Observations on Intergeneric Hybrids in Tilapias

K.J. RANA[a,c]
B.J. McANDREW
G. WOHLFARTH[b,d]
I. MACGOWAN
[a]*Institute of Aquaculture*
University of Stirling FK9 4LA
Scotland, U.K.

[b]*Fish and Aquaculture Research Station*
Dor, D.N. Hof Hacarmel
30820, Israel

RANA, K.J., B.J. McANDREW, G. WOHLFARTH and I. MACGOWAN. 1996. Observations on intergeneric hybrids in tilapias, p. 391-397. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

This study describes the hatchability, growth, sex ratios and reproductive behavior of intergeneric hybrids between *Tilapia zillii* (Linneus) and *Sarotherodon galilaeus* (Linneus), and between *T. zillii* and *Oreochromis andersonii* (Castelnau), *O. aureus* (Steindachner), *O. macrochir* (Boulenger), *O. mortimeri* (Trewavas), *O. mossambicus* (Peters), *O. niloticus* (Linneus), *O. placidus* (Trewavas), *O. spilurus* (Günther) and *O. tanganicae* (Günther). Only hybrid crosses using *T. zillii* as the maternal parent were successful.

Mean fertilization rates of the pure *T. zillii* cross and the hybrid crosses were similar and ranged from 91 to 98%. Hatching rates varied from 67 to 91%. The growth of the juveniles varied among the hybrids, but the growth patterns were similar to *T. zillii*. The sex ratios of the hybrids also showed marked differences and ranged from all males in the *T. zillii* x *O. andersonii* and *O. tanganicae* to all females in the *T. zillii* x *O. niloticus*, *O. placidus* and *O. aureus*. By convention, the female parent is named first. The hybrids in all crosses produced mature gonads. The characteristics of the eggs in all crosses were closer to the maternal parent.

Introduction

In recent years, many approaches have been used to resolve the problems of relatively low-farmed tilapia yields and the low proportion of marketable fish caused by uncontrolled breeding in poorly managed tilapia production systems. To minimize or eliminate this unwanted reproduction, the central aim of all approaches has been to produce all-male tilapia seed for culture. Techniques such as hand-sexing, x-rays, chemical sterilization, interspecific hybridization and hormone therapy have been used with varying degrees of success. Hybridization has also generated considerable interest and controversy concerning the sex-determining mechanism of tilapias.

In the last decade, interspecific hybridization between mouthbrooding species such as *Oreochromis niloticus*, *O. aureus*, *O. mossambicus* and *O. urolepis hornorum* has been widely used to produce predominantly male offspring for culture (Hulata et al. 1983).

[c]Current address: FAO of the United Nations, Viale delle Terme di Caracalla, 00100 Rome, Italy.

[d]Dr. Wohlfarth, one of the pioneers of research on fish culture and genetic improvement of fish in Israel, was killed in a car accident on 22 August 1994.

These species have compatible breeding behavior, readily hybridize under culture conditions and therefore are easily produced and studied. In contrast, there appears to be little information on the biology of *intergeneric* tilapia hybrids, especially hybrids between the substrate spawners and mouthbrooders. Investigations on intergeneric hybrids between the female *Tilapia tholloni* and the males *O. niloticus* and *O. mossambicus* suggested that all F_1 hybrids were female and that reciprocal crosses were unsuccessful (Heinrich 1967; Bauer 1968). A study by Fishelson (1988) on intergeneric hybrids between *Sarotherodon galilaeus* and *O. niloticus* suggests that even though these hybrids were easy to produce, their reproductive performance was reduced due to various levels of gonadal deformity.

In view of the incompatibility of the breeding behavior of substrate spawners and mouthbrooders, their progeny may not breed naturally. Previous studies have indicated biased sex ratios in some crosses and such hybrids may help to elucidate the basis of sex determination in tilapias.

In the present study, intergeneric hybridization between the substrate spawning *T. zillii* and 10 mouthbrooding tilapias were conducted to determine if such crosses are able to produce viable offspring and to gain information on growth, sex ratios and reproductive biology of these hybrids.

Materials and Methods

Procurement of Tilapia Eggs and Sperms

The substrate spawner *T. zillii* served as the dam in all the intergeneric hybridization trials. A different female was used for each trial; the numbers of crosses and trials for a given species pair are given in the Results Section (Tables 3 and 4). Six attempts to produce hybrids from all the reciprocal crosses were unsuccessful.

Single pairs of *T. zillii* (100-200 g) were introduced in 140-l glass aquaria supplied by a recirculatory system maintained at 28°C and checked daily for spawning activity. Prespawning females were removed and held in a covered 10-l plastic aquarium containing clean aerated water at 28°C.

Three spermiating *S. galilaeus, O. andersonii, O. aureus, O. macrochir, O. mortimeri, O. mossambicus, O. niloticus, O. placidus, O. spilurus* and *O. tanganicae* were transferred into separate containers until required.

Manual Stripping and Fertilization of Gametes

To avoid cross-contamination of sperm during hybridization, disposable pipettes and Petri dishes were used for each cross and were immediately discarded. Approximately 150-300 eggs were stripped onto labelled 25x5 cm-perspex slides and spread into a monolayer with a fine paint brush. Milt was collected from three conspecific males, pooled in a Pasteur pipette, and then spread over the eggs and activated with warm water (28°C). Samples of the diluted milt mixture, collected from the slide, were then checked under a microscope to ensure the presence of sufficient motile sperm. After 5 minutes, the eggs were gently rinsed with water (28°C) and incubated in individual 2-l containers of a recirculatory incubating system containing UV-treated water (Rana 1986).

Since the eggs were sequentially stripped from the female for each cross, the pure *T. zillii* cross was carried out last to ensure that all the eggs stripped from the female were viable.

Fertilization rates in all the crosses were determined within 24 hours. The number of pigmented eggs in a random sample of 50-100 eggs was counted on each slide using a dissecting microscope. The eggs were kept submerged in a Petri dish for this procedure before being returned to the incubator.

The time to 50% hatching was noted for each cross. After completion of hatching, each slide was shaken to dislodge the larvae. To estimate the hatching rate, the numbers of unhatched and empty egg shells adhering to the slide were counted. The pre-swim-up larvae (three-day-old) were transferred and reared in 20-l plastic tanks before being used in growth trials.

Survival, Growth and Sex Ratios of Intergeneric Hybrids

Thirty fry from each of the above crosses were transferred to duplicate 30-l plastic rearing tanks served by a recirculatory system. They were fed three times a day at a daily rate of 10% body weight on a trout feed containing 40% protein (No. 4, Edward Baker Ltd., Bathgate, Scotland). Random samples of 10 fry were bulk-weighed every two weeks and feeding rations adjusted, after accounting for mortalities. The growth trials were terminated after eight weeks.

The validity of significance was established using ANOVA after arc sine transformation of data. The fish were weighed and each cross transferred to 60-l tanks for ongrowing to sexual maturity and then killed in an overdose of benzocaine. The fish (50-150 g body weight) were sexed by examination of the genital papillae, internal identification of the gonads and, in uncertain cases, by gonadal squash preparation. Fish were weighed and gonads were removed, then weighed and representative samples fixed for histology.

Breeding of Hybrids

For each hybrid cross, hybrid females were introduced, with either male *T. zillii* or a male of the *Oreochromis* species used as the hybrid parent, into 120-l aquaria for observation of spawning activity.

Results and Discussion

Fertilization, Hatching Rates and Morphological Differences

Mean fertilization rates of the pure *T. zillii* and the intergeneric hybrids were similar (P<0.05) and ranged from 91 to 98%. The hatching rates, however, were variable and ranged from 67% in the *T. zillii* x *O. mossambicus* cross to 91% in the pure *T. zillii* (Table 1). Hatching times of the hybrids were similar and eggs from all the crosses hatched within 48 hours of fertilization. The length of the hybrids and *T. zillii* larvae at hatching were not significantly (P<0.05) different and ranged from 4.8 to 5.0 mm.

T. zillii and all the hybrid larvae possessed two pairs of head glands, but the amount of mucus produced by the hybrids was reduced resulting in the hybrids being free of the substrate a day earlier. The intensity of melanophore pigmentation on the yolksac epithelium also varied between the hybrids, but *T. zillii* controls showed the greatest intensity. The hybrids and *T. zillii* fry began feeding four to five days posthatching (at 28°C).

Early Fry Survival and Growth

The survival and growth of the various hybrids are given in Table 2. The mortality patterns between the replicates were similar, suggesting a genetic basis. Survival of fry between hybrids

Table 1. Means (and SE) for the fertilization and hatching rates of intergeneric hybrids between *Tilapia zillii* females and males of various *Sarotherodon* and *Oreochromis* species.

Male parent	Fertilization rate (%)	Hatching rate (%)
T. zillii^{b} (control)	96.8 (1.4)	91.0 (3.29)
S. galilaeus^{c}	98.7 (0.86)	87.7 (2.82)
O. andersonii^{b}	91.1 (4.54)	89.0 (5.02)
O. aureus^{d}	95.0 (2.9)	88.0 (2.65)
O. macrochir^{c}	93.3 (3.75)	78.3 (8.43)
O. mortimeri^{b}	96.0 (1.84)	84.5 (8.48)
O. mossambicus^{d}	93.8 (2.85)	67.1 (3.10)
O. niloticus^{d}	93.5 (2.05)	78.6 (2.45)
O. placidus^{b}	95.0 (1.70)	85.0 (4.03)
O. spilurus^{d}	95.0 (1.7)	83.5 (4.25)
O. tanganicae^{a}	92.1 (-)	87.4 (-)

a: N=1; b: N=2; c: N=3; and d: N=4 (no. of attempts).

Table 2. Means (and SE) for the growth characteristics of intergeneric hybrid tilapia juveniles: all with *Tilapia zillii* as the female parent and various *Sarotherodon* and *Oreochromis* species as the male parents.

Male parent	Initial weight[1] (g)	Final weight[1] (g)	SGR (SE)[1,2] (%·day^{-1})	Survival[1,2] (%)
T. zillii (control)	0.076	2.38	6.4^{a}	81.5^{cd}
	(0.003)	(0.035)	(0.099)	(1.61)
S. galilaeus	0.132	3.49	6.1^{a}	81.5^{cd}
	(0.027)	(0.11)	(0.179)	(1.66)
O. andersonii	0.072	3.24	7.1^{ab}	98.5^{d}
	(0.006)	(0.057)	(0.198)	(1.5)
O. aureus	0.087	2.49	6.2^{a}	98.5^{d}
	(0.004)	(0.134)	(0.198)	(1.50)
O. macrochir	0.096	3.06	6.18^{a}	76.5^{cd}
	(0.006)	(0.10)	(0.18)	(3.5)
O. mortimeri	0.093	3.19	6.55^{a}	50^{ab}
	(0.007)	(0.028)	(0.049)	(9.9)
O. mossambicus	0.170	5.02	6.25^{a}	37^{a}
	(0.003)	(0.30)	(0.148)	(-)
O. niloticus	0.058	3.68	7.65^{b}	48.5^{a}
	(0.002)	(0.636)	(0.247)	(1.5)
O. placidus	0.115	3.33	6.25^{a}	61.5^{abc}
	(0.002)	(0.010)	(0.049)	(8.5)
O. spilurus	0.070	2.29	6.45^{a}	88.5^{cd}
	(0.002)	(0.042)	(0.049)	(11.5)
O. tanganicae	0.093	3.79	6.9^{ab}	78.5^{bcd}
	(0.003)	(0.071)	(-)	(1.5)

[1]Means based on duplicate treatment.
[2]Means within columns having the same letters are not significantly ($P<0.05$) different.

spawner parent may, in themselves, be of some advantage in possible future uses of these hybrids.

Acknowledgements

The authors are grateful for the facilities provided by the British Overseas Development Administration and to Prof. R.J. Roberts for his financial support.

References

Bauer, J. 1968. Vergleichende Untersuchungen zum Kontaktverhalten verschiedener Arten der Gattung *Tilapia* (Cichlidae, Pisces) und ihrer Bastarde. Z. Tierpsychol. 25:22-70.

Fishelson, L. 1988. Behavior and gonadal structure of intergeneric (*Oreochromis-Sarotherodon*) tilapia hybrids, p. 159-167. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) The Second International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 15, 623 p.

Heinrich, W. 1967. Untersuchungen zum Sexualverhalten in der Gattung *Tilapia* (Cichlidae, Teleostei) und bei Artbastarden. Z. Tierpsychol. 24(6):684-754.

Hulata, G., G. Wohlfarth and S. Rothbard. 1983. Progeny-testing selection of tilapia broodstocks producing all-male hybrid progencies - preliminary results. Aquaculture 33:263-268.

Majumdar, K.C. and B.J. McAndrew. 1983. Sex ratios of interspecific crosses within tilapias, p. 261-269. *In* L. Fishelson and Z. Yaron (comps.) Proceedings of the First International Symposium on Tilapia in Aquaculture. Tel Aviv University, Tel Aviv, Israel.

McAndrew, B.J. 1993. Sex control in tilapiines, p. 87-98. *In* R.J. Roberts and J. Muir (eds.) Recent advances in aquaculture. Blackwell Scientific Publications, Oxford, UK.

Rana, K.J. 1986. Parental influences on egg quality, fry production and performance in *Oreochromis niloticus* (L.) and *O. mossambicus* fry. Institute of Aquaculture, University of Stirling, Stirling, Scotland. 295 p. Ph.D. dissertation.

Sodsuk, P. and B.J. McAndrew. 1991. Molecular systematics of three tilapiine genera *Tilapia, Sarotherodon* and *Oreochromis* using allozyme data. J. Fish Biol. Suppl. 39:301-308.

at the end of the trial varied significantly (P<0.05). Hybrids with *O. mossambicus* and *O. niloticus*, and *O. andersonii* and *O. aureus* had the lowest and highest survival rates, respectively. Despite these differences, the growth rates of the hybrids at the end of the trial were not significantly (r=-0.145, df=21, P<0.05) correlated with the survival rates.

Mean initial weight of the different hybrid groups varied between 0.06 and 0.17 g and were significantly correlated with the final weight (r=0.712, df=21, P<0.05) and weight gain (r=0.699, df=21, P<0.05). This association presents a problem since these growth estimators may result in growth being biased by variation in initial weights. In an attempt to reduce this bias, weight data were transformed and growth of the hybrids was compared as specific growth rate (Table 2). Hybrids of *O. tanganicae*, *O. andersonii* and *O. niloticus* showed the highest growth rates. The high apparent growth rates of *O. niloticus* hybrids, however, may have been influenced by their low survival rate.

Reproductive Behavior, Gonadal Development and Sex Ratios

All the hybrid females lacked the typical breeding behavior of either of their parental species and no spawning occurred in the breeding tanks. One "accidental" spawning did occur with a *T. zillii* x *O. mossambicus* female. This female was held on her own in a plastic tank and therefore no information on the viability of the eggs was obtained. The eggs were laid on the tank bottom. They were partially adhesive and similar to those of a substrate spawner. In addition, the female guarded and periodically fanned the eggs.

From the observations performed to determine the sex of the hybrids, not all fish could be classed as male or female. In some crosses, between 1 and 6% of the fish were sterile (Table 3). These fish lacked any gonadal tissue and contained instead a clear, viscous fluid.

Depending on their paternal species, the sex ratios of hybrids fall into three main groups: predominantly male, pre-

Table 3. Sex ratios of intergeneric tilapia hybrids having *Tilapia zillii* as the female parent and various *Sarotherodon* and *Oreochromis* species as the male parents.

Male parent	Trial 1			Trial 2			Trial 3		
	M	F	S	M	F	S	M	F	S
T. zillii (control)	15	8	-	10	16	-	14	34	-
O. niloticus	0	21	3	0	23	1	0	27	2
O. placidus	0	32	2	0	46	1	0	65	2
O. mortimeri	0	27	4	-	-	-	2	27	1
O. aureus	2	52	1	-	-	-	0	53	-
O. mossambicus	5	17	1	0	50	-	0	33	-
O. spilurus	7	36	-	-	-	-	0	25	-
S. galilaeus	9	24	-	-	-	-	12	13	-
O. macrochir	81	36	-	-	-	-	-	-	-
O. tanganicae	31	0	-	-	-	-	22	0	-
O. andersonii	52	0	-	-	-	-	49	0	-

M: male; F: female; and S: sterile fish (see text).

dominantly female and an approximately equal number of both sexes (Table 3). It is difficult to see any obvious pattern related to the presumed sex-determination system from other studies (McAndrew 1993) as *O. macrochir* and *O. aureus* (homogametic males) occur in different groups. There is no firm evidence that the observed sex ratios are based on any phylogenetic relatedness, as suggested by Sodsuk and McAndrew (1991) who found *O. andersonii* to always be closely grouped with *O. mossambicus* and *O. mortimeri*. In this study, *O. andersonii* appears to be clearly separated from the two other species.

The gonads of hybrids, where recognizable, were of particular interest. In all cases, the gonadosomatic indexes of both sexes were significantly ($P<0.05$) lower than those of pure *T. zillii* progeny, and ranged between 0.005 and 0.36% for the males and 0.32 and 1.74% for the females (Table 4). The majority of the hybrid female ovaries contained relatively few oocytes compared to those of pure *T. zillii*. The majority of the gonads examined contained previtellogenic oocytes up to stage 3, and few gonads contained mature oocytes at stages 5 and 6. Even though the testes of the hybrids with *O. andersonii* and *O. tanganicae* were significantly smaller than those of pure *T. zillii*, they contained gametes at all stages of development. In some fish, mature spermatozoa were present in the tubules of the testes.

It is difficult to derive any hypothesis from this study on sex determination in tilapia. Hybrid sex ratios are a notoriously difficult analytical character (Majumdar and McAndrew 1983). As a result of the difficult statistical problems in identifying and separating expected sex ratios and our limited knowledge on the genetic mechanisms involved in sex determinations in pure species, the consequences for hybridization are impossible to predict. The observed sex ratios, the reduced reproductive performance and the phytophagous feeding habit of the substrate

Table 4. Means (and standard deviations) for the gonadosomatic indexes (GSI) of intergeneric tilapia hybrids from *Tilapia zillii* female parents and various *Sarotherodon* and *Oreochromis* species as male parents.

Male parent	No.	Sex	Mean body weight (g)	GSI* (%)
T. zillii (control)	11	M	78.5 (36.2)	1.47 (0.47)[a]
	6	F	51.5 (23.3)	2.8 (0.20)[1]
S. galilaeus	7	M	101.7 (20.6)	0.36 (0.30)[b]
O. andersonii	10	M	136.6 (42.6)	0.02 (0.013)[b]
O. aureus	21	F	101.2 (46.6)	1.26 (0.96)[1]
O. macrochir	33	M	142.7 (52.7)	0.19 (0.05)[b]
	8	F	125.0 (31.9)	0.50 (0.56)[2]
O. mortimeri	14	F	103.9 (22.8)	0.67 (0.22)[2]
O. mossambicus	6	M	67.0 (34.4)	0.02 (.005)[b]
	5	F	64.1 (55.5)	0.32 (0.10)[2]
O. niloticus	16	F	132.4 (29.4)	1.74 (1.28)[1]
O. placidus	11	F	53.7 (16.7)	0.35 (0.12)[2]
O. spilurus	25	F	74.4 (38.8)	0.51 (0.53)[2]
O. tanganicae	10	M	75.5 (30.5)	0.05 (0.026)[c]

*Means with different letters (for males) and numbers (for females), respectively, are significantly ($P<0.05$) different.