A CONTRIBUTION TOWARDS AN UNDERSTANDING OF THE INTENSIVE TANK CULTURE OF AN ORNAMENTAL CICHLID, *AULONOCARA BAENSCHI*, FROM CHIPOKA, LAKE MALAWI.

THESIS

Submitted in fulfilment of the
requirements for the degree

of

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by

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'We believe that there is a great future in this branch of aquaculture (ornamental fish). We can improve our output greatly if more research effort is put into certain problem areas' (Myburgh* 1987).

* F. Myburgh is a co-owner of Amatikulu Hatchery (Pty) Ltd. (P.O. Box 272, Gingindlovu, 3800), South Africa's largest producer of ornamental fish

FRONTISPIECE

Plate 1. Nuptial colouration of territorial male *A. baenschi*.

Plate 2. *A. baenschi* mouthbrooding female.

CONTENTS

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ABSTRACT

The intensive tank culture of ornamental mouthbrooding cichlids poses several problems which limit their aquaculture potential. This project addressed some of these problems for *Aulonocara baenschi*. The production of juveniles in 250l aquaria was accelerated when: a) aquaria were equipped with refuges, b) females of less than 70mm in standard length were used as broodstock, c) mouthbrooding females were replaced with gravid females at seven day intervals, and d) embryos were removed from the mouths of females at replacement times for artificial incubation. Two sex ratios also accelerated juvenile production. The sex ratio (male:females) 1:30 yielded the highest spawning returns per tank, and therefore represented the most effective utilization of aquarium space (a critical consideration for the small-scale culturist). Contrastingly, the sex ratio 1:12 yielded the highest clutch sizes and a high percentage female spawning return, and therefore represented the most effective utilization of broodstock (an important consideration for culturists inhibited by financial constraints or having an abundance of culture vessels).

The reproductive behaviour of *A. baenschi* was described. Emphasis was given to aspects of reproduction of relevance to culture, for example; spawning times and seasons, clutch size and its relationship with female size, age and size of sexes at first spawning, embryo development rate and size of first swimming juveniles.

The slow growth rate of juveniles, combined with a late attainment of marketable size ($\pm$ seven months) was a major limitation affecting the culture potential of *A. baenschi*. Two factors favouring the culture of this species was the high survival rate recorded for both adults and

juveniles, and the comparatively high prices fetched by fish on domestic wholesale markets (R4,00 per fish).

It is recommended that *A. baenschi* should not be cultured exclusively for the relatively small South African ornamental fish market. A more profitable strategy for domestic culturists should involve a major production effort with *A. baenschi* and other desirable species of *Aulonocara* (e.g. *A. ethelwynnae*; *A. hansbaenschi*; *A. stuartgranti* & *A. maylandi*) for foreign markets (in particular, the U.S.A.; Western Europe & Japan). Not only are these markets massive, but prevailing exchange rates of the Rand with these currencies favour such a strategy.

CHAPTER 1: INTRODUCTION

Ornamental fishes are defined by Conroy (1975) as those fishes which, because of their attractive colouration, size and other characteristics, are suitable for maintenance in captivity, as 'pets' in aquaria, garden pools and similar artificial environments. The majority of popular ornamental fish species are native to the tropical and subtropical regions of the world (Bruton & Impson in prep.).

Aquaculture of ornamental fishes in South Africa

Until 1984, approximately 95% of freshwater ornamental fish traded in South Africa were imported , primarily from Hong Kong and Singapore (Bruton & Impson in prep.). By 1986, two major producers of ornamental fish, and approximately 15 to 20 specialist goldfish producers were operating in South Africa (M. Willman, Tru-Go Aquarium, Johannesburg, pers. comm.). The considerable interest shown in domestic production of ornamental fish (Polling 1985) has resulted in noticeable strides being made towards import replacement. For example, Myburgh (1987) estimated that in 1986, Amatikulu Hatchery (Pty) Ltd. would supply 60% of the domestic demand. Several factors have contributed to the growth of the ornamental fish aquaculture industry in South Africa. The most important of these factors are probably:

a) a realization by domestic producers of the value of the domestic and foreign ornamental fish markets. The retail value of the domestic market for 1986 was estimated at R7 million (Bruton & Impson in prep.). In comparison, UNCTAD (1979) estimated the retail value of the world trade in ornamental fish at U.S. $ 7.2 billion.

b) The high cost of importing fish from foreign sources, due to airfreight costs and exchange rates.

c) The availability of cheap labour and suitable areas for culturing ornamental fish in South Africa.

Although these factors have allowed domestic producers to become increasingly effective in the South African ornamental fish trade, the possibility exists that the situation could be reversed if the Rand

and U.S. Dollar again achieved parity (D. Bevan, Waterlife Aquarium, Port Elizabeth; M. Willman, pers. comm.). It is imperative, therefore, that South African producers become more competitive.

The uncertain future of ornamental fish culture in South Africa is exacerbated by two facts. Firstly, domestic culture in South Africa is in its infancy, unlike many of the highly specialised culture operations in South-East Asia which have been in existence for decades (M. Willman, pers. comm.). Secondly, the ornamental fish culture industry is extremely competitive. For example, Axelrod (1971, in Courtenay & Robins 1973) estimates that there are 450 ornamental fish farms and importers in the U.S.A. alone. A consequence of this competition is a dearth of scientific information on the commercial culture of ornamental fish (Freer *et al.* 1987).

The mouthbrooding cichlids of Lake Malawi: their potential as candidate species

Many species of cichlids are popular ornamental fishes, particularly in the U.S.A., Canada and Western Europe (Freer *et al.* 1987). There are at least 29 serial publications in Europe and America which cater specifically for the interests of cichlid aquarists (A.J. Ribbink, J.L.B. Smith Institute of Ichthyology, Grahamstown, pers. comm.).

The mouthbrooding cichlid species of Lake Malawi are especially popular with aquarists (Conroy 1975; Loiselle 1985). Their popularity is attributable to the ornamental appeal, complex behaviour, comparative rarity and the speciose nature of this group. Ribbink *et al.* (1983) have estimated that 400 to 500 species of cichlid are endemic to Lake Malawi. The rock- frequenting Mbuna (term after Fryer 1959), form the basis of an export trade from Lake Malawi which by the mid 1970's was exporting more than 400 000 fish annually (Ribbink *et al.* 1983). The value of this trade was estimated at R275 000 in 1977 (Ribbink 1980).

A further factor enhancing the culture potential of Malawi cichlids are the high prices fetched per fish, compared to other ornamental fish species. Table 1 illustrates this fact for the domestic market. Moreover, many species of Malawi cichlid regularly fetch $20 to $50 per fish on U.S. markets, with certain rare species fetching more than $100 per fish (Ribbink, pers. comm.).

Table 1. Wholesale price on a domestic market as of 25/11/1987 for several popular species of ornamental fish in comparison with two species of Malawi cichlid (source: D. Bevan).

SCIENTIFIC NAME	COMMON NAME	PRICE PER FISH IN RANDS
A. stuartgranti	blue peacock Aulonocara	R6,00
Cyprinus carpio	koi carp (6cm)	R2,10
Paracheirodon innesi	neon tetra	R0,30
Poecilia reticulata	guppy	R0,70
Pseudotropheus zebra	Malawi zebra cichlid	R2,50
Pterophyllum scalare	angelfish	R1,00
Symphysodon aequifasciata axelrodi	brown discus (4cm)	R2,50
Xiphophorus helleri	swordtail	R0,70

The South African market for these fishes is, however, small as they require specialist attention. This requirement is primarily due to the complex behaviour and aggressive disposition of Malawi cichlids. The export market, in comparison, offers a more lucrative market to South African producers because of the size of markets (for example, Shotts & Gratzek (1984) estimate that 16.3 million U.S.A. households contain ornamental fish) and favourable exchange rates.

Limitations affecting the culture of mouthbrooding cichlids in intensive systems

Three characteristics of the reproductive behaviour of mouthbrooding cichlids (mouthbrooding, polygamy and aggression) offer opportunities, under controlled conditions, for the experimental manipulation of juvenile production. However, these behavioural patterns pose a dilemma for culturists in choosing appropriate manipulative techniques in order to accelerate the production of juveniles. Accordingly, these behavioural characteristics may become limiting factors in the intensive culture of such species. The challenges posed by each of these behavioural characteristics are described as follows:

Characteristic 1: Mouthbrooding. The key question to be addressed here is: should females mouthbrood embryos until the juvenile stage is attained, a technique advocated by Caulton (1979) and Loiselle (1985); or should embryos be removed from the females mouth for artificial incubation as recommended by Rothbard & Hulata (1980); Hepher & Pruginin (1981) and Mires (1982). The advantages and disadvantages of each technique as a means of accelerating juvenile production are elaborated in Experiment 1.

Characteristic 2: Mouthbrooding cichlids are frequently polygamous (Turner 1986). As males are relieved of parental care duties, and can therefore fertilize several females in a day, the key question to be addressed is: how many females should be kept with a male in a given space to accelerate juvenile production.

Characteristic 3: The aggressive dispositions of sexually active mouthbrooding cichlids (Baerends & Baerends van Roon 1950; Fryer & Iles 1972), particularly under aquarium conditions (Jackson & Ribbink 1975; Hepher & Pruginin 1981; Loiselle 1985). This reproductive characteristic poses the following three limitations in intensive culture: a) loss of condition (see Freer 1987, for *Pseudocrenilabrus philander*), b) mortality of broodstock (Mires 1977; Hepher & Pruginin 1981), and c) the channelling of energy into aggression in preference to egg and sperm production. As a consequence of these limitations, techniques need to be developed which would reduce the aggression level of broodstock in intensive systems.

These problems have not been addressed by South African aquaculturists as no species of Lake Malawi mouthbrooders have been cultured to date on a commercial scale in South Africa. The principal objective of this research project was to address the problems posed by characteristics 1, 2 and 3, thereby fulfilling the primary research need listed by Safriel & Bruton (1984) for the development of ornamental fish culture in South Africa.

Three reasons were identified in motivating the choice of tanks for culturing *A. baenschi*:

a) Approximately 12 000 aquarists in the U.S.A., Canada and Europe breed cichlids in aquaria to supplement their incomes (data from cichlid associations in various countries). This small-scale approach to ornamental fish culture holds considerable potential for South African aquarists.

b) The growing trend in intensive, large-scale, ornamental fish culture; both in South Africa (pers. obs.) and in South East Asia (Bevan; Willman, pers. comm.), involves the spawning of adults in aquaria.

c) Manipulation and precise monitoring of fish for experimental purposes is simplified when aquaria are used.

Aulonocara baenschi

Aulonocara Regan 1921 is a haplochromine genus endemic to Lake Malawi (Meyer *et al.* 1987). The fish have small mouths; compressed bodies; enlarged infraorbital line cavities; enlarged orbits and scales on cheek rows 0 to 4 (Meyer *et al.* 1987). Sixteen species of *Aulonocara* have been described (Meyer *et al.* 1987), all inhabiting the sand-rock interface in Lake Malawi at a depth range of 4 to 75m (Grant *et al.* 1987). *A. baenschi* exists as several populations in allopatry at the following localities (Chipoka, Maleri, Nkhomo & Usisya)(Meyer *et al.* 1987). A taxonomic description of *A. baenschi* is provided by Meyer *et al.* (1987).

The Chipoka population (Figure 1) of *A. baenschi* is found on the Chindunga Rocks reef in 10m depth (Grant *et al.* 1987). This species was chosen because of its availability, favourable price and the inherent desirability (Ribbink 1976) and popularity of several species of *Aulonocara* on domestic and foreign markets. In addition, Safriel & Bruton (1984) identified *Aulonocara* as a high priority candidate genus for culture in South Africa.

Associated objectives and structure of the thesis

The materials and methods used for reproduction, the artificial incubation of embryos and the rearing of juveniles are described in chapter 2. Thereafter, the reproductive behaviour of *A. baenschi* is described, with priority given to aspects of reproduction of relevance to culture (chapter 3). In chapter 4, the problems limiting the culture potential of mouthbrooding cichlids are investigated for *A. baenschi*. This involved the use of various techniques for the experimental manipulation of juvenile production (experiments 1 to 4). According to Hecht (1985), growth and survival of juveniles, and marketability of the product are important considerations in establishing the culture potential of a species (chapter 5). The final chapter summarizes and evaluates the main findings of the project and offers recommendations for the intensive culture of *A. baenschi*. It is suggested that these guidelines should also be useful for the intensive culture of other mouthbrooding cichlid species from Lake Malawi.

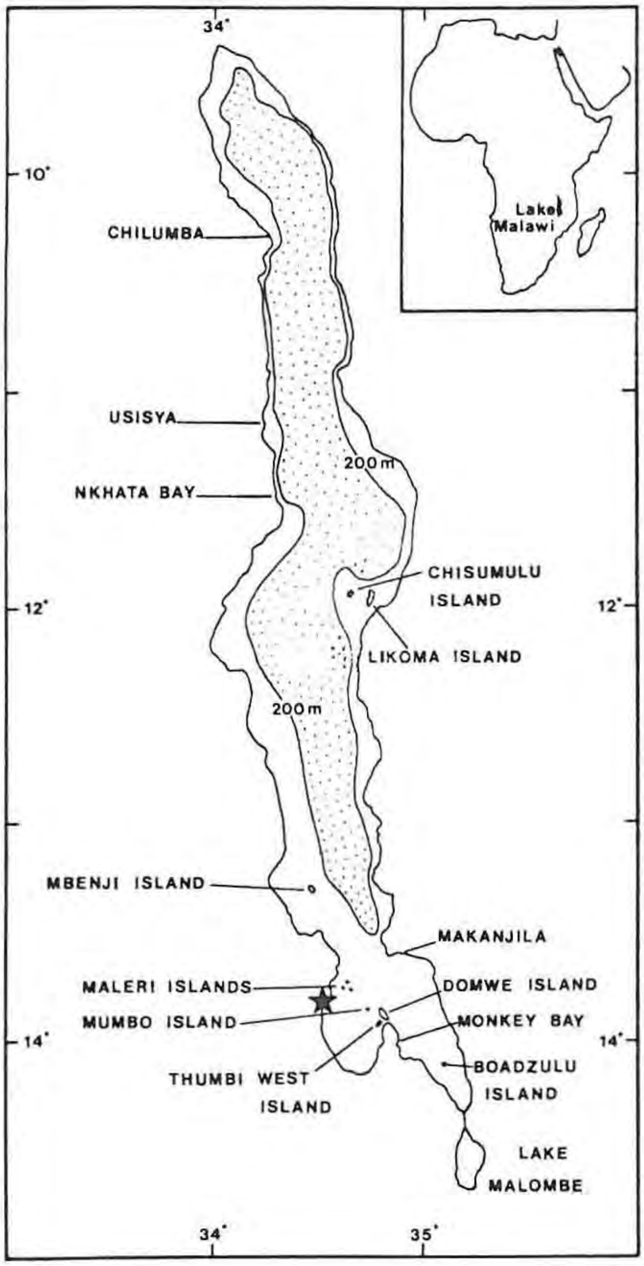

Fig. 1. A map of Lake Malawi with an insert of Africa showing the position of the lake. The locality of Chipoka is indicated with an asterisk. A 200m depth contour is illustrated (diagram from Ribbink *et al*. 1983).

CHAPTER TWO: GENERAL MATERIALS AND METHODS

Hatchery system for broodstock

1. *A. baenschi*

Thirty males (SL - 72 ±3mm; n = 13) and 120 females (SL - 67 ±2mm; n = 13) were purchased from Mr. S. M. Grant, a Lake Malawi - based exporter of ornamental cichlids.

2. Tanks for broodstock

According to Loiselle (1985), *Aulonocara* spp. should be maintained in tanks of 250 to 350 l capacity. Twelve asbestos tanks with glass viewing apertures were used (Plate 3). The eight tanks used to compare experimental variables for broodstock were connected in series, with a submersible pump recirculating the water. The average flow rate through the system was approximately two litres per minute, yielding

Plate 3. An aquarium used to spawn broodstock (100cm × 50cm × 50cm, viewing aperture dimensions: 56cm × 37cm).

an average turnover time of approximately 17 hours.

3. Tank equipment

Aulonocara spp. inhabit the transition zone in Lake Malawi where rocky and sandy shores merge (Fryer & Iles 1972; Meyer *et al.* 1987). Here aquatic vegetation is often scarce or absent altogether (Ribbink, pers. comm.). Caves, cracks and overhangs are the favoured microhabitats (Staeck 1984; Tins 1985). Nests are constructed by the male in the sand (Ribbink *et al.* 1983), usually next to or under some kind of structure. Accordingly, each aquarium unit was supplied with a gravel bottom and a brick which was situated at a rear corner of the aquarium to form a cave. Bricks were placed in this position to dictate male choice of nest site so that spawning behaviour could be observed.

4. Undergravel filter system

Each aquarium was equipped with a 50cm * 50cm * 3cm undergravel filter which had one or two rear - corner mounted airlifts. The undergravel filter was covered with 3 $\pm$1cm of gravel which had a variable grain size (e.g. largest grains 10mm * 5mm, smallest grains 1mm * 1mm). Compressed air was supplied to tanks via plastic piping. Hoseclamps were used to regulate airflow. The flow rate through filters averaged 2 l per minute.

5. Water temperature and water changes

A water temperature of 26 $\pm 1^{\circ}$C was maintained using one 220W thermostatically controlled immersion heater per aquarium. This was within the temperature range recommended by Ribbink (1980)(23 to 28°C) for the culture of Malawi cichlids.

A 10% water change with tap water was performed once a week. The chlorine concentration of the tap water was 110ppm (P. Brits, Dept.

Ichthyology & Fisheries Science, Rhodes University, pers. comm.).

6. Photoperiod

Lake Malawi is in the tropics (9°30 - 14°30S / 33°52 - 35° 20E), and has a diel light cycle where the daylength and nightlength are approximately equal. A photoperiod of 12:12 was used, with the light cycle starting at 08h00.

7. Feeding regime

In Lake Malawi *Aulonocara* spp. are known to feed on insect larvae, molluscs, ostracods and possibly small fish (Meyer *et al.* 1987). Aquarium observations have shown that *Aulonocara* spp. accept all standard aquarium foods (Staeck & Terver 1977; Wolstenholme 1978; Axelrod *et al.* 1984). Each day fish were fed a mixture of Tetra staple flake diet (MAT NR 213 975IN) and Tetra conditioning flake diet (MAT NR 213 913US). Mixed tetramin was fed three times per day; at 09h00, 14h30 and 17h00. Broodstock were fed to satiation. Finely shredded oxheart was fed weekly.

Hatchery system for incubation and rearing

a) Incubation

1. Mouthbrooding

The mouthbrooding of embryos took place in aquaria containing the broodstock.

2. Artificial incubation

Embryos were removed from the mouth of brooding females by hand (Appendix 1). They were then transferred in glass jars to Zuger-type funnels (Rothbard & Pruginin 1975; Hepher & Pruginin 1981) for artificial incubation. The closed system artificial incubator used (Figure 2) was comparable to that developed by Rothbard & Hulata (1980), and Impson (1984) for the artificial incubation of embryos from mouthbrooding cichlids.

The incubator was inspected several times per day. Specific attention was paid to embryo rotation rate, and the presence of dead or diseased embryos, which were removed. Embryos must be rotated to ensure normal development (Fryer & Iles 1972). Dead unhatched embryos were opaque to white in colour and occasionally had a fungal coat.

Once a month, the entire system was emptied and thoroughly cleaned. When refilled, Tetra Fungistop was added at a concentration of 5ml per litre of incubator water. The addition of a bactericide is an essential prophylactic as developing cichlid embryos are susceptible to microbial attack (Loiselle 1985).

Every seventh day all embryos were transferred from the incubator to a 5 l white bucket where individual rates of development could be ascertained. Embryos beyond the tenth day of development (approximately three days post-hatch) were isolated, and then transferred to an embryo grow-out unit (Figure 3). The design of this unit allowed embryos at similar stages of development to be housed together in one of several incubation chambers until the juvenile period was reached. The rationale behind the development of the embryo grow-out unit is explained in Experiment 1.

Ten percent of the water was replaced weekly with tap water in both incubators. Water temperature in the incubators was maintained at 26 $\pm 1^{\circ}$C, a temperature recommended by Axelrod *et al.* (1984) for hastening embryo development in *Aulonocara* spp. The use of this temperature also avoided temperature shock to embryos when they were transferred from aquaria in which they had been spawned.

Eleutheroembryos, at the beginning of the rapid yolk absorption phase (Balon 1985a), were collected by siphoning out the contents of an incubation chamber into a bucket. Embryos were transferred at this stage of development to aquaria for rearing because they were easy to

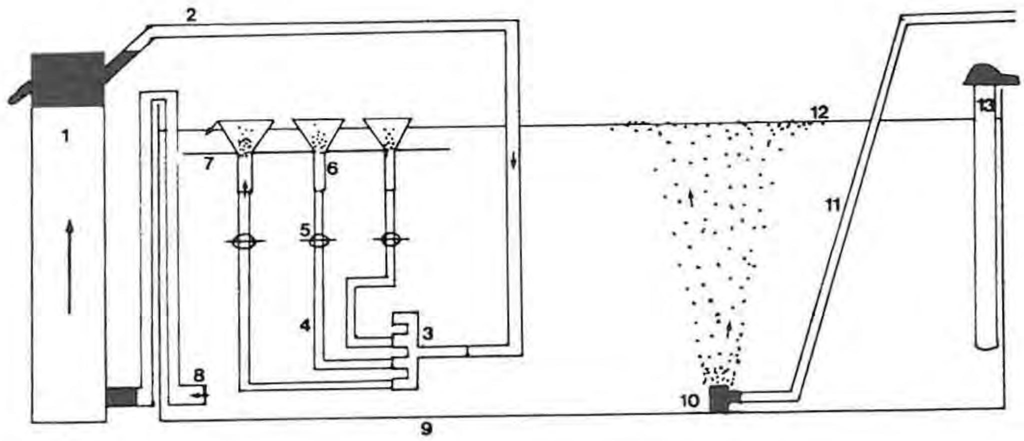

Fig. 2. Diagram of the closed system incubator

Key: 1 - Eheim-type 2013 incubator, 2 - outlet pipe, 3 - four way water channelizer, 4 - plastic pipe, 5 - clasp type flow regulator, 6 - glass funnels containing embryos in suspension, 7 - suspended grid supporting funnels, 8 - intake pipe, 9 - 60cm x 30cm x 30cm glass aquarium, 10 - airstone, 11 - air supply pipe, 12 - water level, 13 - 200W immersion thermostatic heater. Arrows indicate direction of water flow.

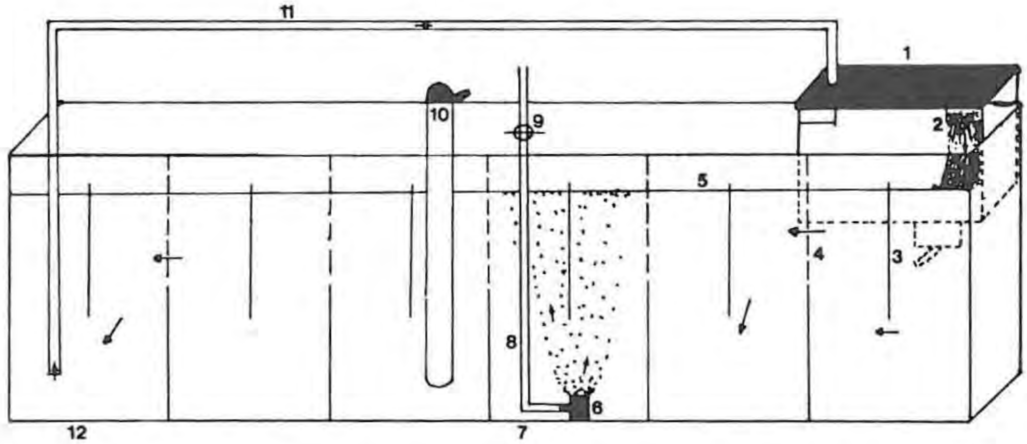

Fig. 3. Diagram of the embryo grow-out unit

Key: 1 - Eheim-type 1010 external filter, 2 - flow outlet, 3 - glass water flow channelizer, 4 - plastic mesh, 5 - water level, 6 - airstone, 7 - 120cm x 30cm x 20cm glass aquarium, 8 - airpipe, 9 - clasp regulating airflow, 10 - 200W immersion thermostatic heater, 11 - intake pipe, 12 - 1 of 6 incubation chambers. Arrows indicate direction of water flow.

capture, resistant to handling and also did not require further maintenance in a specialised incubator.

b) Rearing

Six glass aquaria (90cm * 30cm * 30cm) were used to rear juveniles for the first three months of growth. Juveniles were then transferred to an asbestos aquarium (150cm * 50cm * 30cm) or a plastic pool (150cm diameter * 50cm depth) for grow-out until saleable size was reached. All units used for rearing were closed recirculating systems. Each rearing unit contained a 2 $\pm$1cm deep gravel bottom, of variable grain size, under which an undergravel filter was situated. Two undergravel filters (28cm * 28cm * 1cm), each connected to an airlift, were supplied to glass aquaria. The asbestos aquarium was equipped with two undergravel filters (50cm * 50cm * 3cm), each connected to a single airlift. Three undergravel filters (50cm * 50cm * 3cm), each connected to a single airlift, and one Eheim junior external filter (type 1010) constituted the filtration system for the plastic pool. Compressed air was supplied to tanks via plastic piping. Hose clamps were used to regulate airflow.

Water temperature in each rearing unit was maintained at 26 $\pm 2^{\circ}$C. Ten percent of the water was replaced weekly with tap water.

Juveniles were fed Tetra growth food (MAT NR 213 951 IN) for the first three months of juvenile growth, with live *Daphnia* fed when climatic conditions for their collection were suitable (clear, warm and still days). For the remaining period, until grow-out to saleable size, juveniles were fed a mixture of Tetra staple and conditioning food. Feeding frequency of Tetramin was the same as used for adults. Juveniles were fed to satiation.

<u>Water quality</u>

Five water quality parameters were monitored (temperature, pH, dissolved oxygen, ammonia, nitrite and nitrate) so as to describe ranges of these parameters for *A. baenschi* under which regular spawning activity and normal embryo development occurred. In addition, two of the eight replicates* (Nos. 4 and 8) used to compare experimental variables were monitored to determine whether the recirculating system ensured a consistent water quality throughout the experimental system. This was done as widely varying water quality between experimental replicates was expected to bias results.

These parameters were monitored for the following reasons. Firstly, according to Spotte (1979), most of the toxicity problems encountered in culture systems are attributable to nitrogenous compounds; unionized ammonia (NH_3) being the most toxic, followed by nitrite (NO_2^-), and nitrate (NO_3^-). Secondly, dissolved oxygen (D.O.) is regarded by Stickney (1979) as being an important parameter of water quality in aquatic systems. Thirdly, temperature and pH are important water quality parameters affecting the culture of Malawi cichlids. This is because Lake Malawi is a tropical aquatic system (surface temperature range: 23 to 28°C, Eccles 1974) with alkaline waters (pH range: 7,7 to 8,6; Fryer & Iles 1972).

The mean concentrations of the five water quality parameters monitored are given in Table 2.

* a replicate was defined as identical units given identical treatments (term after Bishop 1966)

Table 2. Water quality in units containing broodstock and embryos (7 samples of each parameter were taken for each category of holding unit, except for the embryo grow out unit where, four samples were measured; standard deviations are given in brackets).

WATER QUALITY PARAMETER	TYPE OF HOLDING UNIT: UNITS FOR BROODSTOCK REPLICATE 4	REPLICATE 8	CLOSED SYSTEM INCUBATOR	EMBRYO GROW-OUT UNIT
TEMPERATURE[1] (°C)	25.4 (0.77)	26.2 (0.92)	26.6 (0.42)	25.1 (0.62)
pH[3]	7.4 (0.05)	7.4 (0.08)	7.4 (0.5)	7.6 (0.2)
D.O.[2] (mg D.O./l)	4.7 (1.4)	4.5 (1.3)	6.2 (1.4)	5.7 (1.1)
AMMONIA[4] (mg NH_3/l)	0.39 (0.33)	0.23 (0.23)	0.09 (0.12)	0.13 (0.09)
NITRITE[4] (mg NO_2^-/l)	0.08 (0.09)	0.03 (0.03)	0.02 (0.02)	0.26 (0.38)
NITRATE[4] (mg NO_3^-/l)	117 (30)	124 (19)	9.2 (3.3)	202 (47)

Monitoring instruments:

1 - thermometer
2 - oxygen probe meter - CG 867
3 - wide range pH indicator solution in conjunction with a Hach type DR EL/4 test kit
4 - auto-analyzer (parameters kindly measured by Miss P. Watson, Institute of Freshwater Studies, Rhodes University)

CHAPTER 3: REPRODUCTIVE BEHAVIOUR

The reproductive behaviour of *A. baenschi* under intensive culture conditions is described with a twofold objective:

a) To determine whether the behaviour was similar to other maternal mouthbrooding cichlid species, thereby evaluating the applicability of the findings of this project for such species.

b) To emphasize aspects of reproductive behaviour relevant to culture, thereby establishing a frame of reference for the experiments in chapter 4.

All descriptions of behaviour relate to fish, kept at sex ratios of 1 male to 6 and to 12 females, and maintained in 250 l aquaria (see Plate 3).

Courtship

Territories were established by males within one day of the introduction of broodstock into aquaria. Boundaries of territories were not demarcated but extended beyond the mating pit (term after Trewavas 1983). Females were actively excluded from male territories by lateral display (term after Baerends & Baerends van Roon 1950) and chasing (term after Nelissen 1985) movements. The focus of each territory was a mating pit constructed by the male alongside or beneath some type of structure (in this case the aquarium wall or a brick). The form of the pit was a simple circular excavation in which the aquarium floor had been exposed. Pit construction involved the movement of gravel and debris by mouth. Females did not assist with pit construction nor with repair at any stage. In establishing a territory the male changed from neutral to nuptial colours. This change involved colour intensification.

A dominance hierarchy was established by females through the use of agonistic behaviour. The formation of social organisation is an early stage of the reproductive process, serving to stimulate and co-ordinate courtship and spawning (Aronson 1957). The behavioural components employed by females in establishing and maintaining the hierarchy involved lateral display; circling (term after Ribbink 1971); mouthfighting, tail beating, biting (terms after Baerends & Baerends van Roon 1950); avoidance swimming, fleeing (term after Vodegel 1978) and chasing movements. Mouthfighting involved two combatants rapidly swimming towards one another with the mouths held open ('mouth rushing'), followed thereafter by brief mouth contact. This did not include mouth gripping (term after Vodegel 1978). Females did not defend distinct territories and were not observed to chase territorial males.

Courtship was initiated by the territorial male. This involved the movement of the male from the nest site towards the periphery of the female school. Here the male performed a side-shake (term after Ribbink 1975) which was usually accompanied by powerful tail beats. Receptive females responded to this signal by moving into the territory of the male. Participation in courtship did not involve colour change in the female. The generalized stimulus / response sequence observed in courtship for *A. baenschi* is described in Table 3.

Spawning

Spawning behaviour was initiated by the same stimulus / response sequence used in courtship. The only difference was that, while courtship culminated in a series of dummy runs by the courting pair, spawning behaviour began with egg-laying and ended when this was completed.

Spawning in the laboratory was aseasonal (Figure 4), but did have a diurnal pattern as fish usually spawned between 08h00 and 12h00 ($p < 0{,}1$; $n = 33$). The duration of spawning was dependent on the clutch size spawned and the number of females ready to spawn at any one

Table 3. Overview of the generalized courtship response sequence for *A. baenschi*.

MALE RESPONSE	FEMALE RESPONSE
1 The male, exhibiting courting colours, remained on the nest site or patrolled the territory chasing intruding females.	A receptive female in the female school moved to the periphery of the males territory.
2 The male swam towards the school of females and performed a lateral display. On return to the nest site the male performed a 'lead swim' (Ribbink 1971).	The female entered the territory of the male. There was no change in colouration, although a submissive posture (term after Ribbink 1975) was adopted.
3 The male swam rapidly towards and then around the female performing a 'courtship dance'. This consisted of an extremely rapid swimming motion around the female involving fin quivering (term after Baerends & Baerends van Roon 1950) and one, two or several powerful tail beats ('turning tail beats', term after Vodegel 1978) at regular intervals.	The female remained motionless maintaining a submissive posture or swam slowly to the nest site.
4 The male swam rapidly towards nest site leading the female by performing a 'lead swim'.	The female followed the male to the nest site and occupied a position on the nest floor. The female picked at bits of debris on the nest floor or the nest floor itself.
5 The male usually repeated the courtship dance and then / or occupied a position on the nest floor in which the body of the male fish was at an obtuse angle to the side facing the female. The body was parallel to the nest site with all fins fully extended, exhibiting quivering movements (the 'horizontal nest'shake, term after Ribbink 1971).	The female positioned herself at right angles to the male with her mouth positioned at the front edge of the males anal fin alongside the genital opening. In this position the female mimicked the oral capture of sperm (after Ribbink 1971).
6 The male remained in position and did not have tactile contact with the female.	the female now occupied a parallel position to the male so that the females head faced the males caudal fin. In this position the female exhibited intention egg laying by skimming (term after Vodegel 1978), thereafter performing a full or half circle to pick up 'eggs'.

time. The usual duration was less than one hour ($p < 0,1$; $n = 7$). Spawning involved a series of visits* by the female to the nest site of the male. The average duration of each visit ($n = 42$) was 103 ± 58 seconds. During each visit one, two or several egg batches (term after Trewavas 1983) were laid. Eggs were released and taken into the mouth of the female before the male attempted fertilization. The female thereafter positioned herself at right angles to the male with her mouth next to the genital opening of the male. The movement and position of the male's body at this stage suggested that sperm was being released. Eggs were therefore fertilized in the mouth of the female. One to eight eggs were laid per batch, with single eggs most commonly laid (Figure 5). The eggs were pear-shaped, light yellow in colour, and did not have filaments or an adhesive layer. The number of batches laid was dependent on the total number of eggs released and also therefore on female size. Females ($n = 5$) between 75 and 85mm laid about 16 ± 3 batches per spawn, with approximately half the eggs released in the first 40% of batches laid.

Clutch size (term after Trewavas 1983; Balon 1985a) was positively correlated to female size ($r = 0,93$; $p < 0,02$; d.f. = 34)(Figure 6), with an average clutch size of 39 eggs ($n = 384$ clutches) being recorded. The lowest and highest clutch sizes recorded were 2 eggs (52mm female) and 109 eggs (84mm female) respectively. The smallest and largest females to spawn were 46mm and 94mm.

After spawning was completed, the female returned to the female school to begin the period of embryo incubation. Polygyny was practiced, as on several occasions males were observed mating with several females in rapid succession.

Post spawning behaviour

A. baenschi is a maternal mouthbrooder. Females did not feed while

* a visit was defined as the time spent by the female in the male's territory for the purpose of laying eggs

mouthbrooding, and during capture did not spit out embryos. Mouthbrooding females were observed to seek shelter and were usually not aggressive towards other fish. Mouthbrooding females were easily distinguished by: a) their lack of participation in certain facets of the behaviour of the female school, such as a refusal to feed; b) an expanded branchiostegal membrane (Plate 2); and c) often a dark region below the eye. According to Fryer & Iles (1972), these changes occur before spawning, and may be triggered by egg maturation. The expanded branchiostegal membrane increased mouth capacity for embryo incubation. The respiratory current of the parent provided the embryos with dissolved oxygen while at the same time removing metabolites. The churning movements of the mouth at regular intervals moved the embryos, a behaviour which, according to Fryer & Iles (1972), keeps the embryos clean and allows for normal development. The regular movement of embryos is believed to prevent the heavy lipids in the embryo from sinking to the lower pole, a process which disrupts the internal organisation of the embryo resulting in developmental failure (Fishelson 1966, in Keenleyside 1979). Moreover, it is likely that embryos obtained considerable protection from fungal and bacterial attack from pharyngeal glands in the females mouth which secrete a bactericidal agent (Breder & Rosen 1966).

Embryos took 5 to 6 days to hatch and a further 14 ± 2 days to attain the juvenile period (term after Balon, 1985b) at 26 $\pm 1^{\circ}$C. The size and weight of juveniles at release was 10,4 $\pm 0,5$mm and 3,0 $\pm 0,4$mg respectively ($n = 15$). Females ($n = 3$) first released juveniles after a 20 ± 1 day brooding period, and thereafter did not take juveniles back into the mouth once they had been released. This behaviour did not change even when females were disturbed. Females kept with juveniles were brown in colour with dark brown bars.

Discussion

Behavioural comparisons

The functional organisation of the reproductive behaviour of *A. baenschi* (in particular, its polygamous reproductive style, and

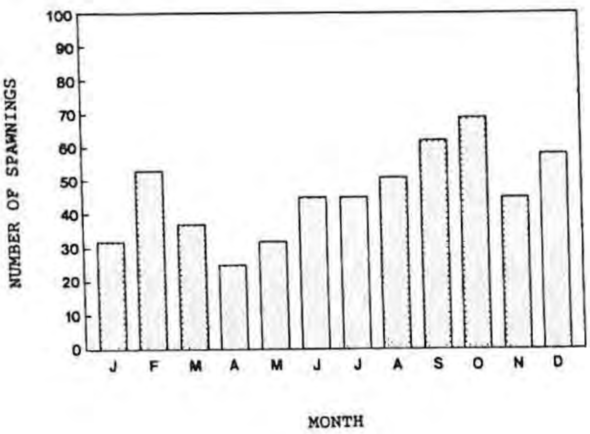

Fig. 4. The number of spawnings recorded per month in 1986. A sustained spawning response was observed (no. of females = 109).

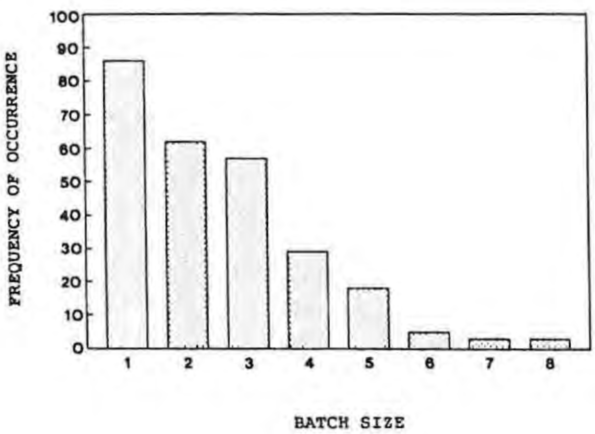

Fig. 5. The relationship between batch size and frequency of occurrence. Single eggs were most commonly released.

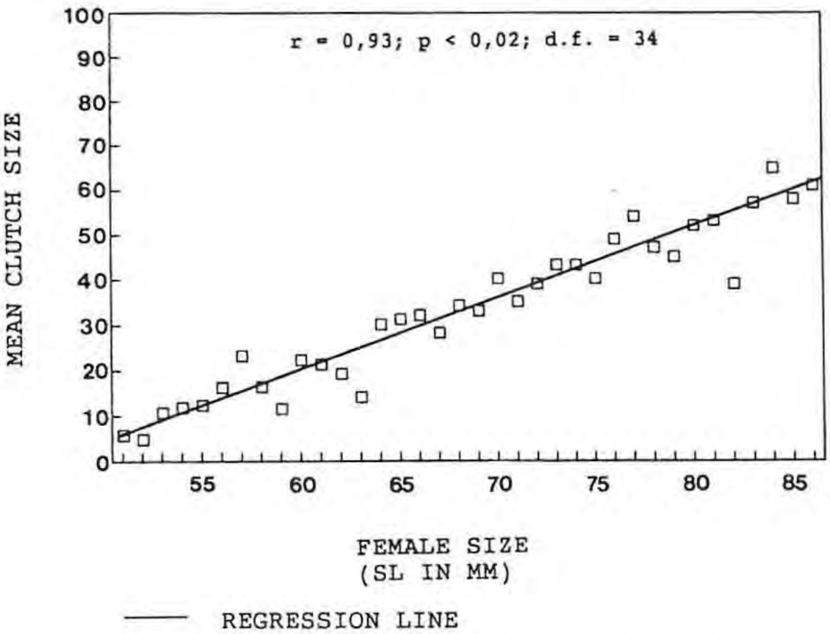

Fig. 6. The relationship between clutch size and female size in *A. baenschi*. This relationship was linear for the size categories 52 to 85mm (n > 7 clutches for each size category).

mouthbrooding and aggressive behaviours), is similar to that of other maternal mouthbrooding cichlids (see Balon 1985a for *Labeotropheus* sp.; Nelissen 1985, for *Melanochromis auratus*; Trewavas 1983, for *Oreochromis*; Vodegel 1978, for *P. zebra*; Ribbink 1971, 1975, for *P. philander*; Baerends 1957, 1986, for cichlids in general; Fryer & Iles 1972 and Jackson & Ribbink 1975, for Malawi mouthbrooders). As such, the techniques identified which accelerate juvenile production in *A. baenschi* (described in chapter 4), may be broadly applicable for use in the intensive culture of comparable mouthbrooding cichlid species.

Behavioural aspects of *A. baenschi* relevant to culture

The aggressive dispositions of sexually active fish posed two constraints to culture. Firstly, there was always the likelihood of mortality of fish as submissive individuals often could not escape the aggressive behaviour of dominant fish. This was because the territories established in aquaria, often were of the same size as the aquarium. This precluded the use of two males in the same aquarium (on three occasions the dominant male killed the submissive male). The occurrence in tanks of aggression-induced mortality in mouthbrooding cichlids is well documented (Mires 1977; Hepher & Pruginin 1981; Axelrod *et al.* 1984; Loiselle 1985). Secondly, the energy used in aggression utilizes a substantial component of the energy budget (Li & Brocksen 1977; Feldmeth 1983, in Wootton 1985; Knights 1985), which could rather be channelled into the production of primary sex products. This scenario was investigated in Experiment 4.

The aseasonal spawning pattern of *A. baenschi* under suitable environmental conditions is not unusual for a species with a tropical origin (see studies of Philippart & Ruwet 1982; Lowe-McConnell 1987; and Rothbard & Pruginin 1975, Hepher & Pruginin 1981, for spawning activities of tilapias under natural and artificial conditions respectively). Consequently a management strategy which encompasses the maintenance of a stable environment must be implemented if a prolonged spawning response is desired. The diurnal spawning behaviour of *A. baenschi* implies that fish must not be disturbed in the morning in a manner which may discourage spawning activity (e.g. introduction of nets and other objects into aquaria or conducting water changes).

An average clutch size of 39 eggs was calculated for *A. baenschi*. This is a common clutch size for Lake Malawi mouthbrooders (Fryer & Iles 1972; Axelrod *et al.* 1986). The low clutch size of *A. baenschi* indicates an extremely precocial life history style (term after Balon 1984), a feature with far reaching implications for the culture of such species. In essence, extreme precociality implies that the supply of juveniles will be a chief factor restricting the culture potential of such species. Consequently, two further assessments are relevant for *A. baenschi*. Firstly, juvenile production is chiefly dependent on the density of females. Manipulation of female density to accelerate juvenile production is described in Experiment 3. Secondly, techniques must be developed which accelerate both the production of juveniles (Experiments 1 to 4), and juvenile survival until marketable size is attained (Chapter 5).

The absence of a levelling off or a reduction in clutch size indicates that females of a size range from 46mm to 87mm are suitable as broodstock. However, Mires (1982) has suggested that small but fecund females may produce more offspring annually than larger females which are infrequent spawners. This suggestion was investigated in Experiment 1.

The finding that females did not feed while mouthbrooding, or spat out embryos during attempts at capture has significant commercial and experimental implications. Essentially, it implies that the mouthbrooding behaviour of *A. baenschi* allows provision for management strategies which necessitate regular and varied manipulation of this behaviour, a crucial finding for the investigations described in chapter 4.

CHAPTER 4: EXPERIMENTAL MANIPULATION OF THE PRODUCTION OF JUVENILES

The commercial culture of mouthbrooding cichlids in intensive systems posed several problems which were investigated in the following four experiments.

In each experiment the particular problem investigated is described. Thereafter techniques used to overcome the problem are defined, described and tested. The results are discussed in comparison to the findings of other workers. The conclusion to each experiment identifies the technique which maximized the production of juveniles.

EXPERIMENT 1 : INCUBATION TECHNIQUES

Introduction

Three techniques were identified by Hepher & Pruginin (1981) for the incubation of embryos of mouthbrooding cichlids. These techniques were tested in this experiment, and the advantages and disadvantages of each technique as a means of enhancing productivity of *A. baenschi* are described in Table 4.

The culture of tilapia in Israeli hatcheries characteristically involves the artificial incubation of embryos in funnels after they have hatched in the females mouth (Hepher & Pruginin 1981). However, there is debate as to the desirability of artificial incubation as a method of enhancing productivity. To quote Loiselle (1985): 'cichlids have had over 60 million years to refine the techniques of buccal incubation; aquarists have been imitating them for two decades. There is no better reason for regarding artificial incubation as an emergency measure only and making every possible effort to encourage ovigerous females to complete a normal incubation sequence'. Moreover, there appear to be no quantified data on the effect

Table 4. Overview of advantages and disadvantages characterizing each of the three incubation techniques used in this experiment ([1]Breder & Rosen 1966; [2]Rothbard and Hulata 1980; [3]Hepher and Pruginin 1981; [4]Lovshin 1982; [5]Mires 1982; [6]Eyeson 1983; [7]Impson 1984 ; [8]Watanabe *et al.* 1984; [9]Loiselle 1985; [10]Freer 1987).

INCUBATION TECHNIQUE USED	ADVANTAGES OF USING TECHNIQUE	DISADVANTAGES OF USING TECHNIQUE
Incubation technique A: The artificial incubation of embryos for the full period of development	-incubators can be reliable, simple, and easy to assemble[2] -greatly reduced interval between spawns e.g. *see for *Oreochromis mossambicus*,[7] *Oreochromis niloticus*,[4] *P. philander*[10] and various tilapias[3] -avoids possibility of paedophagy and premature ejection of embryos	-high embryo mortalities at unhatched stage e.g. *see for *O. mossambicus*,[7] *O. niloticus*[8] & *P. philander*[10] -higher incidence of congenital deformity[9] -cost of constructing and maintaining the incubator -labour intensive -handling stress
Incubation technique B: Embryos are mouthbrooded until hatching occurs and then artificially incubated	-avoids high embryo mortality during unhatched stage -the possibility of females prematurely terminating brooding duties is reduced -reduces brooding period by approximately 70%; and thus substantially reduces the interval between spawns e.g. *see for *O. mossambicus*,[7] *O. niloticus*[5]	-cost of constructing and maintaining incubator -handling stress -labour intensive
Incubation technique C: Embryos are mouthbrooded for the full period of development	-eggs protected from infection by secretions of bactericidal and fungicidal substances produced from glands situated in the roof of the mouth in brooding females[1] -avoids cost of constructing and maintaining incubator -avoids handling induced stress on broodstock -not expensive in labour	-paedophagy by the female on her own embryos - it has been observed that the number of embryos mouthbrooded decreases with time[6] -high incidence of premature termination of brooding, either by swallowing or ejecting embryos, particularly under aquarium conditions[6] -greatly lengthened interval between broods e.g. *see for *O. mossambicus*,[7] *O. niloticus*,[4] *P. philander*[10] & various tilapias[3]

technique on female spawning frequency* or embryo survival.

This experiment investigated the effect of incubation technique on two variables for *A. baenschi*:

a) female spawning frequency, and

b) embryo survival during incubation

Moreover, the effect of female size on spawning frequency using a specified incubation technique was also investigated. This aspect of the experiment was conducted as Mires (1982) has suggested that small but fecund females may produce more offspring annually than larger females that are infrequent spawners.

By investigating these two factors, the incubation technique which yielded the highest production of *A. baenschi* juveniles was determined.

Materials and methods

Experimental system

Four of the eight interconnected aquaria were used to spawn females, each containing broodstock which comprised 1 male and more than 10 females. Three of these aquaria contained broodstock from Lake Malawi, of an unknown age; although all females were larger than 70mm. The other aquarium was used to spawn laboratory reared females which were smaller than 70mm and less than a year old.

The remaining four interconnected aquaria were used to determine female recovery time for each incubation technique and contained one male and 12 spent females. Three aquaria (one for each incubation technique) contained spent females larger than 70mm, while the other

* defined as the mean interval between broods for several females

aquarium contained spent females smaller than 70mm (incubation technique A used).

Embryos selected for incubation techniques A and B were incubated in the closed system incubator and embryo grow-out unit. Collection of juveniles from the former incubator could only be accomplished when both the Eheim pump was switched off, and all the components of the incubator were removed. To overcome this time consuming operation the embryo grow-out unit was developed. Survival of embryos in this unit was assessed.

Monitoring of experimental variables

The monitoring techniques used to determine spawning frequency of females were dependent on the incubation technique and are described in Figure 7.

The survival of embryos for incubation techniques A and B was determined by conducting several incubation trials. A trial consisted of counting the number of embryos to be incubated (a), and then counting the number of these embryos surviving to the juvenile period (b) at the end of the trial. Percentage embryo survival (PES) per trial was determined using the following equation:

$$PES = (b)/(a) \times 100\%$$

A different technique was required for determining embryo survival for incubation technique C. This was because females mouthbrooded embryos for the full period of development, and therefore the original number of embryos incubated had to be estimated. This was accomplished by extrapolating the relevant clutch size value ($y = mx + c$) for each spent female from a female size / clutch size regression (see Fig. 6). These values were summed (c) for females that had mouthbrooded embryos until the juvenile period was reached. The number of juveniles

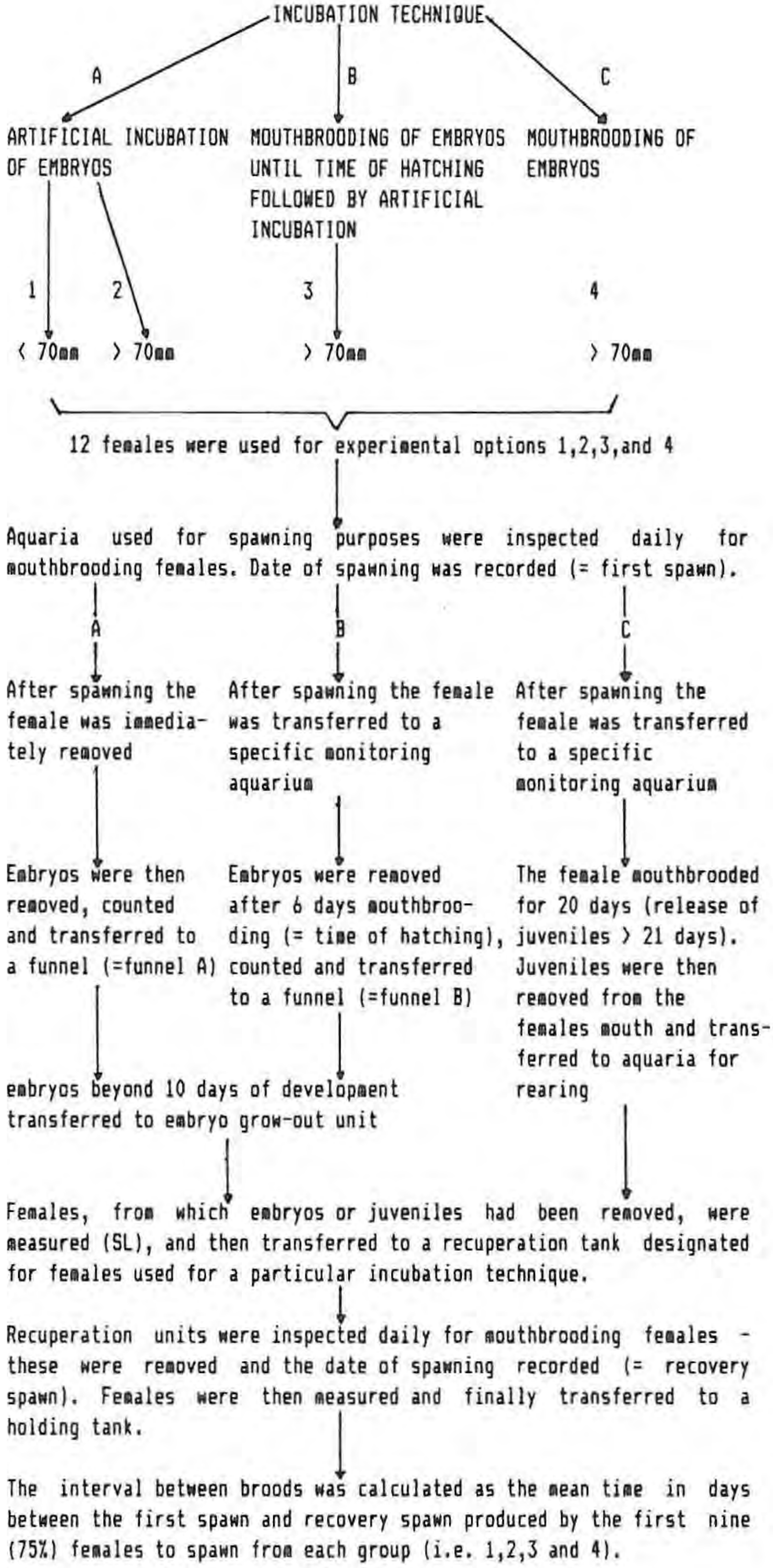

Fig. 7. Monitoring strategy used for each incubation technique

removed from all 12 females was counted (d).

PES for incubation technique C = (d)/(c) x 100%.

Results

1. Female spawning frequency

The artificial incubation of embryos resulted in a reduction in the interval between broods (Figure 8)(ANOVA: $F (3;32) = 39,4$; $p < 0,001$). This figure also shows that female size affected spawning frequency, with females smaller than 70mm showing a 70% reduction in the interval between broods compared to larger females ($F (3;32) = 39,4$; $p < 0,001$). Furthermore, the standard deviations of the mean in Figure 8 reveal that a more uniform interval between broods was attained when smaller females were used. Projected annual spawning frequencies using these incubation techniques and females of different size are illustrated in Table 5. The difference between the means for the category 'interval between broods' were significant (95% Scheffe test).

A positive correlation between female size and clutch size was calculated for *A. baenschi*. The expected clutch size of spawned females (from the clutch size / female size regression, see Fig. 6) was multiplied by the observed spawning frequency to determine whether the advantage of using young females with high spawning frequencies was counteracted by the higher clutch sizes of older females (Table 6). These data confirmed that the use of females less than 70mm yielded a 19% increase in projected annual juvenile production compared to when females over 70mm were used.

2. Embryo survival

Embryo survival was estimated at 85% (Table 7) using incubation technique B. This represented a significant improvement in the embryo survival compared to values obtained for incubation techniques A and C. The 25% increase in embryo survival obtained for incubation

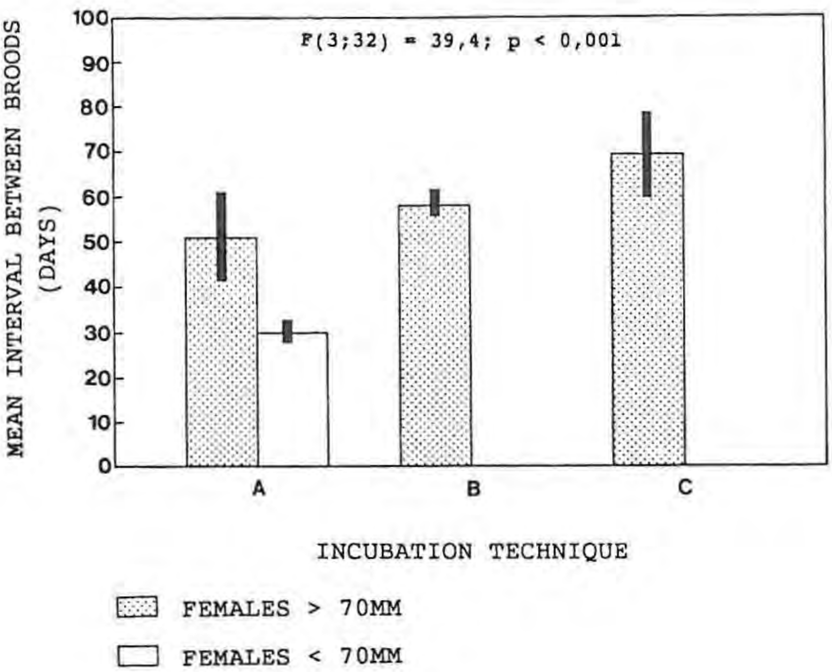

Fig. 8. The relationship between incubation technique, female size and interval between broods. The combined use of incubation technique A and females less than 70mm in SL yielded the lowest mean interval between broods (n = 12 for each treatment, standard deviations represented by line).

<u>key</u>: A - embryos removed from mouthbrooding female within 6 hours of spawning for artificial incubation; B - embryos removed at time of hatching for artificial incubation; C - female mouthbroods embryos

Table 5. Projected annual spawning frequency of females was highest using incubation technique A and females smaller than 70mm (n = 12 for each treatment).

INCUBATION TECHNIQUE	SIZE FEMALES USED	MEAN INTERVAL BETWEEN BROODS (days)	PROJECTED ANNUAL SPAWNING FREQUENCY PER FEMALE
ARTIFICIAL INCUBATION	> 70mm	51	7.1
	< 70mm	30	12.2
ARTIFICIAL INCUBATION AT TIME OF HATCHING	> 70mm	59	6.3
EMBRYOS MOUTHBROODED	> 70mm	69	5.3

Table 6. The relationship between female size, spawning frequency (using incubation technique A), and expected clutch size (data extrapolated from Fig. 6). It is projected that the use of females smaller than 70mm will accelerate juvenile production.

FEMALE SIZE CATEGORY	SIZE OF SPAWNED FEMALE SL (mm)	EXPECTED CLUTCH SIZE	PROJECTED NUMBER OF SPAWNS PER YEAR	PROJECTED ANNUAL JUVENILE PRODUCTION PER FEMALE	AVERAGE
LESS THAN 70mm	63 (*2)	25		305	
	65 (*2)	28		342	
	66 (*3)	29	12.2	354	361
	68	32		390	
	69	34		415	
GREATER THAN 70mm	71 (*2)	37		263	
	72 (*2)	39		277	
	73 (*3)	40	7.1	284	303
	77	46		327	
	80	51		362	

* number of females used for each size category

Table 7. The relationship between incubation technique and embryo survival during incubation. Incubation technique B gave the highest survival.

INCUBATION TECHNIQUE	NUMBER OF INCUBATION TRIALS	NUMBER OF EMBRYOS INCUBATED	NUMBER OF JUVENILES OBTAINED	PERCENTAGE SURVIVAL OVERALL	RANGE
ARTIFICIAL INCUBATION	5	1870	1123	60	52-65
ARTIFICIAL INCUBATION AT TIME OF HATCHING	3	680	575	85	73-94
EMBRYOS MOUTHBROODED	-	*2104	1510	72	-

* an estimation based on the sum of the expected clutch sizes (data extrapolated from Fig. 6, $y = mx + c$), for the sizes of females spawned

technique B compared to technique A was due to high embryo mortality prior to hatching. Embryo survival in the grow-out unit was 89%.

Discussion

Two factors affected the production of juveniles in this experiment - female spawning frequency and embryo survival during incubation.

1. Female spawning frequency

The interval between broods was affected by the incubation technique used and female size. The interval between broods was shortest (30 days) when females of less than 70mm were used as broodstock and embryos were artificially incubated for the full period of development. The artificial incubation of embryos is known to substantially increase spawning frequency in several species of mouthbrooding cichlids (Table 8).

Table 8. The effect of incubation technique on the interval between broods in several species of mouthbrooding cichlids.

SPECIES	INTERVAL BETWEEN BROODS (IN DAYS) ARTIFICIAL INCUBATION OF EMBRYOS AT TIME OF:		MOUTHBROODING OF EMBRYOS	REFERENCE
	SPAWNING	HATCHING		
O. mossambicus	23d	28d	39d	Impson 1984
O. niloticus	13 - 18d		30 - 60d	Lovshin 1982
		34d		Mires 1982
P. philander	35d		54d	Freer 1987
various tilapias	30 - 36d		±73d	Hepher & Pruginin 1981

The decreased interval between broods exhibited by females less than 70mm is in contrast to the findings of Watanabe *et al.* (1984) for *O.niloticus* who found that the mean interval between spawnings, when embryos were artificially incubated, was similar for yearling and two to three year old broodstock.

The considerable variation in interval between broods exhibited by females for all treatments was not unusual. Wide variations in the interval between broods have been recorded for *O. niloticus* (Mires 1982; Watanabe *et al.* 1984) and *Poecilia latipinna* (Snelson *et al.* 1986).

Although increased spawning frequency using young broodstock was correlated with a reduced clutch size, the use of small females yielded an estimated 19% increase in relative fecundity when compared to older females. This finding supports Mires (1982) belief that small but fecund females should produce more eggs annually than bigger fish that are infrequent spawners. A further advantage of using young adults is that it allows culturists to utilise most of, if not the entire reproductive stage of each fish.

2. Embryo survival

Incubation technique B yielded the highest percentage survival of embryos (85%). This technique allowed for the mouthbrooding of embryos during the vulnerable unhatched stage, when mortality under artificial conditions was shown to be highest (40%). Impson (1984) and Watanabe *et al.* (1984) recorded comparable embryo mortality rates during this period for *O. mossambicus* (50%) and *O. niloticus* (35 - 54%) respectively.

The transference of 10 day old embryos (± 3 days post-hatch) from the closed system incubator to an embryo grow-out unit yielded a favourable survival rate of 89%; and it provided for efficient collection of juveniles

3. Estimation of productivity

The preceding discussion recommends the use of incubation technique A to accelerate spawning frequency, and incubation technique B to maximize embryo survival. To overcome these conflicting findings, juvenile production over an annual basis was projected by combining these variables and using results from females larger than 70mm (Table 9).

Table 9. The relationship between incubation technique, embryo survival and female spawning frequency. It is projected that the production of juveniles will be accelerated if incubation technique B is used.

INCUBATION TECHNIQUE	SURVIVAL PER 100 EMBRYOS	ANNUAL SPAWNING FREQUENCY	PROJECTED ANNUAL PRODUCTION OF JUVENILES PER FEMALE
ARTIFICIAL INCUBATION	60	7.1	426
ARTIFICIAL INCUBATION AT TIME OF HATCHING	85	6.3	536
EMBRYOS MOUTHBROODED	72	5.3	382

Conclusion

Production of juveniles was accelerated when females smaller than 70mm were used as broodstock and when embryos were removed from mouthbrooding females six days after spawning had occurred for artificial incubation.

EXPERIMENT 2: REPLACEMENT PERIODS FOR MOUTHBROODING FEMALES

Introduction

Experiment 1 established the effectiveness of a particular incubation technique (the artificial incubation of embryos at time of hatching) as a means of enhancing juvenile production. This technique involved the monitoring of individual females which were usually maintained in separate aquaria. However, from a commercial viewpoint this method was unsuitable, as it was both labour intensive and costly in terms of effective utilization of aquarium space. The replacement of mouthbrooding females with gravid females at a suitable time period was identified as a possible solution to this problem.

Few authors have documented the necessity for periodic removal of mouthbrooding females. Silvera (1978, in Guerrero and Guerrero 1985) recognized that the frequency of removal of mouthbrooding females was a factor affecting the production of *O. niloticus* juveniles. Tave (1985) described the removal of mouthbrooding *Oreochromis aureus* and *O. niloticus* females every four days from plastic pools. Snow *et al.* (1983) compared four removal periods for *O. aureus* and found that the removal of brooders every 10 to 12 days from plastic pools yielded the highest production of juveniles. However, no author appears to have described the replacement of mouthbrooding females.

To manipulate the findings of experiment 1, and to simplify management, three replacement periods were chosen for experimentation. These were the replacement of mouthbrooding females: a) within six hours of spawning, b) every seven days, and c) every 21 days.

A 21 day replacement period is the longest period that can be effectively used for mouthbrooding *A. baenschi* females as embryos attain the juvenile period after 20 $\pm$2days of development. Snow *et al.* (1983) and Tave (1985) have shown that allowing mouthbrooding females to release juveniles into intensive spawning units is not a commercially feasible proposition as the adults cannibalize juveniles.

Four variables were expected to affect the production of juveniles in this experiment. The first two variables were embryo survival during incubation and female spawning frequency, the principal factors affecting juvenile production in Experiment 1. Female spawning frequencies for replacement periods B and C were not determined due to the time required for the successful completion of such a study. The projected annual spawning frequencies determined in Experiment 1 for incubation techniques B (artificial incubation at time of hatching) and C (embryos mouthbrooded) (6,3 & 5,3 spawnings respectively) were used as a guideline for replacement periods B and C. The embryo survival (60%) and female spawning frequency (7,1 spawnings) obtained for incubation technique A (artificial incubation of embryos) in Experiment 1 are applicable for replacement period A as both techniques involved the removal of embryos from females mouth at the same time.

The remaining variables were the number of spawnings obtained per replicate over the experimental period (also referred to as spawning success), and the labour input in terms of time expenditure required at female replacement periods.

Embryo survival, spawning success and the labour input required at replacement times were investigated in the following experiment to determine which of the replacement periods yielded the most cost effective production of *A. baenschi* juveniles.

Materials and methods

The duration of each experiment was 21 days. Seven replicates were used for each treatment.

Experimental system

The experimental system comprised eight 250 l aquaria, connected in series by a recirculating system. Experimental aquaria contained 1 male and 6 females. A further aquarium contained one male, and gravid females for replacement of spawned females. Gravid females were identified by morphological cues such as the appearance of papillae and / or the presence of an enlarged area between the pelvic fins prior to feeding. The remaining aquarium was used as a recovery aquarium for females removed at replacement periods.

The embryos obtained at replacement periods were transferred to one of three units, depending on the estimated level of development. Embryos younger than 10 days were placed in the closed system incubator, while those between 10 and 15 days old were transferred to the embryo grow-out unit. Embryos estimated to be older than 15 days were transferred directly to rearing units.

Monitoring of experimental variables

Aquaria were inspected daily for mouthbrooding females. Daily inspections were expected to improve the accuracy of spawning records as mouthbrooding females of *O. mossambicus* (Robles 1980); *O. niloticus* (Tave 1985) and *Sarotherodon melanotheron* (Eyeson 1983) may consume or spit out embryos at any time while mouthbrooding. Spawning records for each treatment were summed, and then averaged to determine the mean number of spawnings obtained per replicate per treatment in 21 days.

Females were replaced in the afternoon in order not to disturb spawning activities, which occurred between 08h00 and 12h00. Replacement involved the removal of mouthbrooding females from experimental aquaria at the specified time, the counting of embryos and their transference to incubators or rearing units. Females were then measured and finally transferred to a recovery aquarium. Females from the conditioning aquarium, which appeared to be in a gravid condition, replaced those removed from experimental replicates.

The labour input was determined as the total time in man hours required to replace females at the specified replacement times. Labour was given a hypothetical cost of R15 per hour so that data could be compared on a monetary rather than time basis. Embryo survival was determined using the same method as for Experiment 1.

Results

1. Spawning success

The mean spawning successes determined for each of the three replacement periods were significantly different (95% Scheffe test). Female replacement period A yielded the highest average number of spawns per replicate (Figure 9). Significant increases in spawning success of 40% and 167% were obtained over replacement periods B and C respectively ($F\ (2;18) = 17{,}38;\ p < 0{,}01$). Moreover, the weekly replacement of mouthbrooding females yielded a 90% increase in spawning success over the 21 day replacement period.

2. Embryo survival

The highest survival of embryos to the juvenile period (78%), was obtained when replacement period B was used (Table 10). This replacement period yielded a 28% and 7% improvement in embryo survival compared to replacement periods A and C respectively.

3. Labour input

The time taken to replace females was lowest for replacement period C (Table 11). The daily replacement of mouthbrooding females required a 48% and 159 % increase in labour effort over that necessary for replacement periods B and C respectively.

Discussion

The replacement of mouthbrooding females within six hours of spawning (A) yielded the most spawnings per replicate (8 $\pm$1,3 spawns) and the highest projected annual spawning frequency of 7,1 spawnings. However, the improvement in productivity was obtained at the expense of the highest recorded levels of embryo mortality (40%) and labour input required for replacement (31 minutes per replicate). The replacement

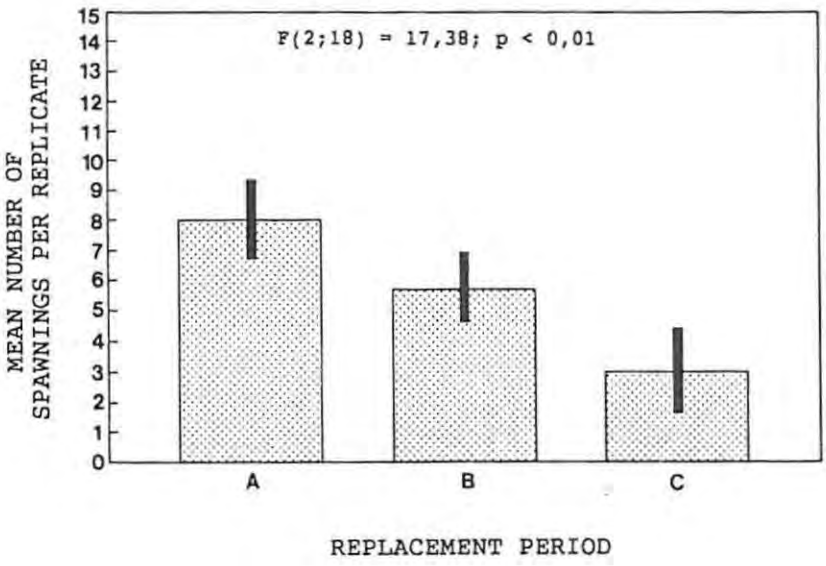

Fig. 9. The relationship between replacement period and spawning success. The replacement of females within 6 hours of spawning significantly improved spawning success (7 replicates were used for each treatment).

Key: The replacement of mouthbrooding females: A - within six hours of spawning, B - every seven days, C - every 21 days.

Table 10. The relationship between replacement periods and embryo survival. Embryo survival was maximized using replacement period B.

FEMALE REPLACEMENT PERIOD	NUMBER OF EMBRYOS INCUBATED	NUMBER OF JUVENILES OBTAINED	PERCENTAGE SURVIVAL OVERALL	RANGE
WITHIN 6 HR OF SPAWNING	1870	1123	60%	52 - 65%
EVERY 7 DAYS	1489	1160	78%	76 - 82%
EVERY 21 DAYS	966	688	71%	69 - 74%

of mouthbrooding females every 21 days (C) represented the opposite extreme, as it combined the lowest labour input required to replace mouthbrooding females (12 minutes per replicate), with an intermediate embryo mortality (29%), a low spawning success per replicate (3 $\pm$1,3 spawns) and the lowest estimated annual spawning frequency per replicate ($\geqslant$ 5,3 spawnings).

The replacement of mouthbrooding females every seven days (B) represented a compromise between the extremes of replacement periods A and C. An intermediate level of spawning success (5,7 $\pm$1,4 spawns) and labour input required for replacement (21 minutes) was recorded per replicate. Embryo survival was accelerated (78%), while estimated annual spawning frequency for females was high ($\geqslant$ 6,3 spawnings).

The commercial potential of each female replacement period over a 21 day period was estimated by combining mean data for the experimental variables: spawning success, embryo survival and labour input (Table 12). This table recommended, for two reasons, the use of the seven day replacement period as a means of enhancing productivity. Firstly, the cost of production was lowest for the seven day replacement period, approximately 18% and 37% lower than that required for the replacement of mouthbrooding females every 21 days and within six hours of spawning respectively. Secondly, the magnitude of juvenile production using the seven day replacement period, was estimated at 8% and 138% higher than production estimates for the replacement of females within six hours of spawning, and every 21 days, respectively.

Conclusion

Production of juveniles was most cost effective when mouthbrooding females were replaced with gravid females at seven day intervals.

Table 11. The effect of different replacement periods on the labour effort required per replicate to replace mouthbrooding females.

REPLACEMENT PERIOD USED	AVERAGE NO. OF FEMALES REPLACED PER UNIT TIME	AVERAGE TIME REQUIRED TO REPLACE FEMALES	COST OF TIME INPUT PER REPLICATE AT R15 PER HOUR
WITHIN 6 HR OF SPAWNING	8,0	31 min.	R 7,75
EVERY 7 DAYS	5,7	21 min.	R 5,25
EVERY 21 DAYS	3,0	12 min.	R 3,00

Table 12. An estimation of the commercial viability of replacement periods A, B and C.

REPLACEMENT PERIOD	MEAN NO. OF SPAWNINGS OBTAINED PER REPLICATE	ESTIMATED PRODUCTION JUVENILES PER SPAWN *	PER REPLICATE	COST OF TIME INPUT PER REPLICATE FOR REPLACEMENT AT R15 PER HOUR	COST TO PRODUCE 100 JUVENILES AS A MEASURE OF THE LABOUR COST OF REPLACEMENT
WITHIN 6 HR OF SPAWNING	8,0	23	184	R7,75	R4,21
EVERY 7 DAYS	5,7	30	171	R5,25	R3,07
EVERY 21 DAYS	3,0	28	83	R3,00	R3,62

* mean clutch size *A. baenschi* = 39 eggs x embryo survival during incubation

EXPERIMENT THREE: BROODSTOCK SEX RATIOS *

Introduction

A major problem facing the culturist wishing to propagate cichlids with polygamous reproductive styles is choice of broodstock sex ratio. A number of sex ratios has been used in the intensive culture of various species of *Oreochromis* (Table 13), a genus of cichlids with a polygamous reproductive strategy (Trewavas 1983). The varied use of sex ratio has confused the choice of sex ratio for culture purposes. According to Mires (1982), the inappropriate choice of sex ratios is a possible reason for the low production of tilapia juveniles in commercial spawning ponds.

Table 13. Sex ratios used in the intensive intraspecific culture of various *Oreochromis* species.

SPECIES	SEX RATIO USED (male/females)	REFERENCE
various *Oreochromis* species	1:2	Caulton 1979
O. mossambicus	3:12	Champion & Travis 1987
various *Oreochromis* species	1:7 to 10	Hepher & Pruginin 1981
various *Oreochromis* species	1:5 to 8	Mires 1974
various *Oreochromis* species	1:10	Rothbard 1979
O. niloticus	1:1 to 3	Watanabe *et al.* 1984

For *A. baenschi* it was postulated that in a given volume, low and high extremes of female to male ratio should reduce

* broodstock sex ratio (also referred to as sex ratio) was defined as the number of females maintained with a male in 250 l aquaria

productivity (see Figure 10). A low female to male ratio is shown to result in unproductive males and excessive aggression directed at females, which is known to result in mortality (Meske 1985). Too high a female to male ratio is shown to lead to a decreased percentage spawning success and excessive interference by females as well as egg eating during egg laying. Mrowka (1987) observed a positive correlation between the occurrence of egg eating and stocking density in *Pseudocrenilabrus multicolor*.

Moreover, there appear to be no quantified findings on the effect of several widely varying sex ratios on productivity for any species of cichlid. The aim of this experiment was, therefore, to quantify for *A. baenschi* which broodstock sex ratio yielded the highest production of embryos in 250l aquaria. To achieve this objective and to test the postulate, the effect of broodstock sex ratio on four variables was investigated:

1) aggression level

2) The number of spawns obtained per replicate (spawning success), with the sex ratio yielding the highest total spawning success representing the most effective utilization of space. This is a critical consideration for the small scale culturist. Alternatively, the sex ratio yielding the highest percentage spawning success represented the most effective utilization of broodstock, an important consideration for culturists inhibited by financial constraints.

3) the clutch size incubated by females

4) the levels of interference* and egg eating at spawning

<u>Materials and methods</u>

The duration of the experiment was 21 days, with five replicates used

* interference was defined as that movement of a female towards a mating pair which resulted in the spawning sequence being temporarily halted

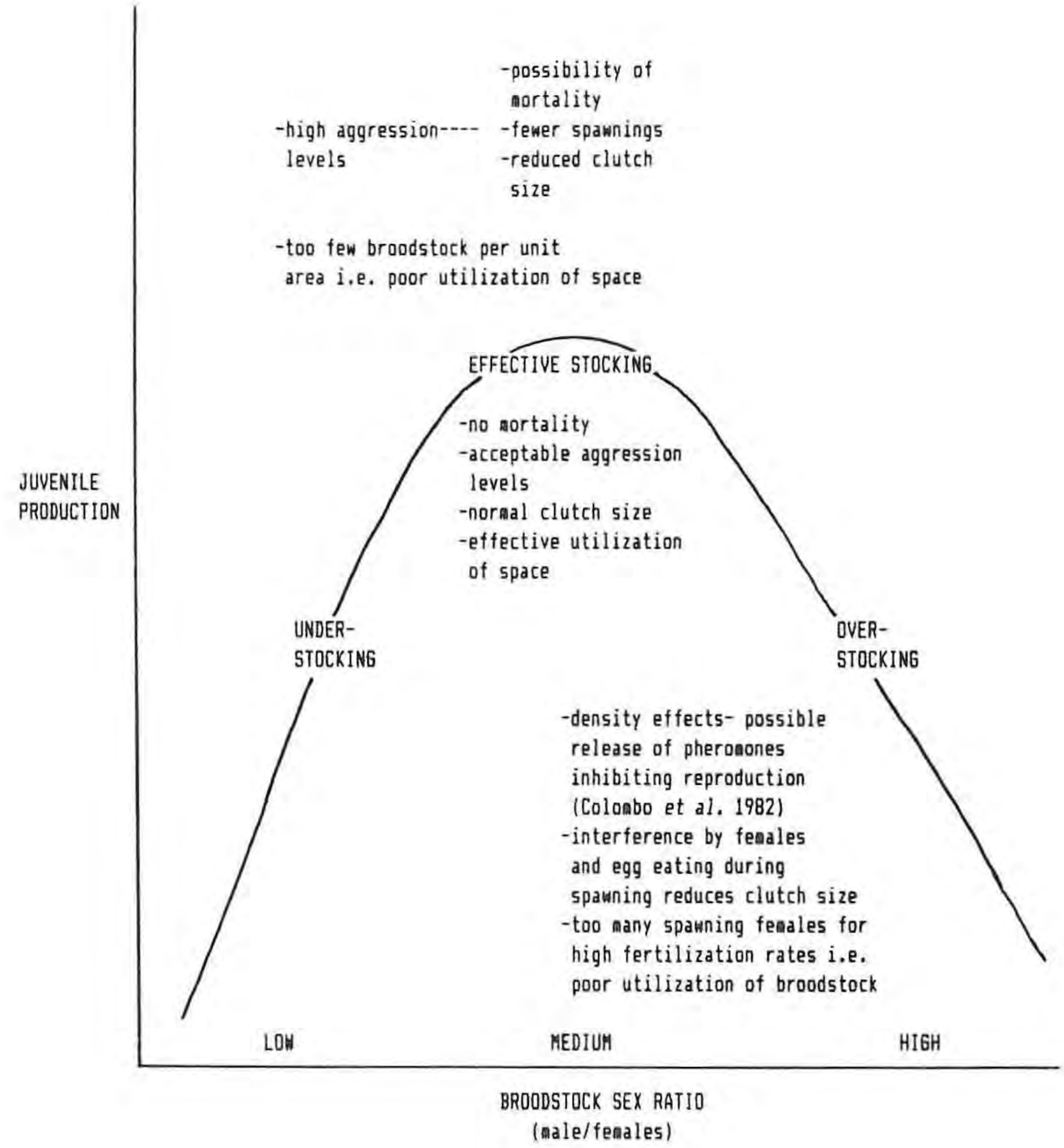

Fig. 10. Postulated relationships between broodstock sex ratio and productivity.

per sex ratio.

Experimental system

The experimental system comprised eight 250 l aquaria which were connected in series by a recirculating system. Two or more sex ratios were tested at any one time. The sex ratios tested were: 1:6, 1:12, 1:18, 1:30 and 1:45.

Monitoring of experimental variables

1. Determining aggression level in broodstock

Aggression was recorded daily as the level of inter / intrasexual chasing. A five-minute observation session per replicate was conducted each day between 09h00 and 11h00. Male and female aggression levels were proportioned by the number of females in each tank. This provided an estimate of how many times each female was chased by the male (male aggression) and by other females (female aggression) in the allotted five minutes. Attention was also focused on the occurrence of aggression-induced mortality.

2. Determining number of spawnings per replicate and female clutch size

Mouthbrooding females were removed from the experimental aquaria at seven day intervals. The number of spawns obtained in each aquarium replicate was counted. Embryos were removed from the mouths of females, counted and transferred to funnels. Females were measured for a clutch size / female size regression analysis, and then returned to their original aquaria. Females were not substituted by other females.

To determine whether deviations in observed clutch size from expected clutch sizes were positive or negative the following test was used, in conjunction with a chi squared test. Firstly, the observed clutch size was subtracted from the expected clutch size for each female size category (the sign of the deviation was retained). The expected clutch sizes (y = mx + c) for each female size category were derived from a female size / clutch size regression (see Fig. 6). Thereafter, the deviations were summed for each sex ratio. This value was then divided by the number of females used in the calculation.

The end result was a value which represented the average deviation in embryo number per clutch from the expected clutch size for a particular sex ratio. It was envisaged that this value would reflect reductions in clutch size due to high aggression levels or high rates of egg eating.

3. Determining levels of interference and egg eating at spawning

To monitor interference and egg eating a minimum of three different females were observed spawning with each of five males at each sex ratio. This was done to avoid a possible bias of results associated with the use of a single male or female. The release of 30 egg batches was observed for each male at a particular sex ratio.

The occurrence of interference was categorized into single interference (one female interfering during egg laying) and multiple interference (several females interfering during egg laying).

Results

1. Aggression level in broodstock

Total aggression levels of males and females increased with increasing sex ratio. However, when the aggression levels were proportioned by fish density then they decreased with increasing sex ratio. Increasing the sex ratio of broodstock from 1:6 to 1:30 significantly reduced male aggression levels ($F (4;520) = 66,12$; $p < 0,001$)(Figure 11). There was no significant difference in male aggression level between the sex ratios 1:30 and 1:45 (95% Scheffe test). Significant reductions in female aggression level were obtained as sex ratio increased beyond 1:12 ($F_{(4:498)} = 85,81$; $p < 0,001$) (Figure 12). Female aggression levels for the sex ratios 1:6 and 1:12 were similar (95% Scheffe test).

These results showed a significant inverse relationship between broodstock sex ratio and both male ($r = -0,81$; $p < 0,05$; d.f. = 4) and female aggression level ($r = -0,95$; $p < 0,01$; d.f. = 4) respectively. No mortalities were observed.

2. The number of spawnings and clutch size

The maximum average spawning success per replicate was obtained at a sex ratio of one male to thirty females ($F_{(4;20)} = 19,26$; $p < 0,001$)(Figure 13). This represented a significant improvement in productivity (95% Scheffe test). However, the sex ratio 1:6 yielded the highest average percentage of females spawned per replicate (60%)(Figure 14). Hereafter, as sex ratio increased, the percentage number of spawns initially decreased slowly to 1:30 (51%), and then dramatically to 1:45 (20%).

There were no significant differences between percentage spawning success per replicate for sex ratios between 1:6 and 1:30 (95% Sheffe test). However, the 20% spawning success obtained for the sex ratio 1:45, represented a significant reduction compared to percentage values obtained for the other sex ratios tested ($F_{(4;20)} = 12,25$; $p < 0,001$).

The relationship between the mean deviation in clutch size and broodstock sex ratio was found to be unimodal (Figure 15). Negative deviations in embryo number from expected clutch size were recorded at low (1:6) and high sex ratios (> 1:18), with deviations increasing rapidly in magnitude as sex ratio increased beyond 1:18. A positive deviation of one embryo per clutch over the expected clutch size was recorded at 1:12. The significance of this small increase is discussed later.

A chi-squared test revealed significant deviations ($p < 0,001$) in

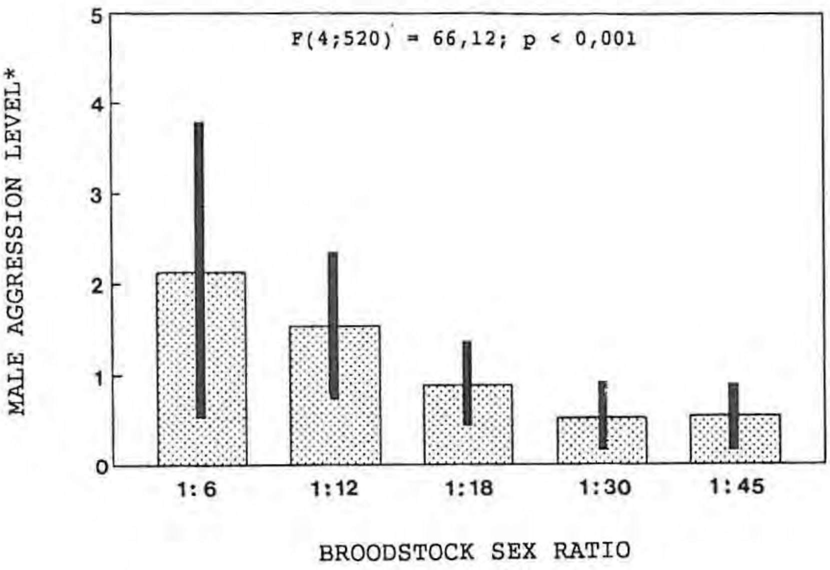

Fig. 11. The relationship between male aggression level and sex ratio. Male aggression level decreased with increasing sex ratio.

* male aggression level = the average number of times each female was chased by the male in a five minute observation period (n = 105 observation periods at each sex ratio)

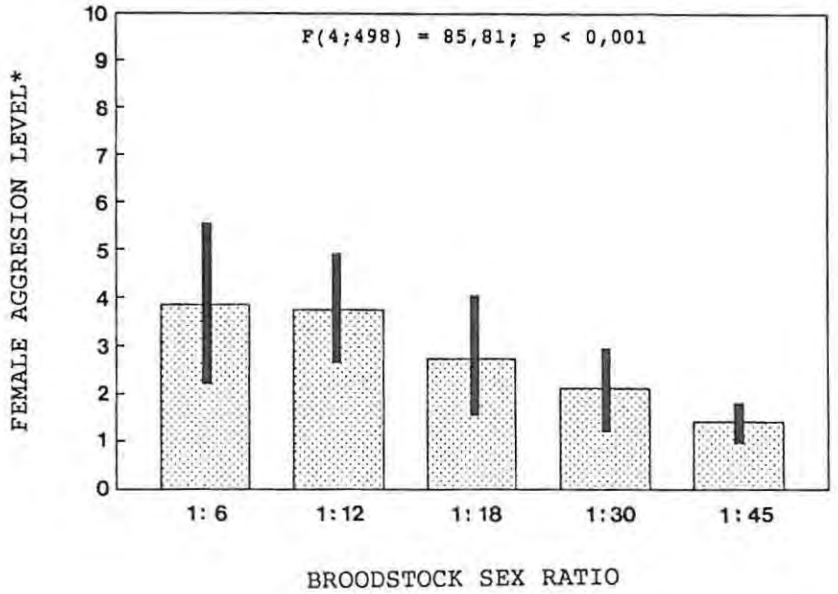

Fig. 12. The relationship between female aggression level and sex ratio. Female aggression level decreased with increasing sex ratio.

* female aggression level = the average number of times each female was chased by other females in a five minute observation period (n = 105 observation periods at each sex ratio)

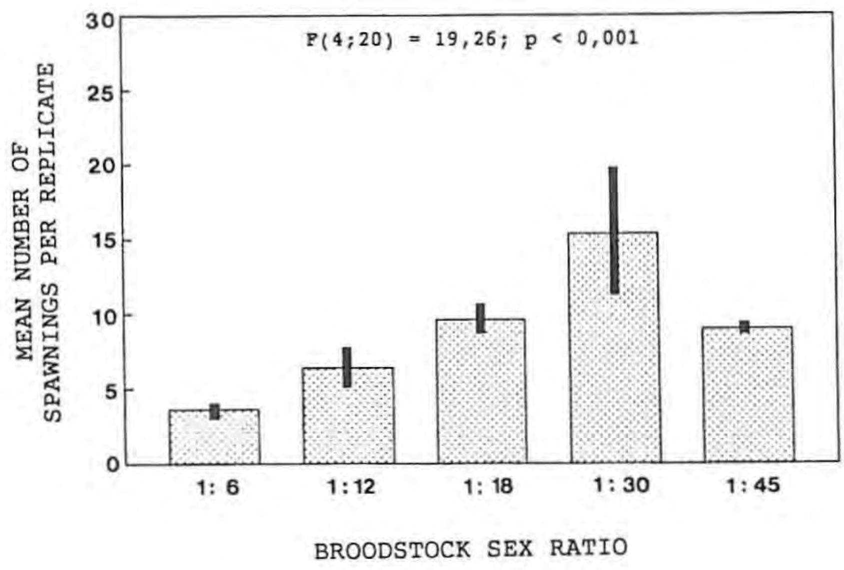

Fig. 13. The relationship between average spawning success and sex ratio. Spawning success was maximized when a sex ratio of 1:30 was used (n = 7 replicates per treatment).

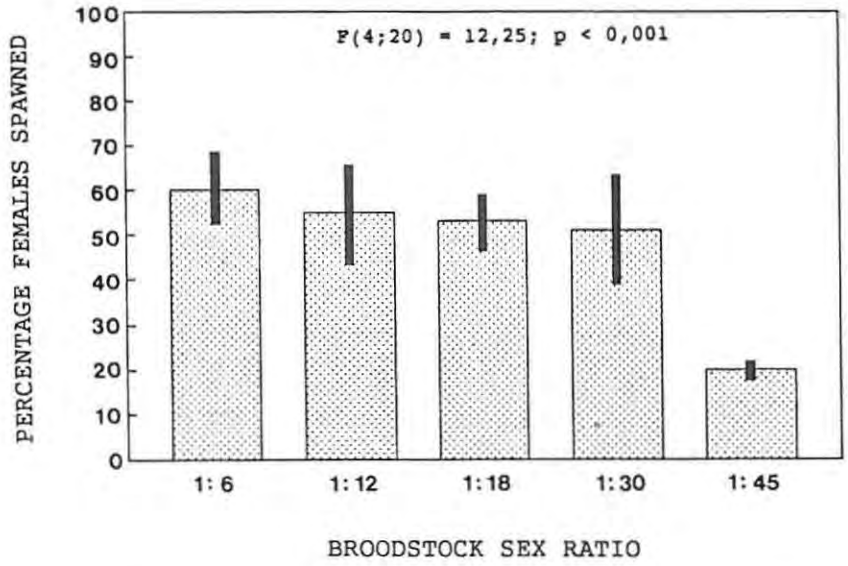

Fig. 14. The relationship between percentage spawning success and sex ratio. The sex ratio 1:45 yielded a significantly lower percentage of spawned females (n = 7 replicates per treatment).

embryo number for observed clutch sizes against expected clutch sizes for all sex ratios (1:6 - $x^2 = 68,0$; d.f. = 24; 1:12 - $x^2 = 79,1$; d.f. = 25; 1:18 - $x^2 = 59,1$; d.f. = 24; 1:30 - $x^2 = 147$; d.f. = 26; 1:45 - $x^2 = 91,3$; d.f. = 25).

3. Interference by females and the occurrence of egg eating at spawning

A significant relationship between the sex ratio and the level of interference ($F\ (4;20) = 6,03$; $p < 0,002$) and egg eating ($F\ (4;20) = 6,02$; $p < 0,002$) was observed. In particular, substantial increases in the level of interference and egg eating (primarily by the male) were observed at the higher sex ratios of 1:30 and 1:45 (Figures 16 and 17, respectively). These results yielded significant positive correlations between broodstock sex ratio and total interference level ($r = 0,97$; $0,01 < p < 0,001$; d.f. = 3) and between broodstock sex ratio and total egg eating ($r = 0,84$; $0,1 < p < 0,05$; d.f. = 3).

Female egg eating was uncommon. Egg eating by the spawning male was found to be a major factor influencing clutch size. For example, at a sex ratio of 1:45, male egg eating was observed in 36 of 150 (24%) egg batches laid. In addition, male egg eating represented 95% (or 63 out of 66) of the incidents recorded.

Moreover, the level of egg eating varied between males, with certain males showing consistently higher levels of egg eating at the higher sex ratios of 1:30 and 1:45 (see for males Nos. 1 & 5, Figure 18).

Discussion

1. Aggression level in broodstock

The results showed a significant positive correlation between sex ratio and aggression level in broodstock. Unfortunately, there have been few studies on the effect of sex ratio on aggression level in other cichlid species. However, Freer's (1987) work on *P. philander* at three densities (1:6; 1:10 and 1:12), showed that the aggression level exhibited per fish decreased with increasing supernumerary density. Moreover, Meske (1985) found that a high stocking density was an effective way of combating aggression in *Tilapia mariae*, kept in small volume tanks.

2. Spawning success

Spawning success, in 250 l aquaria, was maximized when a sex ratio of 1:30 was used. Of the sex ratios tested, this sex ratio therefore represented the most effective utilization of aquarium space. However, in terms of the most effective utilization of broodstock, the sex ratios 1:6 to 1:30 yielded similar percentage spawning successes ($51\% \leqslant x \leqslant 60\%$). A sex ratio of 1:45 yielded a significantly lower percentage of spawned females (20%).

The sex ratio recommended for effective utilization of aquarium space for *A. baenschi*, contrasts sharply with some of the sex ratios used for the intensive culture of *Oreochromis* species (Table 12). This suggests that several of the sex ratios used by other workers may have been ineffective in terms of space utilization.

With reference to percentage spawning success, the trends presented in this experiment conform with the findings of several authors, i.e. a reduction in the percentage of females spawned, with increasing sex ratio. The beneficial effects of male pressure (i.e. low sex ratios) have been well documented for *O. niloticus* by Hughes and Behrends (1983), Guerrero and Garcia (1983) and Watanabe *et al.* (1984). Moreover, Lovshin (1982) noted that the percentage of spawning *O. niloticus* increased with a decrease in the number of females per spawning area.

The reason for the lack of significant differences in percentage spawning success for the sex ratios from 1:6 to 1:30, may have been due to the combination of beneficial factors such as the reduction in the level of aggression, an increasing concentration of sex

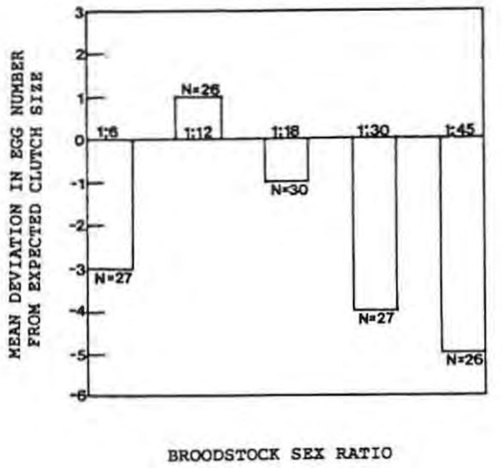

Fig. 15. The relationship between mean deviation in egg number from expected clutch size (= 0 for the purposes of this graph) and sex ratio. The sex ratio 1:12 yielded the highest average clutch sizes (n = no. clutches used in calculation).

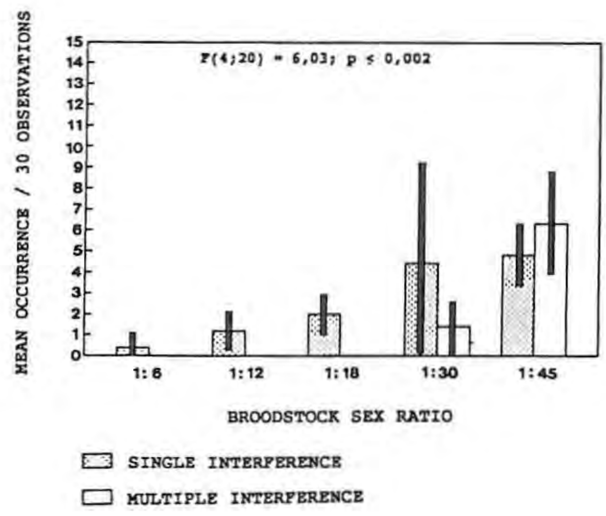

Fig. 16. The relationship between sex ratio and interference level. The level of interference increased as sex ratio increased.

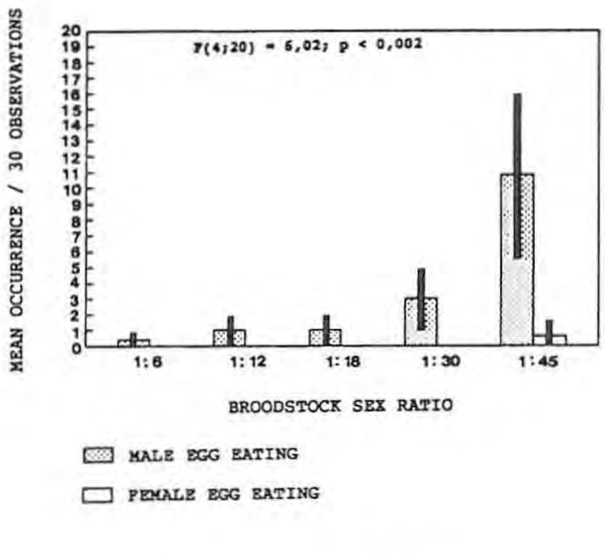

Fig. 17. The relationship between sex ratio and level of egg eating. Egg eating increased as sex ratio increased.

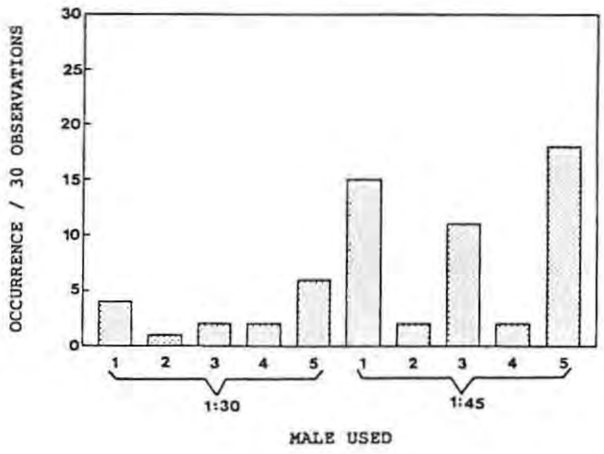

Fig. 18. The relationship between the male used at two sex ratios and the level of egg eating. Males No. 1 & 5 showed higher levels of egg eating at the sex ratios 1:30 and 1:45.

pheromones (Colombo *et al.* 1982); and detrimental factors, such as density-related stressors.

The reason for the significant reduction in spawning success for the sex ratio 1:45, is perhaps more easily explained. According to Billard *et al.* (1981), overcrowding affects productivity via food availability, visual interactions and pheromone release. Balarin & Haller (1982) suggested that the effects of crowding in tilapias adversely affected reproduction due to increased social interactions and a breakdown of territorial behaviour. Moreover, Rose (1959, in Colombo *et al.* 1982) discovered that in the guppy *P. reticulata*, the number of juveniles produced per female was inversely proportional to the number of females per tank. In addition, Swingle (1953, in Colombo *et al.* 1982) showed that the spawning of fish was inhibited if they were exposed to holding water from a high density stocking. However, in this study, Swingle~s finding was not supported as lower density units yielded significantly higher spawning returns despite sharing water with high density units. Further studies to clarify the relationship between density of spawners, pheromone concentration and juvenile production are, therefore, recommended.

3. Clutch size

The use of a 1:12 sex ratio yielded the highest average clutches from females with an average increase of one egg per clutch being calculated over the expected clutch size. All other deviations (excluding that for 1:18) yielded average reductions of three or more eggs per clutch. The positive deviation in clutch size for the sex ratio 1:12 assumes an added significance when the extreme precocial reproductive style of *A. baenschi* is considered. This is because a reduction of four eggs per clutch represents a 10% reduction in mean clutch size for *A. baenschi* (mean clutch size 39 eggs).

The sex ratio 1:6 yielding an average reduction of three eggs per clutch. This result was postulated as it had been suggested that at the sex ratio 1:6 high aggression levels would channel energy away from reproduction into aggressive activity and so reduce clutch size. Aggression level was highest at 1:6, and it is believed that this stress-inducing factor was responsible for this substantial reduction in clutch size. Egg eating was not a factor.

It was postulated that a sex ratio 1:12 would yield the highest clutch sizes due to substantially lower aggression levels and a negligible incidence of egg eating. This was validated. Thereafter, the expected trend of a reduction in clutch size, as sex ratio increased beyond 1:12, was confirmed. This relationship was probably due to the positive correlation between deviation in clutch size from expected values and average level of egg eating ($r = 0{,}70$; $0{,}73 < p < 0{,}81$; d.f. = 4) for the sex ratios 1:18 to 1:45. Furthermore, the possibility of a reduction in egg number due to other density-dependent stressors cannot be discounted. Fryer & Iles (1972) have suggested that female Mbuna may respond to stressful high density conditions by reducing clutch size, either by laying fewer eggs or by resorbing eggs which remain in the ovaries.

The application of a x^2 test yielded inconclusive results, with all sex ratios characterized by significant deviations between observed and expected clutch sizes.

4. Interference and egg eating at spawning

Significant positive correlations were observed between broodstock sex ratio and the levels of interference and egg eating. It was postulated that as female density rose the levels of interference and egg-eating would increase. Mrowka (1987) observed a similar trend for *P. multicolor*, in which high density conditions were associated with an increased occurrence of egg eating. Egg eating in *Cyrtocara eucinostomus* leks, in the Cape Maclear region of Lake Malawi, was also associated with a high density community (McKaye 1983).

A further observation was of relevance - in *A. baenschi* and, under the conditions in this experiment, the territorial male is responsible for egg eating. Female egg eating was negligible. This contradicts the findings of Mrowka (1987) for *P. multicolor*. Chan (1987) also observed no egg eating by territorial male *P. philander*. In *P. multicolor*, only non-territorial males, non-reproductive females and mouthbrooding females were egg eaters (Mrowka 1987); whereas in *P. philander*, only females ate eggs (Chan 1987).

The adaptive significance of egg eating is evident from the studies by McKaye (1983; 1984) and Mrowka (1987). According to Mrowka (1987), the eggs of *P. multicolor* are large (about 2mm in diameter), contain a large amount of yolk and should therefore constitute a profitable energy source, especially at high densities of fish when food is limited. McKaye (1983) observed, in *C. eucinostomus*, that approximately 5% of eggs were eaten by territorial males. McKaye (1983) suggested that the energy obtained from these potential offspring would allow the male to occupy a territory for a longer period, thereby enabling the male to fertilize more eggs and thus increase his fitness.

Conclusion

A sex ratio of one male to thirty females yielded the highest average number of spawnings per replicate, a high percentage spawning success and an acceptable clutch size from females. As such, it is recommended for use by small-scale and other culturists, who place emphasis on effective space utilization. However, a sex ratio of one male to twelve females yielded the highest average clutch sizes and a high percentage spawning success. This sex ratio is recommended for use by culturists who have an abundance of culture vessels or are limited by finances.

EXPERIMENT 4: REFUGES

Introduction

Aggression is one of the traits that has been selected for during the evolution of cichlid fishes (Fryer & Iles 1972; Jackson & Ribbink 1975). Aggression and territoriality are two of the principal constraints to the successful commercial production of cichlids. Although few cichlid species practice persistent territorial defence under natural conditions (Loiselle 1985), manifestations of aggression are exacerbated under artificial conditions, particularly under intensive conditions where space is a limiting factor.

Increased aggression levels in adult cichlids were considered to have three detrimental effects with regard to juvenile production:

a) A reduced reproductive output, as the energy used in aggression utilizes a substantial component of the energy budget (Li & Brocksen 1977, Feldmeth 1983 in Wootton 1985, Knights 1985), which could rather be channelled into the production of primary sex products.

b) Mortality of adults, as increased intraspecific aggression amongst aquarium-maintained tilapiines has been reported to frequently result in mortality (Mires 1977; Hepher & Pruginin 1981). Loiselle (1985) highlighted the need to employ effective prespawning management for polygamous cichlids as a means of avoiding mortality. Furthermore, considering the cost of acquiring ornamental fish broodstock, mortalities should be reduced as far as is possible.

c) Increased stress exhibited by mouthbrooding females. Stress has been known to lead to ejection or consumption of embryos, for example in *S. melanotheron* (Eyeson 1983), *O. niloticus* (Tave 1985) and *O. mossambicus* (Robles 1980). Under natural conditions, mouthbrooding females of the 'mbuna' group of cichlids often form independent shoals (Ribbink 1976), and seek 'brooding grounds' (Jackson & Ribbink 1975). These females appear quiescent and adopt a disruptive colour pattern (Jackson & Ribbink 1975).

Under normal aquarium conditions, mouthbrooding females cannot find solace away from fish engaged in reproductive activities. Increased aggression directed towards these females could lead to ejection of embryos and/or embryo cannibalism, both of which would reduce juvenile production.

Increasing the sex ratio of broodstock was shown to be a method of significantly reducing the level of aggression in broodstock. However, a technique is required which will reduce aggression levels at the relatively low sex ratio 1:12. This sex ratio was recommended in Experiment 3 for two categories of potential culturists of *A. baenschi* - those with an abundance of culture vessels or those culturists hampered by financial constraints.

Pierce (1980), identified the provision of refuges as one factor required for the successful indoor culture of *O. mossambicus*. Loiselle (1985) observed that the presence of cover had a substantial effect upon the level of aggression exhibited by cichlids. Ribbink (1980) advised the use of rocks as refuges for the culture of Malawi cichlids. In addition, Caulton (1979) advised the use of refuges as a means of reducing aggression for the intensive culture of tilapia in tanks. In all of the above-mentioned cases, the results were not quantified.

The aim of this experiment was to determine if the use of refuges influenced productivity. It was suggested that in *A. baenschi* productivity and aggression would be negatively correlated (Figure 19). Moreover, the use of effective refuges should reduce aggression level and increase productivity. To test this postulate, the following

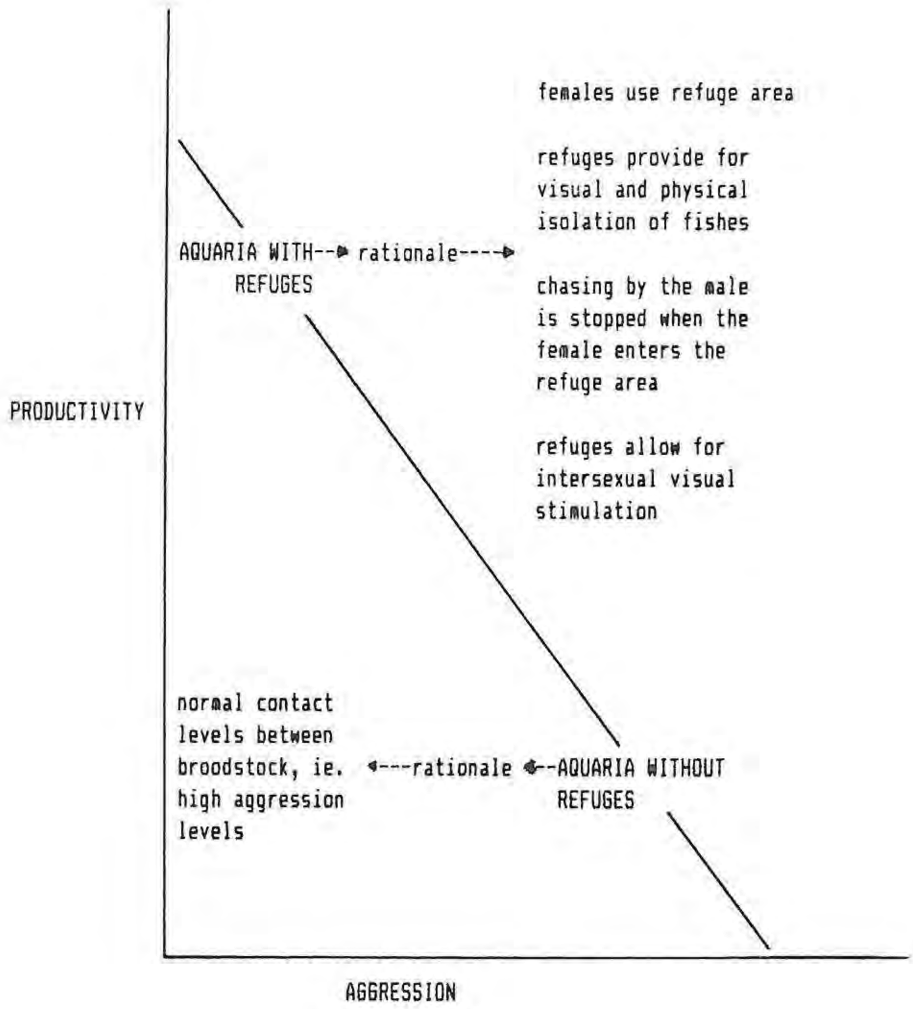

Fig. 19. The suggested relationship between aggression and productivity, and the possible effect of the use of refuges on this relationship.

questions were investigated:

1) Does a refuge area provide an effective barrier to the aggressive behaviour of the male?

2) Would refuges be used by female broodstock as a means of avoiding aggression? Refuge usage was examined in two ways:

-the percentage occupancy of the refuge area

-whether females found sanctuary in refuges when chased by the male and other females.

3) The effect of refuges on aggression level in broodstock.

4) The effect of refuges on productivity, i.e. the number of spawns obtained and clutch size.

Materials and methods

The duration of the experiment was 10 days. Seven replicates were used for each treatment.

Experimental system

The experimental system comprised twelve 250 l asbestos aquaria, five aquaria serving as holding tanks for spent females and for substitute females to replace spawned females. The remaining seven aquaria were used as control or refuge aquaria, and were connected in series by a recirculating system.

Control aquaria were equipped in the standard manner. The layout of an aquarium containing refuges is illustrated in Figure 20 & Plate 4. Each aquarium contained broodstock comprising a sex ratio of one male and twelve females.

Monitoring of experimental variables

1. Use of refuges by females, and the effectiveness of this area as a barrier to male aggression

A five minute observation session per replicate was conducted daily

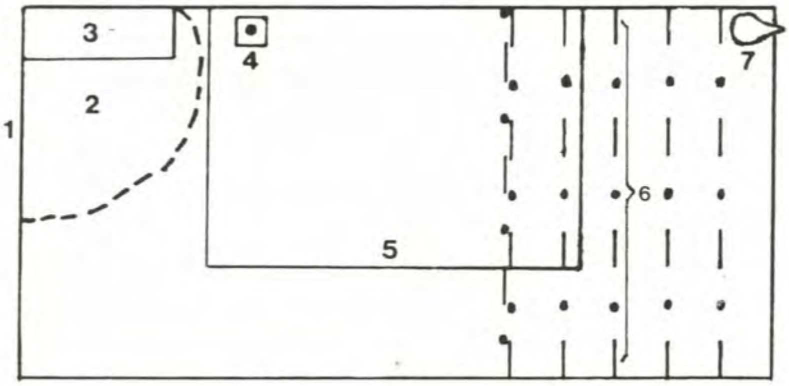

Fig. 20. A plan diagram of an aquarium with refuges (top view).

Key: 1 - asbestos aquarium as shown in Plate 3; 2 - mating pit; 3 - brick; 4 - airlift; 5 - undergravel filter; 6 - 1 of 6 vertically positioned refuge units; 7 - 220W immersion heater-thermostat.

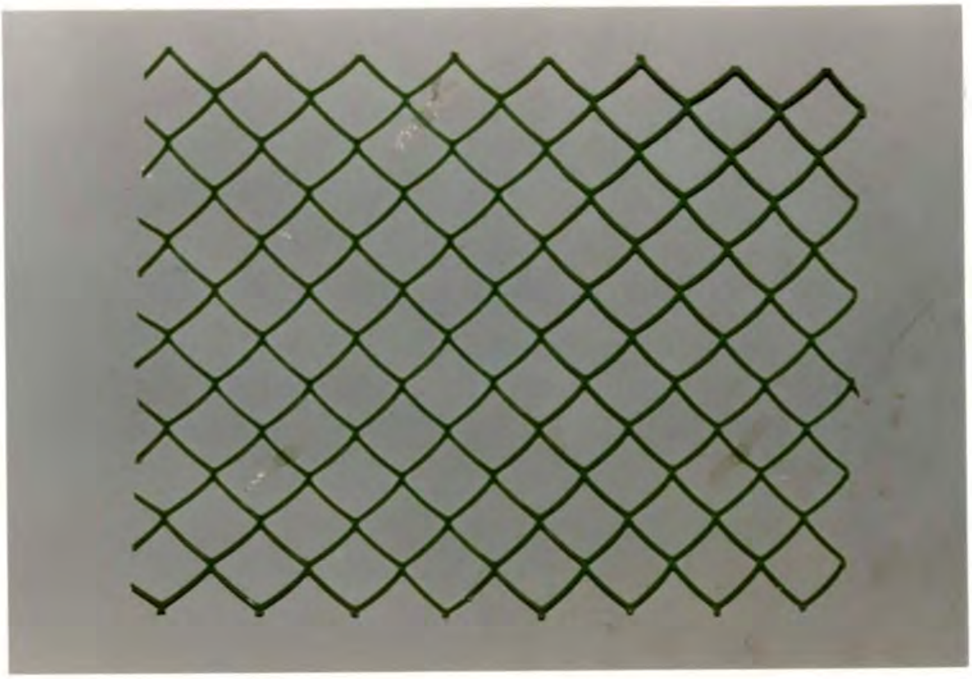

Plate 4. A refuge unit in vertical position (dimensions 52cm x 38cm, plastic 3mm thick, holes 6cm x 6cm).

between 12h00 and 13h00 to determine:

a) the percentage occupancy of the refuge area. At the end of each minute of the observation period, the number of females inside the refuge area was counted. This was compared with the expected occupancy, calculated as being four females (number of females in the tank = 12, multiplied by the area of the tank covered by refuges = 35% or 0.35).

b) whether females, occupying positions in the non-refuge area, found sanctuary in refuges when chased by the male and / or other females. This was determined by observing the number of times females entered refuges when they were chased by the male or other females.

The effectiveness of the refuge wall, in discouraging the male from access to a fleeing female entering the refuge area, was determined by observing how many times the male entered the refuge area in pursuit of females.

2. Determining the effect of refuges on total aggression level in broodstock

Total aggression level was defined as the summed aggression levels exhibited by the male, and females in each replicate in 50 minutes. This was recorded during five minute observation periods (n = 10) conducted once daily, between 11h00 and 12h00, per replicate. Aggression levels were monitored in the same manner as in Experiment 3.

3. Determining the effect of refuges on spawning success and clutch size

Aquaria were checked daily for mouthbrooding females. These were removed immediately (the date of spawning was recorded), whereupon the number of embryos per clutch and size of spawned female was recorded. Spawned females were then transferred to holding units. These females were immediately replaced by females which appeared to be in a gravid condition.

Results

1. The effectiveness of refuges as a barrier to male aggression

Refuges were very effective in providing the female with a barrier to escape male aggression (Table 14). The number of times males stopped chasing females when they entered refuges, varied from 83% to 100%. No result was obtained for replicate 1 as the incidence of male aggression in this tank was negligible.

2. Use of refuges by females

a) Occupancy

Occupancy of the refuge areas varied between replicates (Table 15), with an average occupancy estimated at 7 ± 2 females. The observed occupancy represented a significant deviation from the expected occupancy of four females ($x^2 = 22$; $0,01 < p < 0,001$; d.f. = 6). Moreover, high male aggression levels outside the refuge area were positively correlated with high occupancy rates ($r = 0,72$; $0,1 < p < 0,05$; d.f. = 5).

b) Use of refuges by females, swimming outside the refuge area, when chased by the male and other females

In replicates 2 to 7 females entered the refuge area on average of 75 ± 12% of male / female chases. Use of refuges by fleeing females was positively correlated with the level of male aggression exhibited outside the refuge area ($r = 0,84$; $0,02 < p < 0,01$; d.f. = 5) (Figure 21).

Females were less likely to use the refuge area when chased by other females. Mean use for all replicates was 48%, and the range varied from 3% (replicate 1) to 65% (replicate 6). There was an insignificant

Table 14. The effectiveness of refuges in preventing male access to fleeing females once they had entered refuges.

REPLICATE NUMBER	NO. CHASES OBSERVED IN WHICH FEMALES ENTERED REFUGES	% NO. TIMES THE MALE STOPPED CHASING AT THE REFUGE BARRIER
1	0	-
2	110	97
3	132	100
4	243	95
5	66	83
6	74	95
7	72	90

Table 15. Occupancy rates of refuges in the different replicates (n = 50 observations per replicate).

REPLICATE	TOTAL MALE AGGRESSION LEVEL #	OCCUPANCY BY FEMALES X	S.D.
1	6	3.3	2.1
2	132	7.4	1.6
3	174	6.4	2.0
4	230	10.1	2.0
5	103	7.6	2.4
6	111	7.9	1.8
7	114	6.2	2.2

total chases by male recorded outside the refuge area in 50 minutes

negative correlation between use of refuges by females and the level of female aggression exhibited outside the refuge area ($r = -0,47$; $p > 0,1$; d.f. = 5) (Figure 22).

3. Total aggression level and productivity

The use of refuges resulted in significant reductions in both male ($F (1;137) = 86,73$; $p < 0,001$)(Figure 23) and female aggression level ($F (1;137) = 81,29$; $p < 0,001$)(Figure 24). This reduction in aggression level in refuge-equipped aquaria was accompanied by an increase of 121% in the mean number of spawns obtained per replicate ($F (1;12) = 13,64$; $p < 0,003$) (Figure 25).

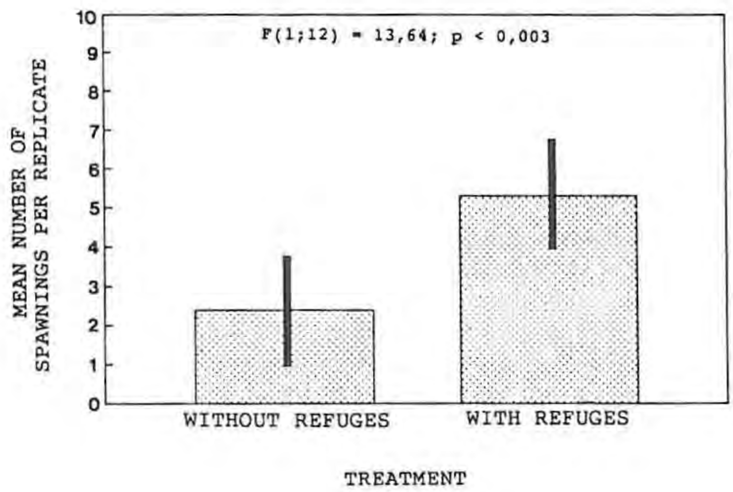

Fig. 25. The effect of refuges on spawning success

The effect of refuges on clutch size was unclear as both treatments yielded average clutch sizes that deviated significantly from expected

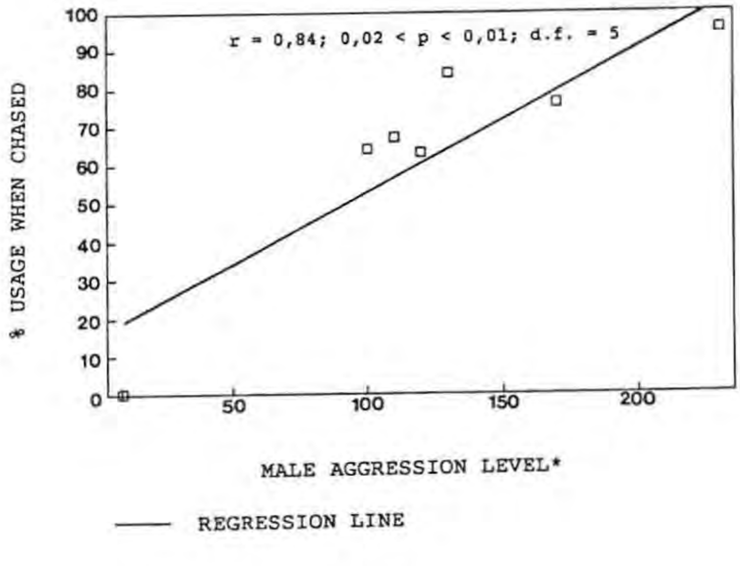

Fig. 21. The relationship between male aggression level and female usage rates of the refuge area when chased. Female usage rates were strongly correlated with male aggression levels.

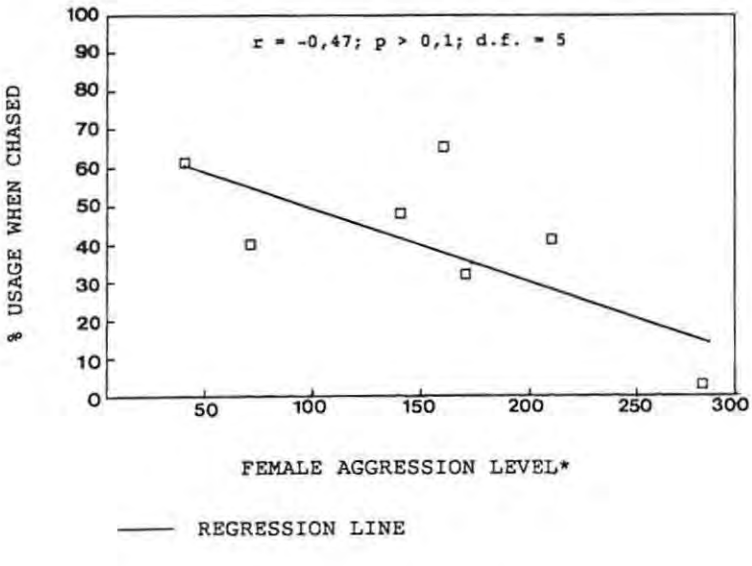

Fig. 22. The relationship between female aggression level and female usage rates of the refuge area when chased. Female usage rates were poorly negatively correlated with female aggression levels.

* male and female aggression levels represent the total number of chases performed by the male and females respectively outside the refuge area in 50 minutes

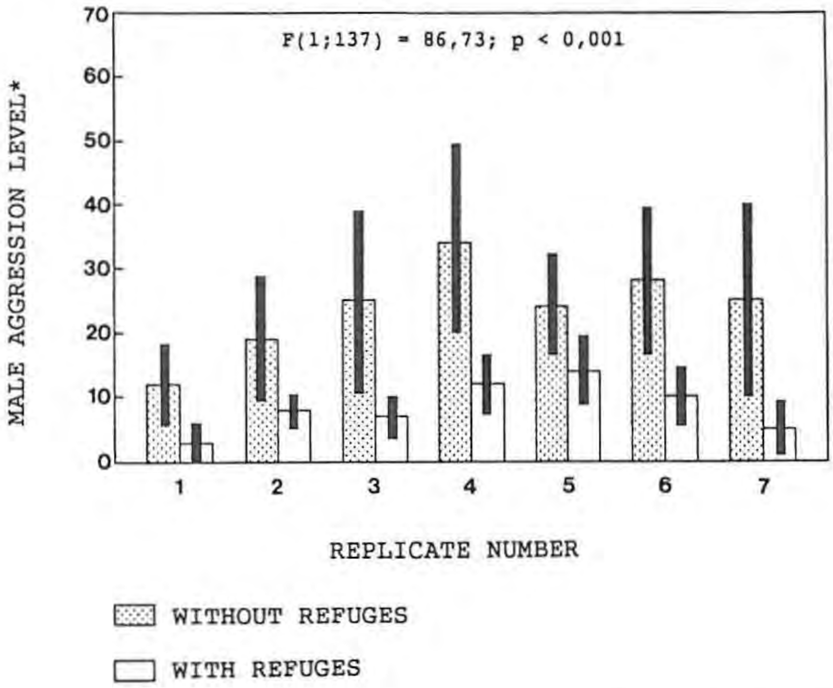

Fig. 23. The effect of refuges on male aggression levels. Each replicate contained a specific male for the duration of the experiment. The use of a refuge area significantly reduced male aggression levels.

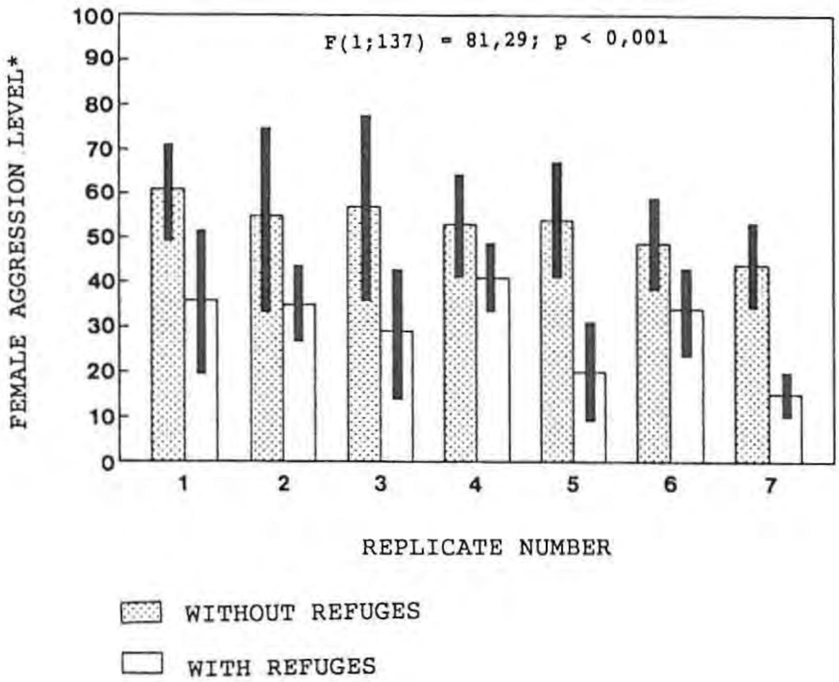

Fig. 24. The effect of refuges on female aggression levels. The use of a refuge area significantly reduced female aggression levels.

* male and female aggression level represent the average number of chases performed by the male and females respectively throughout the tank in 5 minutes (n = 10)

levels (x^2 = 65,4; p < 0,001; d.f. = 28 for refuge treatment, and x^2 = 58; p < 0,001; d.f. = 14 for control).

Discussion

Much of this discussion is speculative in nature as there is a lack of information on this field of research.

1. Use of refuges by females, and the effectiveness of this area as a barrier to male aggression

Refuges significantly reduced male contact with fleeing females once females had entered the refuge area. This reduction had two advantages. Firstly, the duration of such chases were decreased. This should have saved energy, which could then have been channelled into reproductive effort instead. Secondly, approximately 30% of the aquarium space (i.e. the refuge area) could be considered a 'male-free zone'. This can be regarded as a major factor behind the significant reduction in male aggression level in refuge-type aquaria. In creating a zone 'free' from male aggression, it was necessary to determine whether females responded appropriately, i.e. increased their use of the refuge area. This was verified. The mean occupancy rate of the refuge area was seven females against an expected occupancy of three fish (see methods). A x^2 test revealed that the observed occupancy was significantly different to the expected value.

Moreover, females made extensive use of refuges when fleeing from the male. Therefore, it is suggested that females perceived the refuge area as an area to which to escape from the aggressive behaviour of the male. This idea is also supported by the mean occupancy rate recorded.

Females entered the refuge area less frequently when chased by other females (48%) and, unexpectedly, usage was negatively correlated with female aggression level. The following suggestions are proposed for this finding. Firstly, in tanks with low levels of male aggression, females were distributed over a large part of the non-refuge area. Females at some distance from the refuge area were unlikely to seek refuge when chased by other females because of the long distance required to swim to the refuge area (this was noted during observations on aggression). Furthermore, females close to the refuge area (as a consequence of high male aggression levels) were more likely to enter refuges when chased by other females. This means that low levels of female aggression were related to high degrees of refuge use by the females which were being chased. Secondly, females may have perceived male aggression as being more intense and vigorous than female aggression, carrying the likelihood of physical damage. This suggestion is supported by observations on aggressive interactions between males and females, and between females. Females rarely injured each other during combat, while males occasionally bit the fins and bodies of females during chasing, particularly during spawning and at low sex ratios (particularly, 1:6).

2. Total aggression level

The use of refuges resulted in significant reductions in the total aggression level of both males and females. It is suggested that male aggression was reduced in two ways. Firstly, 30% of the aquarium was characterized by negligible levels of male aggression, and secondly, males did not usually enter refuges when chasing females, so the duration of such chases was decreased. Refuges were therefore effective in physically and visually isolating females from the resident male.

Female aggression was reduced in two ways - by high male aggression levels and high occupancy rates of refuges. Occupancy of refuges was significantly positively correlated with male aggression level. By forcing high refuge occupancy, high male aggression levels physically and visually isolated females, which lead to significant reductions in female aggression. Moreover, by forcing females (outside the refuge area) to occupy positions in or near to the refuge zone, high male aggression levels indirectly caused females to use refuges when chased by other females. In these circumstances, female aggression increased refuge use, and therefore played an important role in reducing total female aggression levels.

3. Productivity

The use of refuges resulted in a significant increase in spawning success which was accompanied by significant reductions in both male and female aggression level. The question is thus posed - was the reduction in aggression the causative agent for the increase in spawning success? The studies of Li & Brocksen (1977); Brett & Groves (1979); Feldmeth (1983, in Wootton 1985); Priede (1985) and Wootton (1985) appear to support such a relationship.

According to Wootton (1985), the energy costs of maintaining a territory take two forms: a) the cost of locomotion in defence of a territory, and b) an energy loss associated with the restricted foraging provided within a territory. The former cost is applicable to *A. baenshi* as females made extensive use of locomotion in defending positions in dominance hierarchies.

The energy used in to activity is very difficult to estimate (Soofiani & Hawkins 1985). However, swimming activity is regarded by Knights (1985) as a major component of energy expenditure. Priede (1985) illustrated the high metabolic power requirement of a rapid swimming motion (comparable to a chasing or fleeing movement). For example, Brett & Groves (1979) describe an attack or escape episode involving 20 seconds at burst speed as being equal in terms of energy expenditure, to 15 minutes of active metabolism, or about 3 hours of standard metabolism. Moreover, Feldmeth (1983, in Wootton 1985) estimated that a territorial male pupfish *Cyprinodon* sp. expended 0,32 kJ during a 16 hour day compared to 0,16 kJ or one half that of an inactive fish.

Therefore, it appears that agonistic interactions, particularly those involving fast swimming motions, constitute a major component of energy expenditure. Consequently, it is probable that the significant reduction in aggression level in refuge equipped aquaria resulted in a greater proportion of available energy being accumulated by the experimental animals. According to Priede (1985), this surplus energy can be used to enhance fitness through an increase in reproductive output. This was verified for spawning success. However, the relationship between clutch-size and aggression level was not clear. This may suggest that the energy 'saved' from the reduction in aggression levels in refuge aquaria, was channelled into spawning frequency, rather than into increased clutch size. The postulated relationship between aggression and productivity was therefore accepted.

Conclusion

A significant negative correlation between productivity and aggression level was observed in *A. baenschi*. Refuges were found to reduce the level of aggression in broodstock, thus contributing to increases in productivity.

CHAPTER 5: GROWTH AND SURVIVAL OF JUVENILES

Introduction

The rearing of juveniles to marketable size is a critical component of any culture programme. Hecht (1985) identified four factors on which the culture potential of a fish species is dependent. Three of these factors are of relevance to this chapter: the availability of sufficient juveniles, adequate growth and survival under culture conditions, and marketability of the product.

The aim of this experiment was therefore to determine growth, survival and time taken for *A. baenschi* juveniles to attain marketable size under intensive culture conditions.

Materials and methods

The duration of this experiment was approximately seven months (2/7/1986 to 21/3/1987).

1. Growth and survival

The experimental system consisted of two interconnected glass aquaria (each 60cm * 30cm * 30cm). Juveniles were reared in glass aquaria for two reasons: a) they allowed for precise monitoring of fish; and b) they simulated the type of rearing environment often used on ornamental fish farms.

One hundred and twenty newly released juveniles (1 to 5 days old) were obtained from the broods of several females. Sixty juveniles were stocked into each aquarium. The experimental conditions in each aquarium were identical to those described in chapter 2 (see 'Rearing'), except that the water temperature was maintained at 28 $\pm 2^{o}$C (Ribbink 1980).

Fifteen juveniles were randomly selected from the two aquaria for the determination of weight. Juveniles were initially weighed weekly (weeks 0 to 2), then fortnightly (weeks 3 to 10), then once every three weeks (weeks 11 to 22), and, finally, monthly (weeks 24 to 38). The fish were measured for length to the nearest mm, and then weighed on an electronic balance to the nearest mg. The mass of each fish was calculated by subtracting the mass of the fish with absorbent paper towel from the mass of the towel.

At monthly intervals, juveniles were removed from the aquaria and counted to assess survival rate. Juveniles were returned to experimental aquaria after being monitored for growth and survival.

2. Marketing

A marketing study was undertaken with Mr. D. Bevan of Waterlife Aquariums to determine product presentation and the prices producers could expect for *A. baenschi*. This study involved the transportation of fish to Waterlife, from where they were sold to other retailers or directly to the public.

Results

1. Growth and survival

Juveniles readily accepted crushed tetra growth diet as a first feed. *Daphnia* was eagerly accepted when supplemented to the diet. The growth rates of juvenile *A. baenschi* are depicted on Figures 26 & 27. Males grew at a significantly faster rate than females (for mass - $T = 10,47$; $p < 0,001$; d.f. = 12 (Fig. 28), for length - $T = 10,55$; $p < 0,001$; d.f. = 12). The fastest growing males started exhibiting male characteristics when approximately 90 days old (male 41mm / 2,06g). Evidence of this change was the appearance of yellow colouration on the body and fins, and the development of a pointed dorsal fin. The first male showed intensification of colour (more yellow on the body, development of blue colour on the jaw region) at 111 days (male 46mm / 2,83g)(Plate 5). Males at this stage of development were suitable for sale. Pit building activity by males was first observed when the fish were 125 days old (male 50mm / 3,82g). In spawning trials these males fertilized the eggs of females.

The average age and size of females at first spawning was 261 days ±21 days and 55 ±4mm respectively (n = 5). Percentage survival of juveniles to marketable size was 100% over the experimental period.

2. Marketing

Juveniles were not marketable because they resembled females in colour (Plate 6), and therefore could not command good prices. Males were sold at an earlier age than females, a consequence of their faster growth rates. The first males were marketable after 111 days of growth. However, the sale of *A. baenschi* on domestic markets was accelerated when pairs were sold. The time taken for 70% of juveniles to become suitable for sale as pairs was approximately seven months (average size - males 5,8g; n = 7: females 5,0g; n = 7). A wholesale price of R4 per fish was paid by Waterlife Aquariums. Approximately 150 fish were marketed.

Discussion

Two factors were examined in assessing the culture potential of *A. baenschi* from a rearing perspective. These factors were the time taken for juveniles to attain marketable size and the survival of juveniles during this period. Comparative data on these two factors for several popular species of ornamental fish has been provided by B. Andrews of Amatikulu hatchery.

1. Marketing

In comparison with several other popular species of ornamental fish (Table 16), the time taken for *A. baenschi* juveniles to attain marketable size is very slow, and is therefore, a considerable limitation affecting their culture potential. This drawback was due to slow growth rates of juveniles and a market preference for colourful

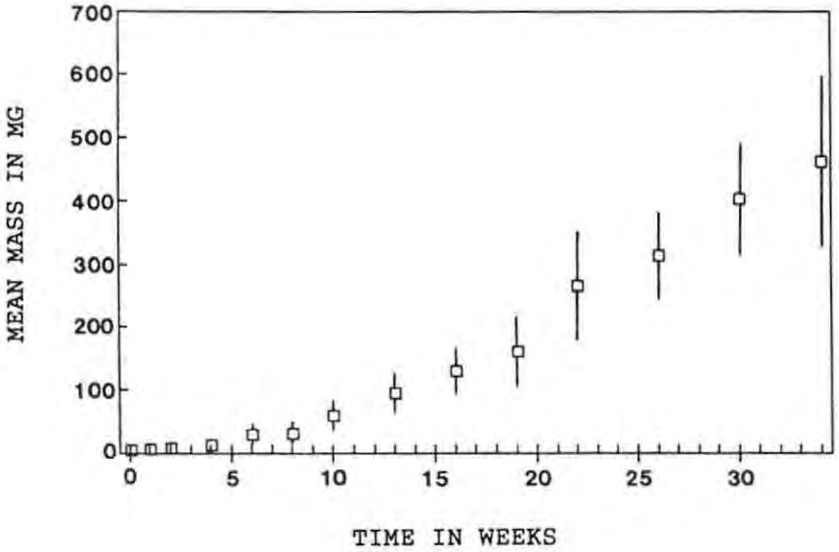

Fig. 26. Growth in mass of *A. baenschi* juveniles.

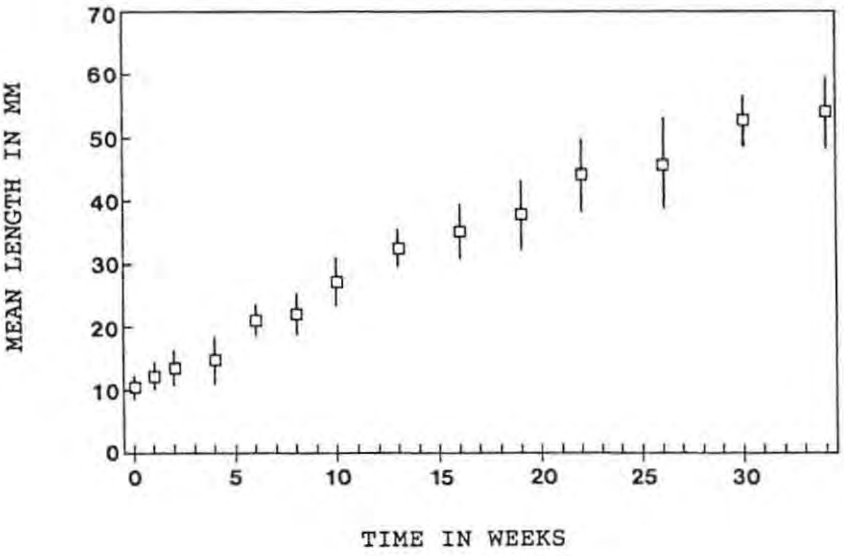

Fig. 27. Growth in length of *A. baenschi* juveniles.

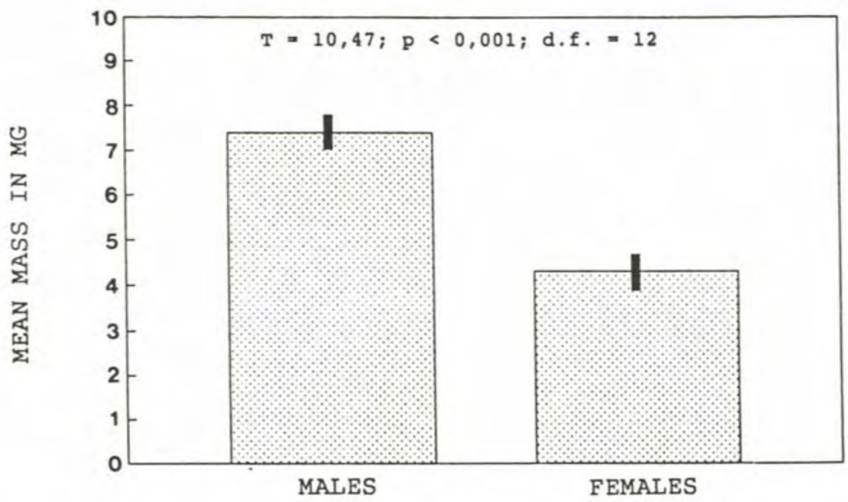

Fig. 28. The relationship between sex of *A. baenschi* juveniles and mass in mg after seven months of growth.

Plate 5. Marketable male *A. baenschi*.

Plate 6. Juvenile *A. baenschi*.

adults sold as pairs. However, the wholesale prices fetched by *A. baenschi* was considerably higher than for several of the species listed in Table 16 (see Table 1).

These findings imply that culturists must use a specific marketing strategy (see Recommendations) if the culture of *A. baenschi* is to become a viable commercial consideration.

2. Survival

The survival of *A. baenschi* juveniles at the experimental density was exceptional when compared with juvenile survival of other cultured species of ornamental fish (Table 17). Moreover, de Villiers (1987) observed a 90 to 94% survival of *A. baenschi* juveniles at widely varying densities (10, 20, 160 & 500 fish per 26 l tank).

However, in any assessment of survival, the life history style of the species must be considered. This is because the rearing-related factor of primary importance to the culturist is the number of marketable fish. For example, embryo yield from a spawning of the ornamental anabantid, *T. trichopteris* (altricial life history style, term after Balon 1984), usually exceeds 10 000 embryos. However, survival of juveniles to marketable size is approximately 30 to 40%. Therefore, from one spawning of *T. trichopteris*, an estimated 3000 to 4000 fish can be marketed. In comparison, the optimal yield of marketable progeny from a spawning of *A. baenschi* (extremely precocial life history style) is less than 100 fish.

Therefore, the extreme precocial life history style of *A. baenschi* necessitates high juvenile survival rates for effective culture. Such a survival rate was obtained at the experimental stocking density and by de Villiers (1987).

Table 16. The average time required for several popular species of ornamental fish to attain marketable size under intensive culture conditions, in comparison to *A. baenschi* (source: B. Andrews, pers. comm.).

SCIENTIFIC NAME	COMMON NAME	TIME REQUIRED
A. baenschi	yellow peacock Aulonocara	±7 months
Brachydanio rerio	zebra fish	±6 weeks
Capoeta tetrazona	tiger barb	±6 weeks
Carassius auratus	goldfish several varieties	±4 - 6 weeks
Cyprinus carpio	koi carp	±2 - 3 months for a 5 - 6cm specimen
Poecilia reticulata	guppy	±12 weeks for males; 6 - 8 weeks for females
Poecilia latipinna	black molly	±2 - 3 months
Pterophyllum scalare	angelfish	±12 weeks
Trichogaster trichopterus	5 varieties	±6 weeks
Trichogaster leeri	pearl gouramy	±8 weeks

Table 17. Percentage survival to marketable size of *A. baenschi* juveniles (under controlled conditions) in comparison to several popular species of ornamental fish cultured at commercial densities (source: B. Andrews, pers. comm.).

SCIENTIFIC NAME	COMMON NAME	PERCENTAGE SURVIVAL
A. baenschi	yellow peacock Aulonocara	±100%
Poecilia reticulata	guppy	±90%
Poecilia latipinna	black molly	±90%
Pterophyllum scalare	angelfish	±30%
Trichogaster trichopterus	5 varieties	±30 - 40%

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

This chapter evaluates the project from several viewpoints and as such has been divided into seven sections.

New knowledge on the biology and ecology of *Aulonocara baenschi* and aggression in mouthbrooding cichlids.

According to Hecht (1985), an important factor in assessing the potential of a finfish species for culture is its suitability for culture, which is established by a thorough knowledge and understanding of its biology under natural conditions. Field studies by Regan (1921), Fryer (1959), Fryer & Iles (1972), Ribbink *et al.* (1983) and Grant *et al.* (1987) revealed that *Aulonocara* spp. were maternal mouthbrooders, unspecialised insectivores, inhabited the sand-rock interface at a considerable depth range (hence the large eyes); with adults exhibiting considerable sexual dichromatism. Aquarium studies on *Aulonocara* spp. by Staeck & Terver (1977); Axelrod *et al.* (1984) and Loiselle (1985) provided preliminary descriptions on recommended tank environments, feeding regimes and embryo development rates. The biology of *A. baenschi*, in particular, under natural or artificial conditions, has received little or no attention.

This study has contributed towards an increased knowledge and understanding of the biology of *A. baenschi* under artificial conditions. Aspects of reproduction of crucial relevance to culturists have been described, for example; spawning times and seasons, clutch size and its relationship with female size, age and size at first spawning, embryo development rates (especially time required for hatching and attaining the juvenile period), and size and environmental requirements of recently released juveniles.

Descriptions of the reproductive biology of *A. baenschi* in tanks has contributed to an understanding of aggression in mouthbrooding cichlids; in particular, the complex interrelationship between aggression, broodstock sex ratio and juvenile production. Moreover, these descriptions provide culturists with an outline of the type of behaviour to be expected from *A. baenschi* in aquaria, which should greatly facilitate the management of broodstock.

New perspectives on the culture of mouthbrooding cichlids in intensive systems

Previous studies on the intensive tank culture of mouthbrooding cichlids have centered on the commercially important food-fishes, the tilapias. It appears that the intensive culture requirements of ornamental mouthbrooding cichlids have not been addressed by applied science.

Several techniques are used for embryo incubation, sex ratio and the removal of mouthbrooding females to accelerate juvenile production in *Oreochromis* spp. (Table 18). Table 18 reveals that there is no stereotyped procedure for culturing species of *Oreochromis*. This assessment perhaps reflects the flexible reproductive behaviour of mouthbrooding cichlids (see James & Bruton 1988, for *O. mossambicus*; and Noakes & Balon 1982, for tilapias in general); a behaviour that provides much scope for manipulating productivity. Moreover, it may show that the breeding behaviour of each species of *Oreochromis* is unique, requiring an independent management approach for effective culture. The above suggestions support the rationale for conducting this study.

Furthermore, research into tilapia culture has not involved investigations into the merits of refuge areas and replacement periods for mouthbrooding females. This is surprising when the aggressive dispositions of these fish and the use of artificial incubation techniques has been acknowledged. This project on *A. baenschi* has provided a new outlook on cichlid culture in intensive systems by verifying the commercial viability of utilizing refuges and a specific replacement period for mouthbrooding females. Moreover, a previously unidentified need for using two sex ratios was identified and verified for *A. baenschi*, one representing the most effective utilization of aquarium space (1 male to 30 females), and the second representing the most effective utilization of broodstock (1 male to twelve females).

Table 18. The techniques used for embryo incubation, sex ratio and removal of mouthbrooding females in the intensive culture of *Oreochromis* spp.

SPECIES	TYPE OF EMBRYO INCUBATION	REMOVAL OF MOUTHBROODING FEMALES	SEX RATIO (MALE: FEMALES)	REFERENCE
O. mossambicus	natural	females captured at night and juveniles removed	3:6	Caulton 1979
O. niloticus & *O. aureus*	artificial	females removed after 3 to 5 days of mouthbrooding embryos	1:7 to 10	Hepher & Pruginin 1981
O. aureus	artificial	females removed every 7 to 10 days	3:9	Snow et al. 1983
Oreochromis species	artificial	females removed every 10 to 20 days	1:2 to 4	Mires 1983

The results of this study confirmed the viability of two techniques frequently utilized for culturing Oreochromis spp., that is: a) the artificial incubation of embryos at the time of hatching (see Hepher & Pruginin 1981), and b) the use of sex ratios which are skewed in favour of females (see Table 13, and Balarin & Haller 1982).

Hatchery techniques to enhance productivity

The following hatchery techniques are recommended for the intensive tank culture of *A. baenschi* as their use under experimental conditions was observed to accelerate the production of juveniles.

The preparation of a tank for spawning purposes must include the provision for a refuge area. The type, dimensions and placement of refuges used in Experiment 4 provide a guideline for effective use. If space is at a premium, tanks should be stocked with a sex ratio of 1 male to approximately 30 females. However, if space for culture purposes is unlimited, or alternatively finances are at a premium, then a sex ratio of 1 male to approximately 12 females is recommended. Once aquaria are stocked then mouthbrooding females must be replaced with gravid females at seven day intervals. The embryos obtained from the mouths of brooding females must then be incubated, initially in funnels until approximately three days post-hatch, and thereafter in an embryo grow - out unit. Embryos at the beginning of the rapid yolk absorption phase are then transferred to aquaria for rearing.

Commercial viability of the culture system used during experimentation

In excess of 500 spawnings were recorded in experimental aquaria. As the hatchery system used yielded a convincing and sustained reproductive response from the experimental animals, it is recommended for the intensive tank culture of *A. baenschi*. The intensive culture of *A. baenschi* in pools is an alternative to tank culture. Culturists wishing to use this approach are advised to consult relevant texts on the culture of mouthbrooding cichlids in extensive or semi-extensive systems (for example Bardach *et al.* 1972; Caulton 1979; Hepher & Pruginin 1981). This project has not examined this approach for culturing *A. baenschi*, and accordingly precise recommendations cannot be offered.

The closed system incubator and embryo grow-out unit were an effective means of incubating the embryos of *A. baenschi*. As the latter incubator was a modified aquarium previously used to determine temperature preferenda, two alterations to the incubator are recommended as a means of simplifying embryo collection (appendix 2). Furthermore, both incubators incorporated expensive component materials such as glass aquaria and Eheim pumps. Cheaper alternatives to these two components are available, for example; asbestos or plastic holding units and unused pumps that are suitable for modification (e.g. washing machine pumps).

The rearing environment yielded excellent juvenile survival but a slow growth rate. Several factors, acting independently or in combination, may have contributed to the slow attainment of marketable size by juveniles. The first possibility may have been an inadequate ration or feeding frequency or that the diet used needs to be improved. Secondly, water quality may have been unsuitable for the attainment of maximum growth. Alternatively, *A. baenschi* is slow growing species.

Assessment of the culture potential of *A. baenschi*

According to Hecht (1985), the potential of a finfish species for culture depends on four factors: a) suitability for culture (established by a thorough knowledge and understanding of the biology under natural conditions), b) availability of sufficient juveniles, c) adequate growth and survival under culture conditions, and d) marketability of the product.

This project has established the suitability of *A. baenschi* for culture by describing environmental conditions under which commercially viable levels of spawning activity, and embryo and juvenile survival were obtained. Moreover, the use of the intensive system recommended in the previous two sections of this chapter should ensure sufficient juveniles for culture purposes. In particular, the use of the two recommended sex ratios must be emphasized, as any species with an extremely precocial life history style incorporating polygamy such as *A. baenschi*, implies that the major factor affecting productivity is the number of females available for spawning.

If growth and survival of *A. baenschi* are considered, then major limitations relating to the former factor become apparent. This is because the time required for juveniles to attain marketable size was more than double the time required for several popular species of ornamental fish. It must be stressed however, that the major objective of this study was not an assessment of this criterion. As such, the results on growth and survival were preliminary in nature. Therefore, it is feasible that under optimal conditions the time required to attain marketable size may be substantially reduced.

The survival of both juveniles and adults under culture conditions was excellent (almost 100%). Moreover, the different life history periods were found to be tolerant of widely varying water quality.

Perhaps the most meritorious culture-related attribute of *A. baenschi* is its marketability. Prices fetched on wholesale markets in South Africa were considerably higher than for several popular ornamental fish species, including species characteristically commanding high prices such as the brown discus *S. aequifasciata*.

However, the market for *A. baenschi* is specialist in nature. As such, a major long term production thrust with *A. baenschi* into the relatively small South African market is not recommended for domestic producers. A more profitable strategy should, therefore, involve a major production effort with several species of *Aulonocara* (in particular *A. stuartgranti*, Plate 7; and the Usisya population of *A. baenschi*, Plate 8) for the large and potentially lucrative foreign markets. An added advantage for South African producers who successfully penetrate these markets is the exchange rate of the Rand with these currencies.

<u>A contribution by applied science</u>

Members of the ornamental fish industry (in particular, producers and wholesalers) frequently ask me what contribution research makes to the industries development. This project has established a scientific foundation for the aquaculture of an ornamental mouthbrooding cichlid species in South Africa. The problems which limit the culture potential of *A. baenschi* under intensive tank culture conditions have been addressed. Specific techniques involving embryo incubation, replacement periods for mouthbrooding females, broodstock sex ratios and the provision of a refuge area have been identified and quantified as a means by which the production of *A. baenschi* juveniles is accelerated. In Chapter 3 it was established that the reproductive behaviour and biology of *A. baenschi* was similar to other maternal mouthbrooding cichlid species. As such these techniques should be applicable for use in the intensive tank culture of comparable species of mouthbrooding cichlids.

Proposals for future research

The culture potential of *A. baenschi* will continue to be negated by the comparatively slow attainment of marketable size. Techniques which might accelerate this process should be investigated in future.

Plate 7. Male *A. stuartgranti* in nuptial colouration.

Plate 8. Male *A. baenschi* from Usisya in nuptial colouration.

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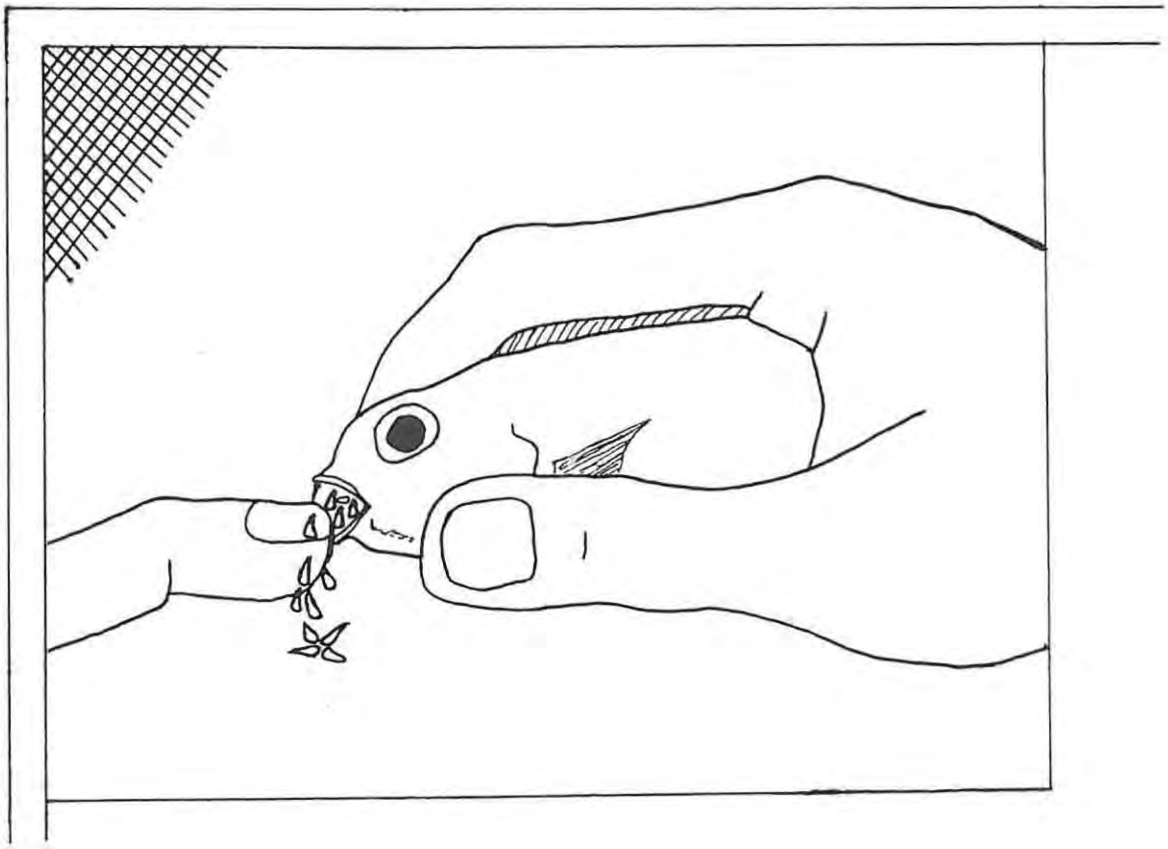
APPENDIX 1: Illustration of the procedure used for the removal of embryos from the females mouth. Gentle intermittent pressure is applied to the lateral side of the gill cover of the mouthbrooding female. As pressure is applied the mouth is held open, thus expelling embryos from the females mouth. The whole operation takes place over a net suspended just below the waters surface. The mosquito mesh of the net prevents the female from escaping during the egg removal procedure and also catches embryos as they are expelled from the buccal cavity. Embryos are collected by using a plastic tube to siphon them into a container.



APPENDIX 2: Recommended embryo grow-out unit for the incubation of *A. baenschi* embryos older than 10 days.

Key: 1 - External filter, 2 - water outlet, 3 - 120cm * 30cm * 30cm glass aquarium, 4 - perforated divider, 5 - water level, 6 - 200W immersion heater thermostat, 7 - airpipe, 8 - airstone, 9 - plastic support for incubation chamber, 10 - plastic gauze (1mm * 1mm) of incubation chamber, 11 - intake pipe, 12 - handle of removable incubation chamber. Arrows indicate direction of water movement.