the 4 management scenarios*

Indicator		Sex-reversed (all males)	Mixed sex (no females removed)	Mixed sex (females removed at 4 weeks)	Mixed sex (females removed at 8 weeks)
Capital costs (US$)					
	Cages and nets (5y lifespan)	16,800	16,800	16,800	16,800
	Durable equipment (10y lifespan)	15,000	15,000	15,000	15,000
	Land and buildings (50y lifespan)	15,000	15,000	15,000	15,000
	Total	46,800	46,800	46,800	46,800
Operating costs (US$ y^{-1})					
	Fingerlings	5,216	5,216	10.432	10,432
	Fish feed	34,667	30,847	41,265	46,988
	Fuel	348	308	426	456
	Maintenance (3% of capital costs)	1,404	1,404	1,404	1,404
	Labour	4,163	3,777	5,888	6,521
	Fixed costs	916	831	1,188	1,316
	Total	46,714	42,383	60,603	67,117
	Costs (US$ m^{-3} $cycle^{-1}$)	31	28	40	45
Income from table fish (US$ $cycle^{-1}$)		51,215	40,697	68,016	72,506
Income from female broodstock (US$ $cycle^{-1}$)				7,614	6,768
Income from other juvenile sales (US$ $cycle^{-1}$)				609	1,804
Total Income (US$ $cycle^{-1}$)		51,215	40,697	68,016	72,506
Net benefit (exclude. depreciation) (US$ y^{-1})		9,001	-3,372	14,826	10,779
Net benefit (US$ m^{-3} y^{-1})		6	-2	10	7
Pay-back period (y)		5.2	-	3.2	4.3
Return on capital costs		19.2%	-7.2%	31.7%	23.0%
Return on operating costs		9.6%	-4.0%	12.2%	8.0%
10-year NPV at discount rates of:	5%	1,603	-87,561	41,836	11,759
	10%	-7,023	-76,962	24,238	491
	20%	-16,333	-62,626	3,934	-12,006
IRR (%) over:	10 y	5.8	-	23.1	10.3

*Annual figures are based on two crops per year

Table 9. Sensitivity assessment showing variation in the 10-year IRR and percentage change as compared to the baseline in parenthesis

Variable	**Sex-reversed (all males)**	**Mixed sex (no females removed)**	**Mixed sex (females removed at 4 weeks)**	**Mixed sex (females removed at 8 weeks)**
Fish sales value (+10%)	38.5 (+564%)	-	60.5 (+162%)	49.8 (+384%)
FCR (-10%)	31.2 (+438%)	-	52.4 (+127%)	42.8 (+315%)
Final harvest fish value (+5%)	22.5 (+288%)	-	41.1 (+78%)	29.5 (+186%)
Feed cost (-5%)	18.2 (+214%)	-	36.8 (+59%)	25.9 (+151%)
Baseline	**5.8**	**-**	**23.1**	**10.3**
Fingerling cost (+10%)	0.6 (-90%)	-	14.7 (-36%)	1.0 (-90%)
Survival rate (-5%)	1.4 (-76%)	-	18.6 (-19%)	5.1 (-50%)
Cost of cage construction (+50%)		-	13.1 (-43%)	0.9 (-91%)
Feed cost (+5%)		-	7.3 (-68%)	-
Final harvest fish value (-5%)		-	3.1 (-87%)	-
FCR (+10%)		-	-	-
No female fish sold for broodstock	5.8	-	-	-
No female fish sold for broodstock, but non-sex reversed fish sold at 5% premium	5.8	-	-	-
No female fish sold for broodstock, but non-sex reversed fish sold at 10% premium	5.8	-	14.7 (-36%)	12.2 (+18%)

Figure legends

Fig. 1: Flowchart showing the experimental design and sequence of events in the growth trial.

Fig. 2: a) Percentage of males and females at the end of the trial according to treatment and b) Final weight of males and females at the end of the trial for MS-300 treatment and final weight of males for the rest of the treatments. Values are mean ± S.E.M.

Fig. 3: Contribution of products to total earnings (a) base case, (b) if all removed females are sold at Thailand prices, (c) if all removed females can be sold at higher Bangladesh prices and (d) if all removed females can be sold as potential broodstock.

Suppl. Fig. 1: A diagram showing the layout of the cages on the river at the grow-out stage. Cages containing the letter A indicate cage contains fish from treatment MS-300; letter B indicate treatment MS-600-R8; letter C indicate MS-600-R4 and letter M indicate M-300. Circles indicate points where water samples were collected for analysis (black) or were used to analyse water current (red).

Mixed-sex tilapia can perform competitively with mono-sex stocks in cage production

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Author Statement

John Bostock: Formal Analysis, Writing – Original Draft, **Amaya Albalat:** Formal Analysis, Writing – Review & Editing, Visualization, **Stuart Bunting:** Formal Analysis, Writing – Review & Editing **Warren A. Turner:** Resources, **Armah Dorcas Mensah:** Investigation, **David C. Little:** Conceptualization, Methodology, Supervision, Funding Acquisition.

Note - Authorship roles:

Term	Definition
Conceptualization	Ideas; formulation or evolution of overarching research goals and aims
Methodology	Development or design of methodology; creation of models
Software	Programming, software development; designing computer programs; implementation of the computer code and supporting algorithms; testing of existing code components
Validation	Verification, whether as a part of the activity or separate, of the overall replication/ reproducibility of results/experiments and other research outputs
Formal analysis	Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data
Investigation	Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection
Resources	Provision of study materials, reagents, materials, patients, laboratory samples, animals, instrumentation, computing resources, or other analysis tools
Data Curation	Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later reuse
Writing - Original Draft	Preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation)
Writing - Review & Editing	Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision – including pre-or postpublication stages
Visualization	Preparation, creation and/or presentation of the published work, specifically visualization/ data presentation
Supervision	Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team
Project administration	Management and coordination responsibility for the research activity planning and execution
Funding acquisition	Acquisition of the financial support for the project leading to this publication

Declaration of interests

☐The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Armah Dorcas Mensaha reports financial support and travel were provided by J Sainsbury plc. Armah Dorcas Mensaha reports equipment, drugs, or supplies was provided by Grobest Group Limited. Armah Dorcas Mensaha reports equipment, drugs, or supplies was provided by Nam Sai Farms Co. Ltd. David Little reports a relationship with Nam Sai Farms Co. Ltd that includes: board membership. Nam Sai Farms Co. Ltd is a commercial tilapia hatchery providing fry and fingerlings to the tilapia farming sector in Thailand - WT

- Tilapia growth trials in cages confirmed there are financial benefits of using sex-reversed monosex fry rather than mixed-sex populations.
- Mixed-sex populations however achieved almost equal growth rates and greater production overall when initially overstocked with an intermediate harvest of smaller females.
- The trials and associated financial analysis showed that the use of mixed sex fish rather than hormone-based sex-reversal could be financially competitive if a premium of at least 8% could be obtained for non-sex-reversed product; or if the small removed females could be sold at a substantially higher price, e.g. as potential broodstock.

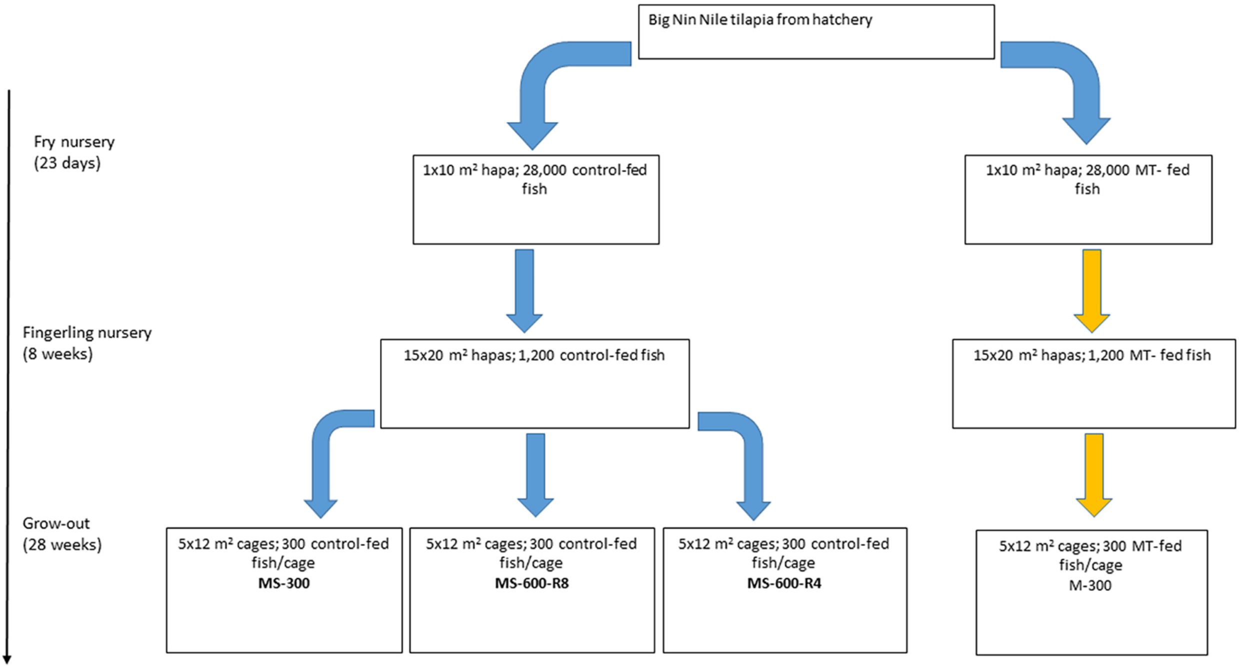
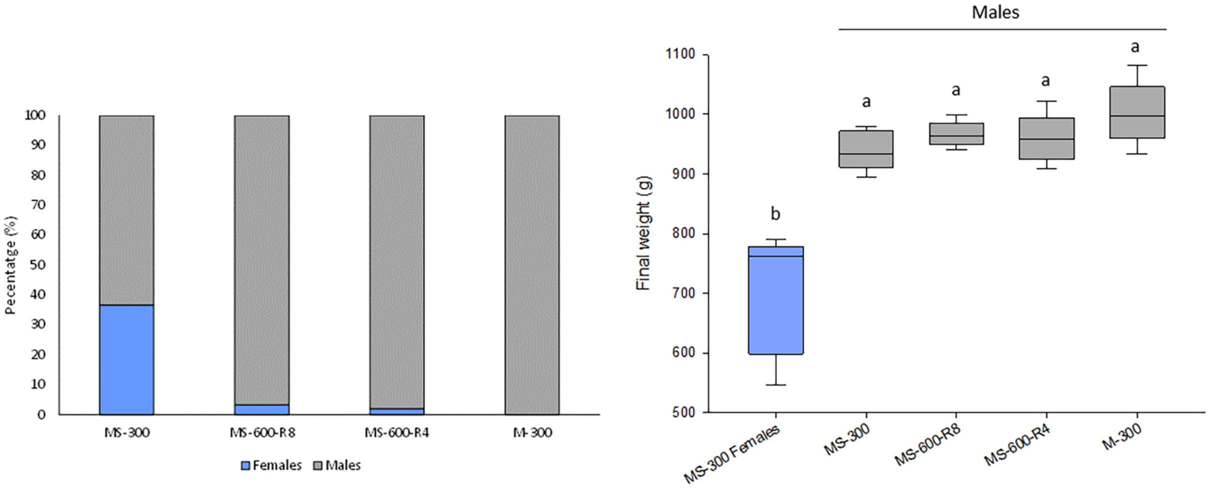
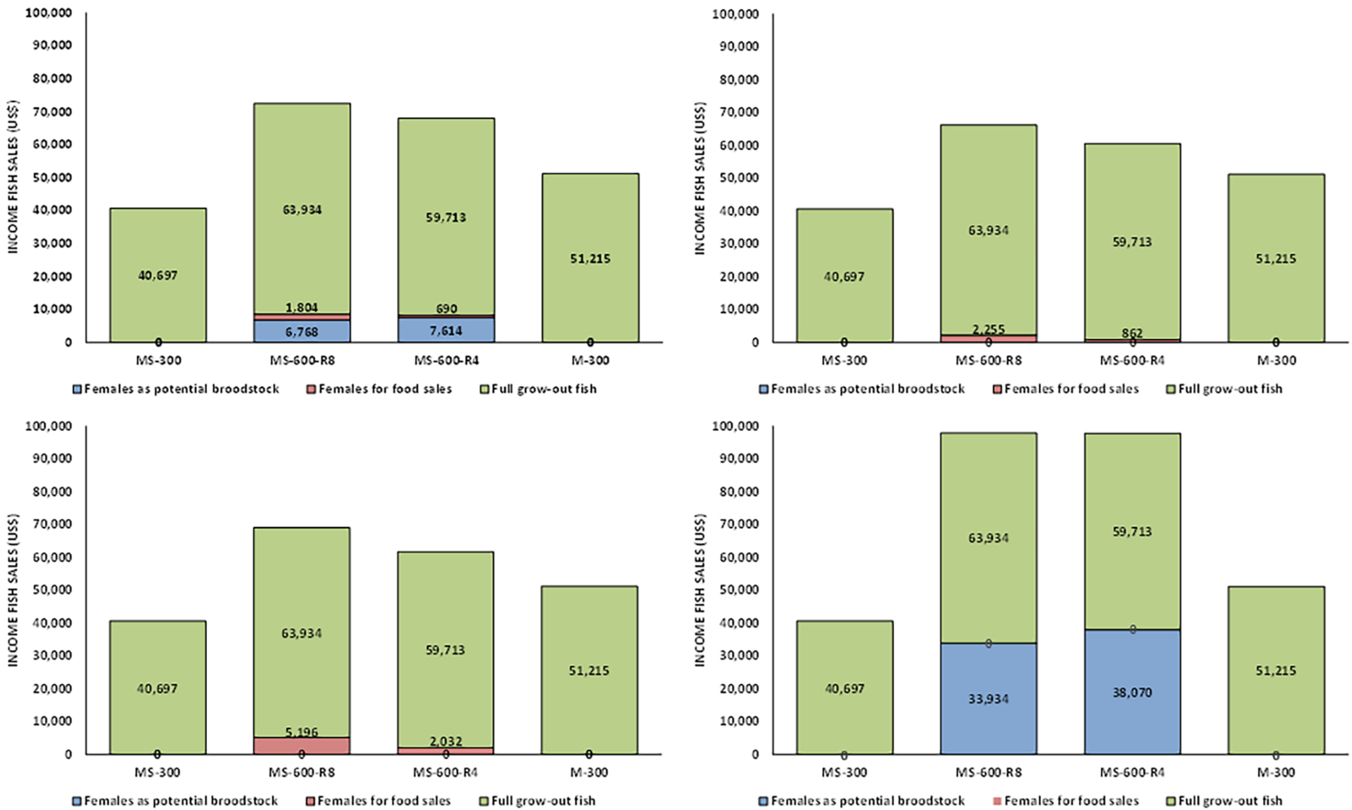

Big Nin Nile tilapia from hatchery
Fry nursery
(23 days)
1x10 m² hapa; 28,000 control-fed fish
1x10 m² hapa; 28,000 MT- fed fish
Fingerling nursery
(8 weeks)
15x20 m² hapas; 1,200 control-fed fish
15x20 m² hapas; 1,200 MT- fed fish
Grow-out
(28 weeks)
5x12 m² cages; 300 control-fed fish/cage
MS-300
5x12 m² cages; 300 control-fed fish/cage
MS-600-R8
5x12 m² cages; 300 control-fed fish/cage
MS-600-R4
5x12 m² cages; 300 MT-fed fish/cage
M-300

Figure 1



Pecentatge (%)
100
90
80
70
60
50
40
30
20
10
0
MS-300
MS-600-R8
MS-600-R4
M-300
Females
Males
Males
Final weight (g)
1100
1000
900
800
700
600
500
a
a
a
a
b
MS-300 Females
MS-300
MS-600-R8
MS-600-R4
M-300

Figure 2



100,000
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
INCOME FISH SALES (US$)
MS-300
MS-600-R8
MS-600-R4
M-300
40,697
63,934
1,804
6,768
59,713
690
7,614
51,215
2,255
862
5,196
2,032
33,934
38,070
Females as potential broodstock
Females for food sales
Full grow-out fish

Figure 3