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Influences of Parental Pairs on Progeny Sex Ratios of Nile Tilapia *Oreochromis niloticus*

Joon Yeong Kwon*, Hyuk Chu Kwon and David J. Penman[1]
Department of Applied Biological Sciences, Sunmoon University, Chung Nam 336-708, Korea
[1]*Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, U.K.*

Sex of the Nile tilapia *Oreochromis niloticus* is mainly determined by an XX/XY system. However, accumulating evidences suggest the existence of additional sex modifying factors including environmental, autosomal and parental influences. In order to investigate the possibility of parental effects on sex ratios of tilapia progenies, in this study, a series of crosses was carried out using gynogenetic clonal fish, neomales, normal males and females, and YY fish. Crosses between clonal XX male and clonal female have yielded only female progenies and no parental influences were observed. However, in the crosses between clonal males and normal females, female parents were significantly associated with the progeny sex ratios (χ^2=20.046, 7 d.f., P<0.01). Progeny sex ratios from the crosses between neomales and normal females (χ^2=60.491, 5 d.f and χ^2=28.072, 2 d.f.) also showed significant association with female parents (P<0.001). The stability of progeny sex ratios from repeated spawns were confirmed by using 6 different parental pairs. In 16 crosses between normal males and normal females, sex ratios of progenies showed clear maternal influences, and further analysis of the results revealed a negative correlation (r^2=0.7718, P<0.05) between the sex ratios of progenies from two different males, indicating a strong paternal influence. No statistically significant relationship between survival rates and sex ratios of progenies was observed in any genotypic groups. Taken together, the influence of parental pairs on progeny sex ratios in this species is evident although the cause of this influence is not clear.

Keywords: Tilapia, Sex determination, Parental influence, Sex ratio

Introduction

It has been proposed that sex in the Nile tilapia *Oreochromis niloticus* is mainly determined by a monofactorial system with male heterogamety (XX/XY system) (Mair et al., 1991a; Mair et al., 1997). However, this simple sex determining system (XX/XY) often fails to explain some unexpected sex ratios in this species. To explain the unexpected sex ratios, environmental influence (Baroiller et al., 1995; Abucay et al., 1999; Baroiller et al., 1999), autosomal influence (Mair et al., 1991b; Hussain et al., 1994), a polygenic system (Wohlfarth and Wedekind 1991) and parental influence (Shelton et al., 1983; Sarder et al., 1999; Tuan et al., 1999) have been suggested.

Amongst these explanations, the possibility of parental influence on progeny sex ratios has not received much attention from fish biologists and aquaculturists, although the possibility was first suggested more than two decades ago (Shelton et al., 1983). The possibility of parental influences on sex ratios has also been proposed in other animals including birds (Bradbury and Blakey 1998; Nager et al., 1999) and many mammals (Moses et al., 1995; Grant 1996; James 1996; Monard et al., 1997; Andersson and Bergstrom 1998; Kruuk et al., 1999; Fisher 1999). Parental influence on sex ratios could possibly be a common phenomenon throughout the animal kingdom including fish.

In tilapia, *O. niloticus,* Wohlfarth and Wedekind (1991) showed the evidence of the stability of sex ratio in repeated spawns of the same parental pair in intraspecific crosses. This implies the possibility of parental influences on sex ratio of progenies. A strong parental influence on the response to high temperature was also noticed in *O. niloticus* (Baroiller et al., 1995).

In contrast, Mair et al. (1991a) produced progeny from 25 different parental combinations and found no evidence for paternal or maternal influences. However, it should be noted that, among the five females that they used, one female produced consistently higher proportion of intersex (5.8~26%) with all five males compared to the proportion of intersexes from the four other females (mostly 0%, except two parental

*Corresponding author: jykwon@sunmoon.ac.kr

combinations giving 3% intersex).

The existence of parental influences on sex ratio of progenies is still in arguement. Furthermore, it is not clear whether the parental influences observed in some species was associated with genetic or non-genetic events. If there are non-genetic parental influences, it might not be easy to recognize the non-genetic parental influences under the presence of autosomal influence and temperature effect. As already mentioned, strong evidence of autosomal influences on tilapia sex determination has been reported, including an epistatic locus (SDL-2, two alleles, SR and sr: Hussain et al., 1994) and rare autosomal recessive sex-influencing genes (Mair et al., 1991a). Temperature also significantly altered sex ratios in this species (Abucay et al., 1999; Kwon et al., 2002). Tuan et al. (1999) proposed the possibility of parental influence in *O. niloticus*, but the experimental temperature was not mentioned in their report. Water temperature would need to be maintained at a range of constant temperatures in which sex determination is not influenced.

Sarder et al. (1999) established clonal lines of *O. niloticus*, and propagated them by means of mitotic gynogenesis and subsequent meiotic gynogenesis. Such gynogenetic clonal fish that should produce all female progeny in theory, even under the interference of an autosomal sex modifying gene such as SDL-2, could increase the chance to recognize non-genetic parental effects.

To investigate the possibility of parental effects on sex ratios of tilapia progenies, a series of crosses was carried out using gynogenetic clonal fish, neomales (sex reversed XX males), normal males and females, and YY fish in recirculating breeding and rearing systems where temperature was controlled.

Materials and Methods

All types of broodstock of *O. niloticus* (originated from Lake Manzala, Egypt) that were used in this study were produced in the Institute of Aquaculture, University of Stirling through a series of sex determination studies on this species. They were maintained individually in glass aquaria within a recirculating system at 28±1°C. They were fed at least twice a day with commercial trout feed (BOCM PAULS Fish Feed Group, Renfrew, UK).

To examine parental influences, a total of 12 males of four different genetic types and 18 females of three different genetic types were tested (Table 1). XX neomales were produced by dietary treatment of all female fry with 17α-methyltestosterone (MT) (SIGMA) at a dose of 50 mg kg^{-1} in diet for 1 month and progeny-tested before use. YY males were produced by crossing YY males and YY females from the YY fish lines in the Institute of Aquaculture, and some YY male fry were sex reversed by the treatment of diethylstilbestrol (DES) (SIGMA) to produce YY females. Fully inbred clonal male and female broodstocks were produced by previous research using mitotic and meiotic gynogenesis (Sarder et al., 1999). The clonal males, CM1 and CM2, were siblings of the clonal females that had produced high percentages of males in meiotic gynogenesis. A clonal male, CM3, and three clonal females were derived from all-female producing lines in previous studies (Table 2). All clonal lines were confirmed as completely homozygous and without any paternal influence by multilocus fingerprinting (Sarder et al., 1999).

Eggs were manually stripped from ovulated females. Milt were collected into glass capillary tubes and either used directly for fertilization or transferred to 1.5 ml plastic centrifuge tubes for short-term storage. After fertilization, eggs were placed into a 1 litre incubation jar and the resultant fry were transferred to an aquarium (5-L volume) in a recirculating system. When fish were 1~2 months old, they were transferred to bigger tanks (25-L volume) to allow for further growth and sexing. Fish were sexed at around 3 months old by the aceto-carmine gonad-squashing method (Guerrero and Shelton, 1974). Temperature was maintained at 28±1°C from the incubation until the end of experiments.

Sex ratio data from all crosses were first analyzed by chi-square goodness-of-fit test to determine any significant differences from 1:1 sex ratios. Data were transformed to arc-sine values when necessary. In order to determine whether parental pairs are independent of progeny sex ratios, sex ratios from the crosses between clonal males and normal females, and from the crosses between normal females and normal males were subjected to three-dimensional contingency table (2×6×3) analysis followed by chi-square tests. Afterwards, the data from single pair crosses and pooled data for each male or female parent with other females or males were again tested by two-dimensional chi-square contingency analysis to further determine which gender of parents is independent of or associated with progeny sex ratio. Statistical differences of sex ratios from repeated spawns of the same parental pairs were determined by t-test and/or chi-square analysis of 2×2 contingency tables when applicable (Zar, 1984). Regression analysis was performed to determine whether parental influences were attributed to skewed sex ratios for some

Table 1. Lists of broodstock used in this experiment

Tag No.	Genetic identity	Phenotypic sex	Identity code
00-0135-EA1B	XY normal	male	NM1
00-013C-BOEC	XY normal	male	NM2
00-013C-AFA5	XX clone, MT treated	male	CM1
00-013E-OA23	XX clone, MT treated	male	CM2
009-783-894	XX clone, MT treated	male	CM3
005-117-817	XX MT treated	male	MTM1
006-023-629	XX MT treated	male	MTM2
014-556-527	YY super	male	SM1
014-571-512	YY super	male	SM2
014-555-315	YY super	male	SM3
010-554-342	YY super	male	SM4
013-554-595	YY super	male	SM5
00-013E-12E1	XX normal	female	NF1
00-013E-3245	XX normal	female	NF2
00-013E-42E4	XX normal	female	NF3
005-831-633	XX normal	female	NF4
013-110-064	XX normal	female	NF5
00-013E-3BFC	XX normal	female	NF6
011-779-353	XX normal	female	NF7
00-013E-3A6C	XX normal	female	NF8
00-012C-13B7	XX normal	female	NF9
147-982-273	XX normal	female	NF10
Tag missing 1	XX normal	female	NF11
Tag missing 2	XX normal	female	NF12
00-013E-2F19	XX clone	female	CF1
00-013E-3466	XX clone	female	CF2
00-013E-OEDE	XX clone	female	CF3
00-013C-AD32	YY DES treated	female	E2-F-1
00-013C-B207	YY DES treated	female	E2-F-2
011-571-283	YY DES treated	female	E2-F-3

Table 2. Identities of clonal fish used in this experiment

Clones	Maternal lines	Paternal lines	Characteristics of clonal lines
CM1	002-046-539		Inbred, Occurrence of males reported*
CM2	002-046-539		Inbred, Occurrence of males reported*
CM3	010-036-092		Inbred, All female line*
CF1	009-356-316	010-036-092	Outbred, All female line*
CF2	006-812-566	010-036-092	Outbred, All female line*
CF3	006-812-566	010-036-092	Outbred, All female line*

*Further details on clonal lines and their founders can be found in Sarder et al. (1999).

crosses, and to examine the relationship between survival rates and sex ratios in three different groups of progenies (all female groups with predicted genotype XX; mixed sex groups with predicted genotype XX or XY; all male groups with predicted genotype XY or YY).

Results

Crosses between an inbred clonal male and outbred clonal females

A clonal male (CM1) was crossed to three different out-

Table 3. Sex ratios from the crosses between a clonal male (XX) and clonal females (XX)

Parents		Progenies		
Male	Females	♂	♀	%♂
CM1	CF1	0	37	0.00
	CF2	0	40	0.00
	CF3	0	16	0.00
Overall		0	93	0.00

bred clonal females. Both maternal and paternal lines of the clonal females were identified as all female producing clonal lines in the previous studies. No male progeny were observed in any of the crosses (Table 3).

Crosses between clonal males and normal females

Three clonal males, CM1, CM2 and CM3 were crossed to eight normal females NF1-8 (Table 4). Under the assumption that CM1 and CM2 are homozygous for a recessive sex modifying allele (i.e., XX srsr), the crosses between CM1 or CM2 and normal females were expected to produce some male progenies depending on the genotype of normal females for the sex modifying locus. As expected, the crosses between CM1 or CM2 and normal females produced some male progenies with variations between females mated (0~33.3% males for CM1; 0~31.8% males for CM2). However, the assumption was undermined by the occurrence of some males (0~17.9% depending on female parents) in the progenies of normal female parents and the clonal male parent (CM3) that was expected to produce 100% female progeny based on the assumption that this clonal line posseses a homozygous dominant genotype to the autosomal sex modifying gene (i.e., XX SRSR).

Chi-square analysis of three-dimensional contingency table (2×6×3) for sex ratio from three clonal males and six normal females, NF1-6 revealed that male and female parents, and progeny sex ratios are not mutually independent of each other (χ^2=90.075, 27 d.f., P<0.001). Further two-dimensional contingency table analyses using pooled data for 3 male parents (2×3) and for 8 female parents (2×8) were performed. Female parents were significantly associated with the progeny sex ratios (χ^2=20.046, 7 d.f., 0.005<P<0.01), whereas male parents were not strongly associated with the progeny sex ratios (χ^2=4.407, 2 d.f., 0.05<P<0.15).

Crosses between neomales and normal females

Two neomales (MTM1 and MTM2) which were not related to the established clonal lines, were crossed to either six normal females or three normal females (Table 5). The proportion of males in the progenies varied with female parents (7.8~59.2% in MTM1; 0-60.9% in MTM2).

Progeny sex ratios from MTM1 (χ^2=60.491, 5 d.f.) and MTM2 (χ^2=28.072, 2 d.f.) showed significant association with female parents (P<0.001), but not with male parents when analysed by 2×2 contingency table using pooled data (χ^2=0.955, 1 d.f., P>0.25). However, the result of this analysis seemed to be biased by an extreme outlier NF10. When sex ratios from 5 females were further analysed by 2×5 contingency table after omitting the data from NF10, the association of female par-

Table 4. Sex ratios from the crosses between clonal males (XX) and normal females (XX). Ratios expressed as male:female, with percentage of males in parentheses

Parents	*Clonal males*			Pooled for each female parent
	CM1	CM2	CM3	
Normal females				
NF1	0:15 (0)	4:26 (13.3)	2:57 (3.4)	6:98 (5.8)
NF2	0:12 (0)	1:10 (9.1)	0:8 (6.7)	1:30 (3.2)
NF3	1: 8 (11.1)	7:15 (31.8)	0:17 (0)	8:40 (16.7)
NF4	5:10 (33.3)	2:16 (11.1)	1:23 (4.2)	8:49 (14.0)
NF5	8:20 (28.6)	0:19 (0)	12:55 (17.9)	20:94 (17.5)
NF6	2:21 (8.7)	0:17 (0)	0:20 (0)	2:58 (3.3)
NF7	NT	0:21 (0)	5:41 (10.9)	5:62 (7.5)
NF8	0: 3 (0)	2:45 (4.3)	NT	2:48 (4.0)
Pooled for each male parent	16:89 (15.2)	16:169 (8.6)	20:221 (8.3)	52:479 (9.8)
	χ^2 for male parents = 4.407, 2 d.f.			χ^2 for female parents = 20.046, 7 d.f.*

NT: not tested.
*Significantly associated with progeny sex ratios (P<0.01).

Table 5. Sex ratios from the crosses between neomales (XX: sex reversed genetic females by MT treatment) and normal females (XX)

♂ parents	♀ parents	Progeny sex ratios ♂	♀	%♂	χ^2 values for female parents
MTM1	NF2	6	71	7.8	
	NF4	34	162	17.3	
	NF7	21	81	20.6	= 60.491, 5 d.f.
	NF10	29	20	59.2	(from 2×6 contingency table)*
	NF11	18	130	12.2	
	NF12	3	28	9.7	
Pooled		111	492	18.4	
MTM2	NF9	0	9	0.0	
	NF10	14	9	60.9	= 28.072, 2 d.f.
	NF12	1	31	3.1	(from 2×3 contingency table)
Pooled		15	49	23.4	
χ^2 values for male parents = 0.955, 1 d.f. (from 2×2 contingency table)					

*Significantly associated with progeny sex ratios (P<0.001).

ents with progeny sex ratio (χ^2=8.178, 4 d.f., 0.05<P<0.10) was not as strong as when NF10 was included.

Sex ratios from repeated spawns

The stability of sex ratios from repeated spawns was examined using XX to XX cross (MTM1×NF2 and MTM1×NF4: all female progenies are expected), XY to XX cross (NM1×NF1 and NM2×NF3: mixed sex progenies are expected) and YY to XX cross (SM1×NF5 and SM3×NF12: all male progenies are expected). None of these repeated crosses produced significantly heterogeneous sex ratios (Fig. 1). All sex ratios of two repeated spawns from MTM1×NF2 and MTM1×NF4 were not significantly different from 100% females (t-test, P>0.05). Chi-square analyses of 2×2 contingency tables for the sex ratios of these crosses also did not show any significant association of different spawning with progeny sex ratios (χ^2= 1.322, 1 d.f.; χ^2=1.695, 1 d.f., P>0.10 for both). Repeated crosses of NM1×NF1 (χ^2=0.035, 1 d.f.) and NM2×NF3 (χ^2= 0.035, 1 d.f.) were not associated with progeny sex ratios (P>0.75 for both), apart from the significantly different sex ratios for NM2×NF3 to 1:1 sex ratios (t-test, P<0.05). Sex ratios from repeated YY to XX crosses were not statistically tested since all data repeatedly showed 100% males. Parental influences were also noticed in XX×XX crosses (χ^2=4.036, 1 d.f., P<0.05) and XY×XX crosses (χ^2=4.856, 1 d.f., P<0.05) as demonstrated earlier and later in this study.

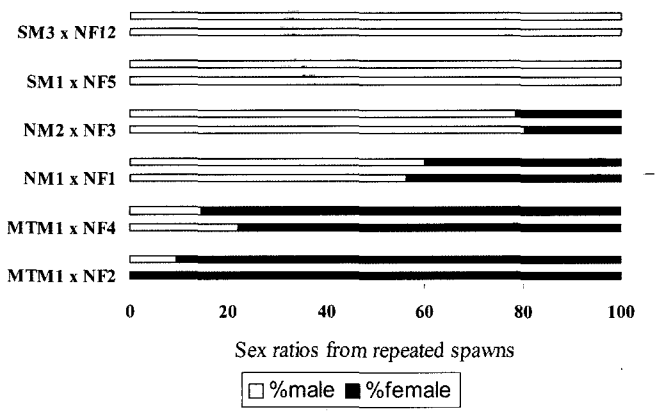

Fig. 1. Progeny sex ratios from repeated spawns. MTM1×NF2 and MTM1×NF4 (XX×XX): progenies are genetically all females; NM1×NF1 and NM2×NF3 (XY×XX): sex ratios of progenies are expected to be *1:1 (genetically mixed sex groups)*; SM1×NF5 and SM3×NF12 (YY×YY): progenies are genetically all males.

Crosses between normal males and normal females

Among 16 crosses between normal males and normal females, sex ratios of progenies from 6 crosses were significantly different to 1:1 sex ratio (P<0.05, 0.01 or 0.001, chi-square goodness-of-fit analysis, χ^2 values not shown) (Table 6). Sex ratios from NM1 with different normal females were homogeneous (χ^2=8.519, 7 d.f., P>0.25, heterogeneity chi-square analysis), while sex ratios from NM2 were heterogeneous (χ^2=30.133, 7 d.f., P<0.001, heterogeneity chi-square analysis), suggesting the possibility of paternal influences in sex determining process.

On the other hand, maternal influence was once again observed among different females. Overall sex ratios from these crosses were significantly different from 1:1 sex ratios (χ^2=18.000, 1 d.f., P<0.001, chi-square goodness-of-fit analysis) and NF3 and NF6 were main contributor to this skewness. Without sex ratios from NF3 and NF6, overall sex ratios

Table 6. Sex ratios from normal cross (XX female×XY male). Ratios expressed as male:female, with percentage of males in parentheses

Female parents (XX)	Male parents (XY)		
	NM1	NM2	Total
NF1	15 : 11 (57.7)	16 : 12 (57.1)	31 : 23 (57.4)
NF2	15 : 14 (51.7)	9 : 9 (50.0)	24 : 23 (51.1)
NF3	7 : 9 (43.6)	59 : 15 (79.7)***	66 : 24 (73.3)***
NF4	10 : 6 (62.5)	16 : 9 (64.0)	26 : 15 (63.4)
NF5	34 : 34 (50.0)	4 : 0 (100.0)^{NT}	38 : 34 (52.8)
NF6	10 : 14 (41.7)	18 : 5 (78.3)**	28 : 19 (59.6)
NF7	13 : 4 (76.5)*	8 : 22 (26.7)*	21 : 26 (44.7)
NF8	NT	24 : 11 (68.6)*	24 : 11 (68.6)*
NF9	12 : 5 (70.6)	NT	12 : 5 (70.6)
Total	116 : 97 (54.5)	154 : 83 (65.0)***	270 : 180 (60.0)***

NT: not tested.
*Significantly different from 1:1 sex ratio ($P<0.05$, chi-square goodness-of-fit analysis).
**Significantly different from 1:1 sex ratio ($P<0.01$, chi-square goodness-of-fit analysis).
***Significantly different from 1:1 sex ratio ($P<0.001$, chi-square goodness-of-fit analysis).

were not significantly different from 1:1 sex ratio ($\chi^2=3.769$, 1 d.f., $P>0.05$, chi-square goodness-of-fit analysis).

Chi-square analyses of 2×8 contingency tables for the sex ratios of NM1 and NM2 with different females also showed that progeny sex ratios were significantly associated with different parental pairs (not in NM1 $\chi^2=8.587$, 7 d.f., $P>0.25$; but in NM2 $\chi^2=33.104$, 7 d.f., $P<0.001$).

Maternal influence on progeny sex ratios was obvious in crosses between clonal males and normal females, and between neomales and normal females. However, no clear paternal influence was observed. Thus, the sex ratio data from the progeny of two normal males (NM1 and NM2) that were mated to 6 normal females (NF1, NF2, NF3, NF4, NF6 and NF7) were further analysed to determine whether the parental influences observed here were caused only by maternal side or also by paternal side (data from Table 6). Regression analysis revealed a negative correlation ($r^2=0.7718$, $P<0.05$, ANOVA) between the sex ratios of progenies from two males (NM1 and NM2), indicating a strong paternal influence on progeny sex ratios (Fig. 2).

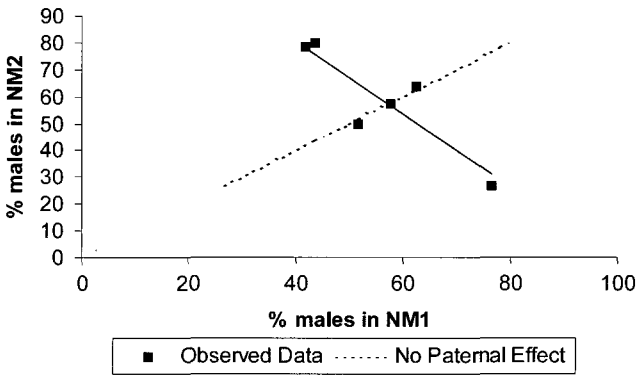

Fig. 2. Regression analysis of progeny sex ratios from two normal males that were mated to 6 normal females (data from Table 6). If there is no parental influence at all, theoretically sex ratios should be 1:1. If there is only maternal influence, the sex ratios between two males should show a positive correlation. This analysis indicates that the sex ratios observed are the consequence of an interaction between maternal factors and paternal factors. The broken line (-----) indicates a theoretical correlation between the sex ratios when there are no paternal influences.

Crosses between YY males and normal females or YY females

All crosses between YY parents or YY and XX resulted in 100% males with two exceptions of 95 and 96.7% (2 out of 18 crosses) (Table 7). Sex ratios were all significantly different from 1:1 ($P<0.001$, χ^2 values not shown). Among 393 progenies sexed, only 2 fish were identified as females. No parental influence was found in any of these crosses.

Survival rates versus sex ratios

Survival rates of progenies from all crosses were 3.7~100.0% (42.1±28.9% in genetically all female groups), 6.7~81.3% (33.2±20.3% in genetically mixed sex groups) and 5.6~100.0% (57.2±27.8% in genetically all male groups) (Fig. 3). No statistically significant relationship between survival rates and sex ratios of progenies was observed in any genotypic groups when the data were subjected to regression analysis ($r^2<0.100$ and $P>0.05$ for all).

Table 7. Sex ratios from the crosses between YY males to normal females (XX) or YY female. Ratios expressed as male:female, with percentage of males in parentheses

Parents		Progeny	Parents		Progeny
♂	♀	Sex ratios	♂	♀	Sex ratios
SM1 (YY)	NF1 (XX)	13:0 (100)	SM1 (YY)	E2-F-1 (YY)	30:0 (100)
SM1	NF2 (XX)	20:0 (100)	SM1	E2-F-2 (YY)	126:0 (100)
SM1	NF3 (XX)	45:0 (100)	SM1	E2-F-3 (YY)	73:0 (100)
SM1	NF4 (XX)	6:0 (100)	SM2 (YY)	NF3 (XX)	15:0 (100)
SM1	NF5 (XX)	41:0 (100)	SM3 (YY)	NF10 (XX)	20:1 (95.2)
SM1	NF6 (XX)	3:0 (100)	SM3	NF12 (XX)	29:0 (100)
SM1	NF7 (XX)	40:0 (100)	SM4 (YY)	NF10 (XX)	14:0 (100)
SM1	NF8 (XX)	30:0 (100)	SM5 (YY)	NF12 (XX)	29:1 (96.7)
Total					391:2 (99.5)

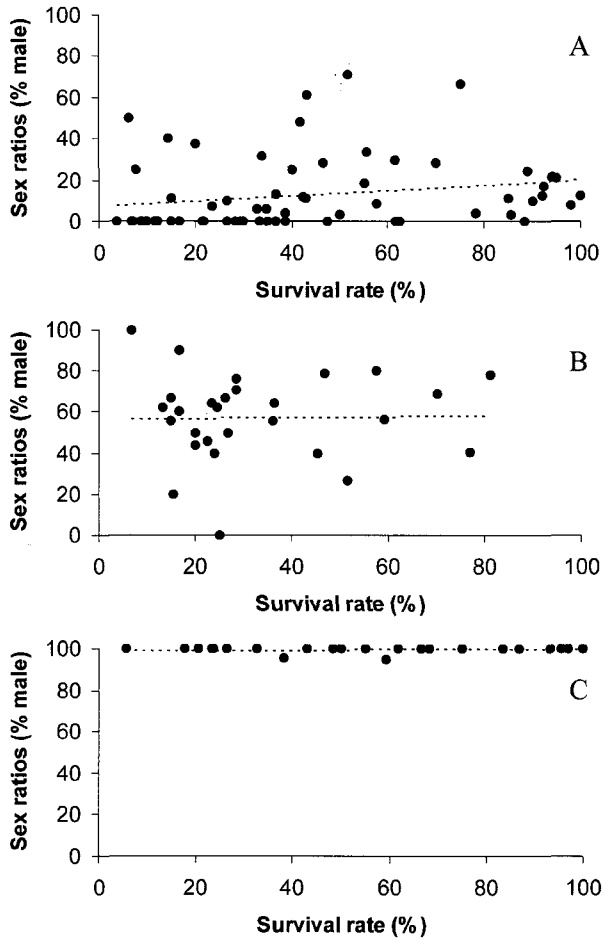

Fig. 3. Regression analysis of progeny sex ratios and survival rates. A: genetically all female progeny; B: mixed sex groups, C: genetically all male progeny.

Discussion

There appears to be strong parental influences on progeny sex ratios in this species. Not only in normal crosses but also in crosses using gynogenetic clonal males or neomales that should produce all genetically female progeny under the same temperature, sex ratios of progenies were significantly associated with parental pairs.

This observation furthers the previously proposed possibility of parental influence on progeny sex ratio from the crosses between normal males and normal females in tilapia species (Shelton et al., 1983; Tuan et al., 1999). Sarder et al. (1999) also observed a strong effect of female parent on progeny sex ratios from one clonal line (006 046 539) of *O. niloticus* where the clonal males CM1 and CM2 belong. In their study, crosses to one of the control females produced only female offspring, while crosses to the mitotic mother of the clonal males and the other control female produced a high percentage of males. It was assumed that this clonal line might have been fixed genetically for some alleles, or combination of alleles at different loci, which cause female to male sex reversal but with limited penetrance. However, this explanation does not exactly fit to the present results, because there were no significant differences between males from this line (CM1 and CM2) and a male from another line (CM3) when their progeny sex ratios were analyzed. Considering that the line from which CM3 came was identified as an all female producing clonal line (e.g., XX SRSR) in their study, 100% female progenies were expected from the crosses between CM3 and normal females. Contradictory to this expectation, 5 females out of 7 that were crossed to this clonal male produced male progenies (3.4~17.9%). CM1 and CM2 showed the same pattern of progeny sex ratios as CM3 when crossed to the same 7 or 8 normal females. On the basis of these observations, it is assumed that CM1, CM2 and CM3 all have the same genotype (e.g., XX SRSR), and that the unexpected males observed here may have been caused by other factors such as environments or genetic/non-genetic parental influences.

In the work of Sarder et al. (1999), the founder of clonal line 006 046 539 and a normal female (11C) showed a high percentage of males and may have been the same cases of extreme maternal influence. NF10 in the present study also showed the extreme case of maternal influence. Excess male progenies, produced from two females, were also observed by Mair et al. (1991a), but these authors concluded these two females were 'naturally sex reversed' XY fish on the basis of progeny testing results. However, if we suppose that the sex ratio altering factor of the female is inherited, progeny testing results should also show the same skewed sex ratios. Cytological or molecular studies in future would have to judge whether these outlier females are 'naturally sex reversed' XY fish or true XX normal female with other sex ratio altering factors. It is not impossible that NF10 was one of the cases of 'naturally sex reversed XY female'. However, other normal females that produced high percentages of female progeny when crossed to clonal males also showed strong parental influence. Thus, it is not likely that the parental influences observed throughout this experiment were caused by 'naturally sex reversed' XY females.

Analysis of sex ratios from repeated crosses and relationship between survival rates and sex ratios also support the possibility of parental influence. Results from repeated crosses of the same parents in this study are consistent with the suggestion that progeny sex ratios in *O. niloticus* are stable and reproducible (Wohlfarth and Wedekind, 1991). Sex ratios of progenies obtained from repeated crosses of the same parents, however, were heterogeneous in another study of the same species (Tuan et al., 1999). This discrepancy may have been created by different experimental conditions. The study of Tuan et al. (1999) was carried out in fertilized earthen ponds where temperature influence is suspected while the present study was carried out in a recirculating system where temperature was maintained at 28°C, well below the TSD threshold for this species. The stability of sex ratios from repeated crosses in this study implies that the varying sex ratios from different parents must have been derived by more than chance. In addition, survival rates did not appear to be the main cause of sex ratio variation in this study. None of the genotypic groups showed significant association of survival rate with progeny sex ratio.

Table 8 summaries the parental influences observed in this study. In the crosses between clonal males and normal females, it seems likely that the sex ratio altering factors were introduced from the normal females, since clonal male × clonal female crosses produced 100% female progenies. However, paternal influence was also noticed from normal male × normal female crosses. In these crosses, a strong effect of the interaction between maternal and paternal factors was observed (Fig. 2). No parental influence was found in the crosses between YY males and normal females (XX) or YY females. This supports an XX/XY sex determining system in this species.

Parental influence appears to override the XX/XY sex determining system in some crosses. However, at the moment, in fish there is no evidence of what the parental influence might be. Parental influences on progeny sex ratios in fish have been noticed in several studies (Conover and Kynard, 1981; Shelton et al., 1983; Wohlfarth and Wedekind, 1991; Sarder et al., 1999; Tuan et al., 1999), and most of them were explained in favour of a polygenic sex determining theory. At least, all of them were discussed on the basis of genetics. However, in other higher vertebrates, several possible causes of non-genetic parental influences have also been suggested based on an XX/XY sex determining system (Hardy, 1997). First, the performance of sperm that carry X chromosomes could differ from that of sperm with Y chromosomes depending on the parents (paternal influence) ("Pre-fertilization control"). Second, eggs may be able to discriminate X-bearing

Table 8. Summaries of parental influences on progeny sex ratios observed in this study

Types of Crosses	Results (progeny sex ratios)	Comments
Clonal males × Clonal females (XX×XX)	100% females	No parental influences
Clonal males × Normal females (XX×XX)	0–33.3% males depending on female parents	Maternal influence No paternal influence
Neomales × Normal females (XX×XX)	0–20.6% males (sex ratio from an extreme, NF10 not included)	Mild maternal influence No paternal influence
Normal males × Normal females (XY×XX)	26.7–79.7% males depending on both male and female parents	Maternal influence Paternal influence
YY males × Normal females (YY×XX)	98.8% males	No parental influence
YY females × YY females (YY×YY)	100% males	No parental influence

sperm and Y-bearing sperm by selectively accommodating sperm (maternal influence) ("Pre-fertilization control"). Third, the composition of X and Y carrying sperms in semen may vary from male to male (paternal influence) ("Pre-fertilization control"). Fourth, when females are in poor condition, they would produce poor quality eggs and the viability of male embryos might differ from female embryos, resulting in sex-biased mortality during very early developmental stages (maternal influence). Lastly, levels of maternal steroid hormones vary with its physiological condition. It would result in differential hormone deposition into eggs, causing hormonal sex-reversal when the hormone contents are extremely high or low (maternal influence).

These ideas could be adopted to explain the parental influences observed in tilapia in this study. The absence of parental influences in crosses between clonal males and clonal females (Table 8) may infer that the parental influences observed in other crosses were the result of genetic differences between parental combinations. However, it could also be interpreted in favour of non-genetic parental influences. As summarised in Table 8, there were no paternal influences when males produce only one type of sperm (e.g., only X-bearing sperm in gynogenetic clonal males and neomales or only Y-bearing sperm in YY males). In this case, selective accommodation of X- or Y-bearing sperms by oocyte and competition between X- and Y-bearing sperms cannot be expected. Strong paternal effect observed in crosses between normal males (that produce both X- and Y-bearing sperm) and normal females support this interpretation. Maternal influences observed in some types of crosses may be the result of differential loading of steroids into eggs by female parents during oogenesis.

Taken together, parental influence on progeny sex ratios is evident in this species although the cause of this influence is not clear. Thus, it is suggested that when sex ratio data from this species is interpreted, parental influence should be considered together with the influences of autosomal factors and temperature. However, it remains unknown whether the parental influences observed here were the consequences of genetic events or non-genetic events. The present findings attract further studies on parental influences on progeny sex ratios in fish.

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