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Environmental sex determination: the effect of temperature and salinity on sex ratio in *Oreochromis niloticus* L.

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Abstract

This paper reports the effects of environmental conditions during the period of sex differentiation on the sex ratio of the Nile tilapia (*Oreochromis niloticus*). Different sex genotypes were exposed to varying temperatures (putative all-female, all-male and all-YY males) and salinities (putative all-female progeny only) for a minimum period of 21 days after first feeding and were on grown prior to sexing by gonad squash. The majority of the putative all-female progeny exposed to high temperature (36.54 ± 0.39°C) produced significantly higher percentages of males compared to controls reared at ambient temperature (27.87 ± 1.40°C). Similarly, at high temperature, some of the all-male and YY male progenies had significantly lower percentage of males compared to controls. Sex differentiation in YY males appears to be more labile than in normal XY males although this could possibly be attributable to different levels of inbreeding. Low temperature (25.78 ± 0.24°C) and varying levels of salinity (11.30 to 26.65 ppt) did not significantly affect sex ratios. The apparent sensitivity of sex differentiation to some environmental factors is considered in the context of a predominantly monofactorial genetic sex determining mechanism. Implications for sex control technologies are discussed.

Keywords: *Oreochromis niloticus*; Sex determination; Temperature; Salinity; Monosex

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1. Introduction

Studies on the mechanism of sex determination of the commercially important tilapia, *Oreochromis niloticus*, have demonstrated that this species exhibits a predominantly monofactorial genotypic system with male heterogamety and female homogamety (Penman et al., 1987; Shah, 1988; Mair et al., 1991; Müller-Belecke and Hörstgen-Schwark, 1995; Mair et al., 1997). In accordance with this system it is expected that crosses of XX♀ × XXΔ♂, XX♀ × YY♂ and YYΔ♀ × YY♂ (the delta prefix denotes functionally sex-reversed genotypes as proposed by Mair et al., 1987) should give all XX female, XY male and YY male progeny, respectively.

However, some of the sex ratios observed from these crosses do not conform completely to expectations. For example, in single pair matings of normal females with sibling XXΔ males Baroiller (1996) observed all-female progenies in only five crosses of the 35 crosses tested, the others having sex ratios ranging from 65 to 99% female progeny. In a similar study, using mass spawning, Calhoun and Shelton (1983) observed that the sex ratio of the progeny of XXΔ males varied depending on the source of the females used. The proportion of female progeny from mothers that were half or full siblings of the XXΔ male breeders (99.9%) were significantly higher than in the progeny from randomly selected females (94.7%). Theoretically, there should have been no difference between the two female groups as they were both carrying XX chromosomes.

Similar results have also been observed from crosses of XX♀ × YY♂. In a series of progeny tests, Mair et al. (1997) observed sex ratios ranging from 69.0 to 100% male from over 100 different YY males. In YYΔ♀ × YY♂ crosses, no females have been observed in the progeny at the time of writing (Mair et al., 1997 and unpublished data). Although the majority of the YY males tested have produced 100% male progeny in crosses with normal females, the presence of unexpected females in the progeny of some YY males could be an indication of the presence of a factor or factors other than the major sex determining genes or sex chromosomes, which may exerting an influence during the differentiation of sex.

In studies of the underlying mechanism of sex determination in some animals, it has been observed that environmental factors, particularly during the stage of sexual differentiation, can influence or determine the sex ratio. For example in reptiles, the direction of development of the sexes depends on the temperature during the egg incubation period (see review by Bull (1980)).

Evidence of the effect of environment on sex ratio has been observed in a number of fish species. A number of studies have reported on the varying degrees of temperature dependent sex determination and its adaptive significance in the Atlantic Silverside, *Menidia menidia* (Conover and Kynard, 1981; Conover, 1984). Other environmental extremes such as low pH have also been observed to affect sex ratio in Cichlids and Poecilids (Rubin, 1985), and in *Apistogramma* (Teleostei, Cichlidae) (Römer and Beisenherz, 1996). A number of studies have also demonstrated effects of temperature on sex ratio in tilapia. In an early study, Mair et al. (1990) observed that at a cold temperature (19°C) the sex ratio of *O. mossambicus* in one experiment had a significant excess of males (89%) compared to the control reared at 28°C (0%). In another

experiment at a warm temperature (32°C) the authors noted that the sex ratio of *O. aureus* had significant excess of females (80%) compared to the control (97%) reared at 29°C. In another study on *O. niloticus*, Baroiller et al. (1996) reared putative all-female (from XX♀ × XXΔ♂ crosses) at elevated temperatures. The proportion of males in the groups treated at 36°C was observed to increase by between 14.1 and 91%, compared to the control reared at 28°C. Similarly, the sex ratio of a group of *O. niloticus* progeny reared at 35 ± 1°C for a period of 60 days, beginning at first feeding, was 80% male as compared to the 1:1 sex ratio of the control reared at 22 ± 1°C (Mbahinzireki and Dabrowski, 1997).

Recognizing the importance of growing monosex progeny in sexually dimorphic species such as the tilapias, there have been long term efforts to develop effective methods of controlling sex including hybridization, sex reversal and genetic manipulation. The success of these attempts to control sex through genetic means depends on a thorough understanding of the underlying sex determination mechanisms existing in the species of interest. This paper presents the results of experiments where we evaluated the effects of temperature and salinity on sex ratio in *O. niloticus*. The implications of these results are discussed in relation to the hypothesis of an underlying predominantly monofactorial genetic mechanism in this species.

2. Materials and methods

The study was carried out using the strains of *O. niloticus* being maintained by the Genetic Manipulation for Improved Tilapia (GMIT) Project at the Freshwater Aquaculture Center of Central Luzon State University in the Philippines. These included Egypt–Swansea (ES), Egypt–ICLARM (EI), Ghana–BFAR (GB) and Kenya–Turkana (KT). Sexually undifferentiated purebred and crossbred putative all XX female, XY male and YY male progeny from crosses of XX♀ × XXΔ♂, XX♀ × YY♂ and YYΔ ♀ × YY♂ were used in the study. The progenies were produced by pooled spawning either in 2 m × 5 m × 1 m fine mesh cages installed in earthen ponds or in 1.5 m diameter circular concrete tanks. Collection of eggs or fry was done 7 days after stocking and every 7 days thereafter until a sufficient number of fry had been obtained. All the fry in the same batch of the same genotype were pooled and randomly divided into sets of two, one set for treatment and the other one for control. The collected eggs were incubated at ambient temperatures in down welling incubators until hatched.

The treatments were applied indoors in 195- and 40-l capacity glass aquaria for testing temperature and salinity effects, respectively. For tests on the effect of temperature, three small fine mesh cages (approximately 18-l capacity) were installed in each aquarium for replication. The desired temperature was maintained using a 14–38°C range Biotherm-2000 thermoregulator and Nimrod thermostatic heater. No changes of water were made during the treatment period and stable temperatures were maintained except during occasional brief power interruptions. A motorized pump filter was installed in each aquarium to prevent the accumulation of waste products and to maintain uniform temperature by continuously circulating the water. For the controls the same system was also set up without a heater and was maintained at ambient temperatures (27.87 ± 1.40°C).

In the first experiment, the effect of high temperature on the sex ratios of purebred and crossbred putative all-female and all-male progeny was evaluated by rearing the treated progeny at elevated (36.54 ± 0.39°C) and the control at ambient temperatures (27.87 ± 1.40°C). The all-female and all-male progeny were produced from crosses of XXΔ and YY males in the ES, EI and GB strains with normal females from the same three strains and KT. Stocking density varied (100–300 fry/cage) depending on the availability of fry and the conditions during treatment. A higher stocking density was used during cold months to allow for higher mortality in the control as the lower temperatures.

In the second experiment, putative all-female (GFT), all-male (GMT) and all-YY males (YY) in the ES strain were reared at low (25.78 ± 0.24°C), normal (29.77 ± 0.26°C) and high temperatures (37.04 ± 0.49°C) at a density of 100 fry/cage to further determine the extent of the effect of temperature on sex ratio. Since the study was conducted during the cold months to enable a low temperature environment it was necessary to maintain an additional normal temperature control (28–30°C).

Following the observed extreme sensitivity of YY males to high temperature (Experiment 2), further testing (Experiment 3) using purebred and crossbred YY male progeny from crosses of YYΔ ♀ × YY ♂ of the ES strain and YYΔ ♀ ES × YY ♂ EI strain, respectively, was carried out following the procedure described in Experiment 1. The stocking density ranged from 50 to 300 fry/cage depending on the availability of fry.

The fourth experiment was carried out as a preliminary trial to determine the effect of different salinities on sex ratio. Two replicated trials were done using putative all-female progeny sired by XXΔ males of the ES and EI strains at a stocking density of 250 and 280 fry/aquarium, respectively. Facilities were not available to conduct similar experiments on all-male or YY male populations.

At the start of each experiment, randomly divided first feeding fry were stocked in cages in each aquarium after which the water was gradually heated up to the desired temperature, to acclimatize the fish. The fry were fed fine sieved fry mash four times a day ad libitum. A feeding ring fixed in the middle of each cage was provided to avoid scattering of food and excess fouling of the cage. A plastic bowl fixed at the bottom of each cage was also provided to prevent food easily passing through the bottom of the cage (similar earlier trials without the plastic bowls had resulted in retarded growth). Daily monitoring of temperature was done using a Jenway digital thermometer (Model 9070). The duration of temperature treatment was from 21 to 30 days encompassing the estimated labile period for sexual differentiation (Nakamura and Takahashi, 1973). The duration of some of the experiments/replicates was unavoidably extended up to 30 days, although the sex was assumed to be differentiated by 21 days after first feeding. It was not possible to determine if there was difference in sex ratio of those replicates treated beyond 21 days but no clear trends were apparent.

In the salinity experiment, after the fry were stocked, the salinity was gradually increased (2–3 ppt/application; once in morning and once in the afternoon or 4–6 ppt/day) by dissolving commercial grade crystallized unionized salt (2.5 g/l of water or a total of 100 g/aquarium) until the desired level was attained. The water used was not changed throughout the experiment but regular siphoning of waste products was carried out. Daily monitoring of salinity level was done using a refractometer to adjust

and maintain the desired salinity level. Fine sieved fry mash was given four times a day ad libitum.

At the end of every experiment, the temperature or salinity levels were slowly brought back to a normal level by adding cool or fresh water, respectively. The fish were then taken out from either the cages or aquaria and counted, after which they were stocked and grown on for sexing in 1 m^3 fine mesh cages installed in earthen ponds. When the fish had reached 60 days old and/or had an average weight of > 2 g, they were sexed by gonad squash (Guerrero and Shelton, 1974).

Prior to statistical analysis, all percentage data were arcsine transformed. The percent decrease of females and males in the treated progeny of XXΔ and YY males, respectively, were expressed as the difference between the sex ratio of the treated and control progeny as a proportion of the sex ratio of the control. The significance of the difference between the survival rate of the treated and control progeny was tested by paired-samples *T*-test. The sex ratio of the treated progeny was compared to the sex ratio of the control using a 2×2 contingency χ^2 test. The effect of female source (strain) on the percent decrease of females in the treated progeny of the XXΔ males was determined by a two-way analysis of variance (ANOVA). The difference between the percent decrease of female progeny of the XXΔ males and between the percent decrease of male progeny of the YY males (Table 1) and the data from experiment involving more than two replicated treatments (Tables 2 and 4) were analyzed by a one-way ANOVA.

3. Results

Table 1 summarizes the percentage survival of the purebred and crossbred putative all XX female and all XY male progeny after temperature treatment and the percentage of males after sexing. For the putative all-female progeny, the mean percentage survival of the treated fish (62.72%) was significantly lower ($P = 0.035$) than of the control (71.86%), however, the difference was not large. The sex ratios of the controls were not all 100% females as expected, with the proportion of males ranging up to 36.84%. High temperature caused a remarkable decrease in the percentage of females of the treated fish compared to the untreated control progeny with differences ranging from 0 to 88.62%. The percentage of females was lower in the treated progeny than in controls in 28 of the 30 replicates tested, with the difference being significant ($P < 0.01$) in 18 of these families.

Comparing the percent decrease of females of the different purebreds and crossbreds, the progenies sired by the XXΔ males in the ES strain (55.77%) were observed to be more sensitive ($P = 0.003$) to high temperature compared to the progeny of the XXΔ males in the EI strain (0.42%). The progeny produced by the GB XXΔ males were not included in the two-way ANOVA as they were crossed to only one of the female strains used in the analysis. The thermosensitivity of the progeny of the two groups of XXΔ males (ES and EI) was not affected ($P = 0.306$) by the source of females (Fig. 1). Comparing the three groups of XXΔ males (regardless of female source), the percent decrease of females in the treated progeny of ES males (55.77%) was significantly

Table 1
Summary of the survival and sex ratio (% ♂) of purebred and crossbred putative all-female and all-male progeny from XX♀ × XXΔ♂ and XX♀ × YY♂ crosses reared at elevated (36.54 ± 0.39°C) and at ambient (27.87 ± 1.40°C) temperatures during sexual differentiation

Strain cross (♀:♂)	Genotypic cross	Replicate	Number stocked	Treated		Control		2×2 χ^2 value
				Percent survival (%)	Percent male (%)[1]	Percent survival (%)	Percent male (%)[1]	
ES×ES	XX×XX	1	100	33.67	49.40 (28)	89.33	2.96 (56)	77.36***
		2	100	80.67	40.0 (33)	79.67	3.85 (43)	52.95***
		3	100	82.67	79.58 (47)	66.98	20.0 (52)	102.9***
		4	100	71.0	63.08 (43)	80.67	26.12 (45)	35.04***
KT×ES	XX×XX	1	100	72.33	42.86 (44)	94.0	21.40 (76)	17.69***
		2	100	85.0	89.58 (48)	92.33	8.46 (67)	222.9***
		3	100	84.67	77.86 (47)	64.33	36.84 (25)	33.98***
EI×ES	XX×XX	1	100	73.81	70.83 (40)	92.38	3.23 (52)	137.4***
		2	100	79.33	28.73 (60)	79.67	4.24 (39)	26.21***
		3	250	31.60	71.43 (21)	9.67	0.0 (5)	5.77***
EI×EI	XX×XX	1	100	61.33	19.50 (53)	78.0	5.53 (66)	15.33***
		2	100	35.0	16.67 (30)	78.0	2.14 (47)	14.07***
		3	100	74.33	0.0 (37)	74.0	0.0 (44)	0.0[ns]
		4	100	64.0	12.23 (46)	92.33	20.81 (58)	3.74[ns]
ES×EI	XX×XX	1	100	53.0	40.0 (45)	54.67	6.02 (44)	28.47***
		2	100	61.06	3.23 (31)	87.61	0.0 (23)	0.02[ns]
		3	175	42.86	16.13 (31)	N/A	0.0 (33)	3.75[ns]
		4	300	46.67	8.82 (34)	N/A	0.0 (34)	1.39[ns]
		5	300	48.0	2.27 (44)	23.0	0.0 (15)	0.32[ns]
KT×EI	XX×XX	1	100	85.0	81.82 (40)	83.0	36.75 (39)	48.34***
		2	100	74.33	22.63 (46)	83.33	7.58 (44)	10.65**
GB×GB	XX×XX	1	100	78.33	22.38 (48)	96.67	3.97 (50)	20.5***
		2	100	–	20.0 (17)	N/A	2.25 (30)	10.64**
		3	100	36.36	12.50 (16)	N/A	3.33 (30)	0.33[ns]
EI×GB	XX×XX	1	100	73.50	11.22 (49)	99.50	1.52 (66)	8.21**
		2	100	35.56	16.67 (6)	55.11	2.04 (49)	0.42[ns]
		3	300	14.0	33.33 (24)	7.33	0.0 (8)	2.0[ns]
		4	200	53.19	12.50 (24)	57.98	0.0 (43)	3.08[ns]
		5	300	27.33	15.09 (53)	N/A	6.90 (29)	0.54[ns]
		6	300	27.33	7.69 (26)	N/A	0.0 (42)	1.18[ns]
ES×ES	XX×YY	1	100	60.0	88.80 (42)	96.67	96.05 (51)	6.52*
		2	100	60.67	61.17 (34)	9.67	98.06 (52)	63.35***
		3	100	48.33	98.89 (30)	92.33	100.0 (58)	6.0*
ES×EI	XX×YY	1	300	40.33	80.95 (21)	N/A	100.0 (24)	7.64**
		2	300	69.33	32.26 (31)	N/A	100.0 (25)	30.06***
EI×EI	XX×YY	1	250	63.03	100.0 (30)	21.01	100.0 (38)	0.0[ns]
		2	300	90.0	100.0 (20)	24.0	100.0 (23)	0.0[ns]
ES×EI	XX×YY	1	300	63.67	100.0 (48)	N/A	100.0 (42)	0.0[ns]
GB×GB	XX×YY	1	150	62.76	100.0 (26)	17.24	100.0 (13)	0.0[ns]
		2	125	76.0	45.45 (11)	44.80	100.0 (16)	14.60***
		3	100	74.44	100.0 (24)	52.22	100.0 (18)	0.0[ns]
		4	300	23.33	100.0 (20)	6.67	100.0 (12)	0.0[ns]
		5	300	36.67	100.0 (21)	69.33	100.0 (15)	0.0[ns]
EI×GB	XX×YY	1	250	53.60	100.0 (39)	N/A	100.0 (7)	0.0[ns]
		2	110	83.64	100.0 (36)	N/A	100.0 (44)	0.0[ns]

Table 2
Summary of results of a study aimed at determining the effect of three temperatures on sex ratio of putative all-female (GFT), all-male (GMT), and all-YY males (YY) in the ES strain

Genotype	Temperature	Average temperature ± s.d. (°C)	Percent survival (%)	Male	Female	Intersex	Percent male (%)
GFT	low	25.51 ± 1.81	89.0[a]	11	79	0	12.22[a]
GFT	normal	29.57 ± 0.23	58.33[b]	21	83	0	20.19[ab]
GFT	high	37.48 ± 0.86	75.00[c]	41	69	0	37.27[b]
GMT	low	25.88 ± 1.29	84.00[ab]	69	0	0	100.0[a]
GMT	normal	30.07 ± 0.27	90.67[a]	98	0	0	100.0[a]
GMT	high	37.12 ± 0.83	70.33[b]	82	13	0	86.32[a]
YY	low	25.96 ± 1.14	84.33[a]	110	0	0	100.0[a]
YY	normal	29.68 ± 0.18	90.67[a]	119	0	0	100.0[a]
YY	high	36.51 ± 1.54	53.00[b]	8	97	0	7.62[b]

The mean sex ratios for each genotype were compared using a one-way ANOVA.
Values marked with the same letter superscript are not significantly different at 5% level.

higher than that of the EI (20.42%) and GB males (14.84%); the latter two were not significantly different.

For the putative all XY males, the treated progeny have a significantly ($P < 0.05$) better mean survival than the control (59.52% vs. 35.09%). In some replicates, the treatment was carried out during cold months, which caused the low survival of the control, unlike the treated groups, which were maintained at elevated temperature. Except for two replicates, all the controls had 100% male progeny. The effect of high temperature was evidenced by a decrease in the percentage of males of the treated progeny in some replicates, but the majority did not show any response. Five of the six replicates, which responded to high temperature, were sired by YY males of the ES strain. The percent decrease of male progeny in families sired by ES YY males (26.61%) was higher than of those sired by the GB (7.80%) and EI YY males in which there was no response. However, in spite of the clear differences, the one-way ANOVA was not significant, even after transformation, due to the high within group variability.

The result of determining the extent of the effect of low, normal and high temperatures on the sex ratio of putative all-female (GFT), all-male (GMT) and all YY male (YY) progeny in the ES strain (Experiment 2) are presented in Table 2. The three genotypes survived differently under the three temperatures. Except for the GFT, where, inexplicably, the lowest survival was observed at normal temperature, the trend was

Notes to Table 1:
Survival data relate the number of fish surviving after a minimum of 21 days temperature treatment. Replicate denotes the number of batches or families tested from each strain cross.
ES = Egypt–Swansea; EI = Egypt–ICLARM; KT = Kenya–Turkana; GB = Ghana–BFAR.
[1]Values in parenthesis are the number of fish sexed.
N/A = Data not available.
χ^2 Value = represents the deviation of sex ratio of the treated progeny from that of the control.
ns = Not significantly different; $*$ $P < 0.05$; $**$ $P < 0.01$; $***$ $P < 0.001$.

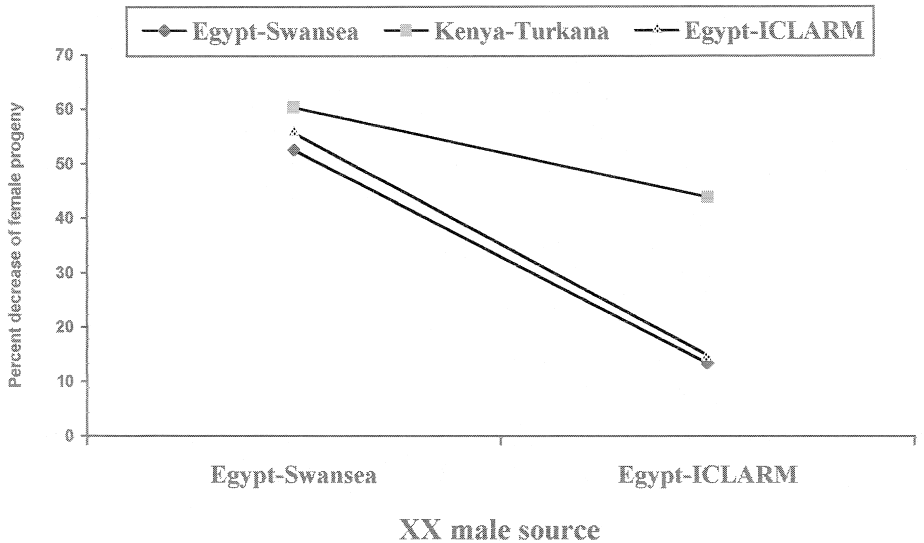

Fig. 1. Graph showing the percent decrease of females in the treated progeny of the XXΔ males in ES and EI strains crossed to females in ES, KT and EI strains. Two-way ANOVA showed the source of females had no effect on the percent decrease of female progeny of the XXΔ males ($P > 0.05$). The percent decrease of female progeny from the two XXΔ male strain were significantly different $P = 0.003$).

similar in both the GMT and YY with survival lowest at high temperature. At low temperature, a sex ratio response was seen only in the GFT with a 39.47% decrease in the percentage of males compared to the normal temperature control but this difference was not significant. At high temperature, the percentage of males of the YY genotype deviated highly significantly ($P < 0.001$) from those reared at normal temperature, with a difference of 92.38%. There was also a marked increase in the percentage of males in the GFT and a decrease in the male percentage in the GMT but the differences (13.68 and 17.08%, respectively) were not significant. This result was contrary to expectation, the YY males had been thought to be the most resistant to temperature effects and to be consistently all-male. The proportion of males in the high temperature treated GMT was significantly higher ($\chi^2 = 124.8$; $P < 0.001$) than that of the high temperature treated YY genotypes, indicating a much greater effect of temperature on sex differentiation in the latter genotype.

It is hypothesized that the YY male line in ES strain is more inbred than the other strains due to bottleneck effects associated with introductions and as a direct result of the breeding programme for the production of YY males. Thus a further testing of the effect of high temperature on sex ratio was carried out on purebred and crossbred YY males to determine any differential effect on inbred and outbred fish (with consideration for possible confounding strain effects). A total of 13 families of pure ES YY males and 10 crossbred YY males from crosses of YYΔ ♀ ES and YY♂ EI were tested at ambient (28.75 ± 0.7°C) and elevated (36.51 ± 0.1°C) temperatures (see Table 3). There were no survival data for the control of the purebred YY males as the families used were part of

Table 3
Summary of survival and sex ratio of purebred and crossbred putative all-YY males from crosses of YYΔ ♀ × YY♂ reared at elevated (36.51 ± 0.10°C) and at ambient (28.75 ± 0.67°C) temperatures during the period of sex differentiation. Replicate denotes the number of batches or families tested in each strain cross

YY × YY cross (♀ × ♂)	Replicate	Number stocked	Treated		Control		2×2 χ^2 value
			Percent survival (%)	Percent male (%)[1]	Percent survival (%)	Percent male (%)[1]	
ES × ES	1	150	33.30	40.54 (37)	N/A	100.0 (47)	37.50***
	2	100	61.0	46.67 (30)	N/A	100.0 (48)	29.95***
	3	100	72.0	71.43 (14)	N/A	100.0 (63)	18.16***
	4	100	50.0	25.0 (20)	N/A	100.0 (35)	39.97***
	5	75	44.0	5.56 (18)	N/A	100.0 (36)	54.10***
	6	100	52.0	100.0 (16)	N/A	100.0 (47)	0.0[ns]
	7	100	48.0	47.22 (36)	N/A	100.0 (54)	36.75***
	8	100	50.0	42.86 (14)	N/A	100.0 (45)	22.87***
	9	100	85.0	95.65 (23)	N/A	100.0 (42)	5.84*
	10	100	79.0	53.85 (13)	N/A	100.0 (27)	18.50***
	11	150	66.0	76.92 (13)	N/A	100.0 (41)	14.90***
	12	60	90.0	25.81 (31)	N/A	100.0 (42)	49.0***
	13	150	37.67	28.57 (42)	N/A	100.0 (100)	142.91***
ES × EI	1	300	67.33	100.0 (12)	70.0	100.0 (54)	0.0[ns]
	2	195	87.18	100.0 (17)	78.46	100.0 (59)	0.0[ns]
	3	280	45.94	100.0 (33)	48.76	100.0 (45)	0.0[ns]
	4	240	90.83	100.0 (56)	49.17	100.0 (87)	5.70*
	5	150	48.67	88.46 (52)	N/A	100.0 (42)	0.0[ns]
	6	150	75.33	100.0 (16)	83.61	100.0 (36)	0.0[ns]
	7	110	55.56	100.0 (22)	75.45	100.0 (50)	0.0[ns]
	8	240	84.49	97.30 (37)	57.14	100.0 (39)	4.16*
	9	150	68.67	100.0 (21)	N/A	100.0 (74)	0.0[ns]
	10	150	51.33	100.0 (35)	N/A	100.0 (54)	0.0[ns]

ES = Egypt–Swansea, EI = Egypt–ICLARM.
[1]Values in parenthesis are the number of fish sexed.
N/A = Data not available.
χ^2 Value = represents the deviation of sex ratio of the treated progeny from that of the control.
ns = Not significantly different; * $P < 0.05$; *** $P < 0.001$.

an on going selection programme. After splitting each family into two, the controls were not reared in the same culture system as the treated fish instead they were stocked directly in cages and sexed manually when they were large enough. For the crossbred YY males, no significant difference ($P > 0.05$) in mean percentage of survival of the treated and the control progeny (72.38% and 66.08%, respectively) was observed. No unexpected females were observed in all the controls including those of the crossbred. In the treated purebred YY male group, females were observed in 12 replicates at significant percentages (4.35 to 74.19%) and only one replicate had no response. Subsequent trial spawning of YYΔ females, sex-reversed by temperature treatment, have shown some to be reproductively viable indicating that high temperatures are inducing functional sex reversal. For the crossbred YY males, females were also observed in significant proportions but only in two treated replicates and the percentages were smaller than for the purebreds (11.54% and 2.7%).

Table 4
Summary of results of an experiment aimed at determining the effect of different salinities on the sex ratio of putative all-female progeny from XX♀ × XXΔ♂ crosses in the ES and EI strains

Strain	Average salinity ppt(±s.d.)	Percent survival (%)	Male	Female	Intersex	Percent female (%)
Egypt–Swansea	12.75 (±0.80)	65.73[a]	4	204	0	98.08[a]
	19.87 (±0.91)	78.13[a]	14	247	0	94.64[a]
	26.75 (±0.13)	79.60[a]	6	120	0	95.24[a]
	0	62.40[a]	23	179	0	88.61[a]
Egypt–ICLARM	11.30 (±0.26)	66.19[a]	51	181	0	78.02[a]
	18.34 (±0.45)	87.74[a]	45	147	0	76.56[a]
	24.34 (±0.88)	71.07[a]	38	172	0	81.90[a]
	0	26.31[b]	26	107	0	80.45[a]

The percentage of survival and percentage of females within strain were compared using a one-way ANOVA. Values within a column marked with the same letter superscript are not significantly different at 5% level.

For the effect of salinity on sex ratio, separate tests were performed using putative all-female progeny from the ES and EI strain. The survival rates of the progeny of ES under the different salinity levels were not significantly different while significant differences were observed for the EI strain ($P = 0.002$). The control groups of EI suffered high mortality caused by bacterial infection during the treatment period. No significant effect of salinity on sex ratio was observed (Table 4).

4. Discussion

The results presented here provide evidence of a significant effect of temperature on sex ratio in Nile tilapia (*O. niloticus*), confirming results obtained for this and other tilapia species in previous studies (Mair et al., 1990; Baroiller et al., 1995; Baroiller et al., 1996; Mbahinzireki and Dabrowski, 1997). Not all the progenies tested were affected by the increase in temperature, supporting the observation of Baroiller et al. (1995, 1996) that different genotypes have different sensitivity to environmental effects on sex ratio. Unlike previous studies where only putative all-female (Baroiller et al., 1995, 1996) or mixed-sex fish (Mair et al., 1990; Baroiller et al., 1995; Mbahinzireki and Dabrowski, 1997) were used, in this study we present evidence that high temperature can influence sex ratio not only in the direction to male but also to female.

The observed sensitivity of the fish used in these studies to high temperature is likely to be related to effects during sexual differentiation. There are two possible developmental pathways whereby temperature can affect this process. First, an environmental shock such as high temperature might disrupt the normal development processes during sex differentiation causing the switch to males for the genetic females and switch to females for the genetically male progeny. Second, the high temperature might have an effect on the structure or action of a hormone or hormones acting during sex differentiation (Hunter and Donaldson, 1983). For example, Wibbels et al. (1994) discuss the potential

effects of temperatures on the action of aromatase (the catalyst for the breakdown of androgens to estrogens) in temperature dependent sex determination in reptiles.

It has been observed by Varadaraj et al. (1994), Abucay (1997) and Dong Soo Kim (personal communication) that elevating the temperature as high as 36°C during hormone treatment can increase the rate of sex reversal. The apparent absence of a salinity effect on sex ratio may be due to lack of influence of salinity on the development processes during sex differentiation. Further investigation to include putative all-male progeny should be carried out to produce a more conclusive result as the present study evaluated only the effect of salinity on sex ratio in putative all-female progeny.

There are numerous observations in the literature on tilapia of sex ratios deviating from the norm of a 1:1 or from ratios predicted from sex-reversed fish (Calhoun and Shelton, 1983; Majumdar and McAndrew, 1983; Shelton et al., 1983; Mair et al., 1991) and these are commonly explained on the basis of autosomal or polygenic effects. As most of the progenies examined in these studies were reared in either controlled or partially controlled conditions with minimal temperature fluctuations caution must be used in attributing these deviations to temperature effects. However, based on our results we theorize that the environment effect may enhance the expression of the effect of genetic sex factors. According to Kirpichnikov as cited by Beamish (1993), male and female genes may be located in many chromosomes and the determination of sex depends on a balanced effect of these genes. A deviation from normal environmental conditions such as the increase of temperature during the labile period of sex differentia-

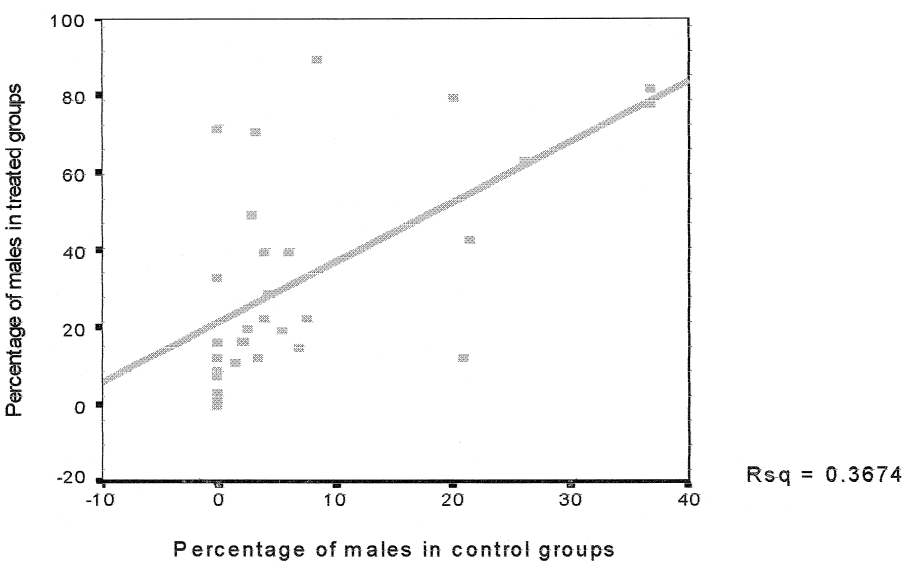

Fig. 2. Scatter diagram showing the relationship between the proportion of males in temperature treated and untreated control of the putative all-female progeny presented in Table 1. The figure demonstrates a positive correlation in the observed percentage of males of the two groups ($r = 0.653$).

tion could result in an unexpected sex ratio among individuals carrying a differential loading of autosomal sex modifying genes.

In Table 1 it can be seen that the majority of the control replicates for the putative all-female progeny contained some males and those replicates that had a high percentage of males also had a high percentage of males in the treated group. The percentage of males for the treated and control groups are plotted in Fig. 2. A highly significant positive correlation pattern ($n = 30$, $r = 0.653$, $P < 0.001$) was observed. The presence of males in the control groups could suggest the presence of a substantial influence of male modifying factors in the progeny causing some to develop as male. In extreme conditions such as high or low temperature, the expression of these factors might be heightened causing more progeny to develop into males. For the progeny of YY males (Table 1), the percentage of males of six replicates in the treated group also decreased, particularly those of the ES strain. However, when plotting the percentage of males for the control and treated group no significant trend ($n = 15$, $r = 0.126$, $P = 0.653$) was observed (Fig. 3), possibly due to insufficient data.

The production of the all-female progeny involved the use of XXΔ males as broodstock. Based on their method of production there is the possibility of the selection for male determining factors in the production of these fish. During hormonal masculinization to produce XXΔ males it is possible that XX progeny that are carrying more male sex modifying genes tend to be sex-reversed more easily compared to other genotypes. Rothbard et al. (1990) demonstrated that newly hatched fry are carrying high

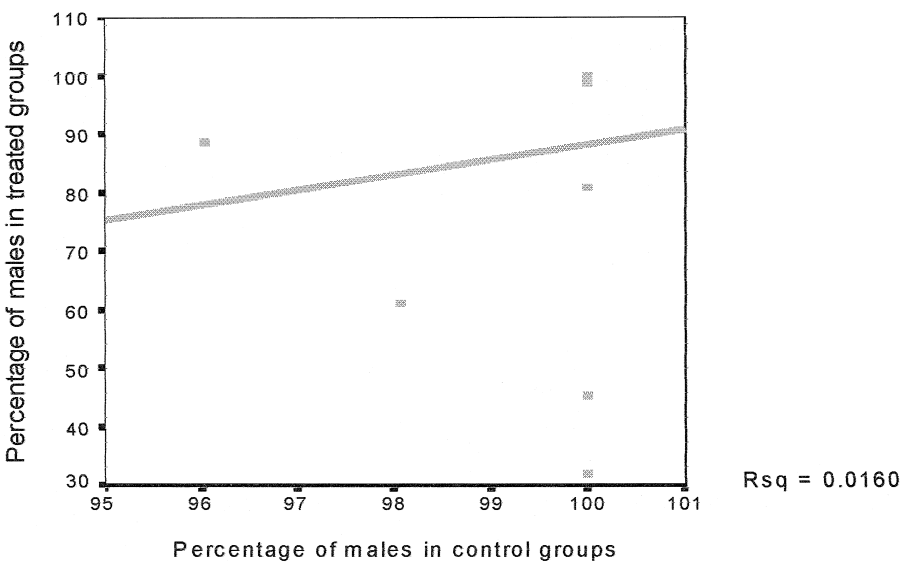

Fig. 3. Scatter diagram showing the relationship between the proportion of males in the temperature treated and untreated control putative all-male progeny presented in Table 1. No obvious trend can be observed as denoted by the low correlation coefficient ($r = 0.126$). The majority of the percentage of males in both groups were plotted at the 100% mark for the untreated controls.

levels of endogenous hormones and these levels differed among individual fish. It is possible that those fry with a higher loading of male autosomal sex modifying genes may have higher level of endogenous androgen. During hormone treatment for sex reversal, the administered exogenous hormone combined with the higher level of endogenous androgen may enable the fish to be masculinized more easily. In his experiments on progeny testing of androgen sex-reversed males Baroiller (1996) observed that the majority of males tested had significant proportions of unexpected males in their progeny. If sex-reversed fish, which are carrying more male modifying genes, are used for breeding, the loading of autosomal sex modifying genes will tend to increase in the next generation and along with this the sensitivity of the progeny to extreme temperatures. The validity of this hypothesis is currently being evaluated experimentally. This effect was the basis for the discussion of Winge (1934) of possible alteration of sex chromosomes into autosomes and vice versa.

For the YY males (Table 3) the same theory could also explain the presence of females in the treated replicates, particularly among the purebred fish. It is possible that female genes are also accumulating in the progeny. During the hormonal feminization of sexually undifferentiated YY genotypes to produce YYΔ females, those progeny that are carrying more female sex modifying genes may tend to be sex-reversed more easily compared to the other genotypes.

In the case of the normal XY males, the accumulation of male autosomal sex modifying genes in all-female and female modifying genes in YY male lines could result in more balanced genetic effect in the progeny resulting from the cross of the XX females and YY males.

In the case of the observed higher sensitivity of the purebred YY males compared to the crossbred, we suspect that the level of inbreeding of the population (ES strain) that we used in the development of the YY males may have affected their fitness and developmental stability. The possible loss of developmental stability coupled with increases in the presence of autosomal sex modifying genes could have made sex differentiation more sensitive to environmental extremes. Price (1984) cited work, which demonstrated the existence of autosomal genes affecting sex in an inbred line of platies. When XXΔ males were outcrossed to normal females the progenies were all-female as expected, however, when intracrossed, both sexes were produced in extremely variable proportions.

Purdom (1993) proposed an alternative hypothesis for the observed atypical sex ratio in inbred gynogenetic progeny. This is that sexual homeostasis is disrupted by inbreeding and that the greater level of inbreeding implicit in mitotic gynogens causes greater deviation from expected sex ratios. The loss of genetic variation in inbreeding reduces the ability of an individual to adapt to different conditions, whilst those individuals with a higher level of heterozygosity have relatively higher levels of individual homeostasis (Mitton and Grant, 1984).

In the case of the study presented here, it is not possible to separate the potential effect of inbreeding with possible strain differences in response of sex differentiation to temperature effects. In their excellent work on temperature dependent sex differentiation in the Atlantic Silverside, Conover and Kynard (1981) and Conover and Heins (1987) demonstrated different sex ratio responses to temperature in different populations of the

species. However, the experiment on temperature effects on XY males in the EI strain did demonstrate a response of sex ratio to high temperature (see Table 1) indicating that this strain is also susceptible to temperature effects. Thus, the hypothesis of reduced developmental stability in the purebred YY males may be the most plausible hypothesis to explain the apparent minimal effect of temperature on the crossbred YY males.

In summary, this paper presents new data on the effect of temperature on sex differentiation in this commercially important tilapia species. High temperature can have profound effects on sex ratio which were clearly seen in putative monosex progeny. This paper demonstrates that high temperature can influence sex in the direction of both male and female and there appears to be a genetic basis to the susceptibility to temperature effect on sex ratio. Further research is required to determine whether normal seasonal and diurnal temperature fluctuations can affect sex ratio but environmental conditions should be considered when interpreting data from sex determination studies in tilapia.

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