

PARENTAL INFLUENCES ON EGG QUALITY, FRY PRODUCTION
AND FRY PERFORMANCE IN OREOCHROMIS NILOTICUS (LINNAEUS)
AND O. MOSSAMBICUS (PETERS)

A thesis presented for the degree of
Doctor of Philosophy to the University of Stirling

by

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1986

5/87

To my parents
and all those who educate me



Oreochromis niloticus



Oreochromis mossambicus

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ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr Donald Macintosh for his assistance throughout my studies and for seeing this thesis through to its conclusion.

My studies were made less arduous by the help and advice of many members of staff at the Institute of Aquaculture and in particular I would like to thank Dr Albert Tacon, Dr James Muir, Dr Hadrian Stirling and Dr Brendan McAndrew. I would also like to extend my thanks to Dr Newbury of the Department of Biological Sciences and Dr Mitchell of the Department of Mathematics, University of Stirling, for their invaluable advice on matters statistical.

I received secretarial and technical help from several people and thank Laura Cummings, Sheila MacEwan, Elizabeth Stenhouse and Ian MacRae. I would also like to acknowledge the technical help of the friendly staff of the Shared Technical Services and in particular Mr Robert Brodie, and Mr Ron Stewart for his invaluable help in photography. A special thanks to the staff of the Tropical House, in particular Mr Ian MacGowan, for their help and co-operation.

For the typing, I would like to thank Joanna Mackay for the excellent transformation of my manuscript. Her expertise and patience were much appreciated.

My friends and colleagues helped in many ways and I would like to thank all the PhD students, Dr Kshitish Majumdar and Dr Tej Singhe for the many lively discussions.

I am greatly indebted to the Africa Educational Trust for their Scholarship making this study possible.

Finally I would like to mention a very special thank you to my family for their help and support from across the oceans and most of all my wife Jenny for her love and encouragement throughout my studies.

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ABSTRACT

Reproductive traits, age of female broodfish and aspects of parental behaviour influencing the production and quality of fry in the mouth-brooding tilapia species Oreochromis niloticus and O. mossambicus were investigated.

Two incubation containers (conical and round-bottomed) and rearing temperature were first studied to ascertain their effects on egg and fry performance. Partial agitation of eggs in round-bottomed containers significantly ($P < 0.05$) improved hatchability and success rate of fry production, by 17% and 25%, respectively, compared with conical containers.

For the temperature trials only O. niloticus were studied. Two egg acclimation conditions were tested; these influenced the temperature range of hatching, hatching success and the upper and lower median temperature tolerance limits of artificially reared eggs. Thermal tolerance of eggs and fry decreased with progressive development and optimum (>90%) survival and growth of swim-up fry occurred at 28°-30°C.

Hatching times were inversely related to temperature ($P < 0.01$) and rates of development to hatching were best described by a curvilinear relationship ($P < 0.01$). Growth rates, gross yolk utilization efficiency to maximum body weight, age at maximal body weight, onset of exogenous feeding and 50% irreversible starvation (point-of-no-return, PNR) were temperature-dependent. At 24°, 28° and 30°C

maximum body weight occurred on days 18, 9 and 6 post-hatching, respectively, four days earlier than fry at 24°C. Similarly, PNR occurred on days 23, 20 and 18 at 24°, 28° and 30°C, respectively.

Reproductive traits of 0+, 1+ and 2+ age-classes of broodfish were investigated. In O. niloticus mean dry egg weight and clutch weight were significantly ($P < 0.05$) different between all three age-classes, and yearlings produced the smallest eggs, whereas for total and relative fecundity only yearling females were significantly ($P < 0.05$) different to older broodfish. In both species broodfish age-class had no effect ($P > 0.05$) on egg:body weight ratio. In both species all reproductive traits were significantly related ($P < 0.01$) to female age, length and weight. The strongest influences were maternal age on egg size and maternal length and weight on total fecundity and clutch weight.

The influence of maternal age and hence egg size on hatching time, and growth, survival, onset of feeding and PNR of fry developing solely on their yolk reserves was investigated. Larger eggs produced longer ($P < 0.001$) and heavier ($P < 0.001$) fry which sustained starvation stress longer ($P < 0.001$). Initial advantages of egg size on growth persisted through to 60 days post-hatching ($P < 0.05$). Feeding success was improved by using fry from 1+ and 2+ females instead of yearlings. For fry from 0+, 1+ and 2+ O. niloticus and O. mossambicus females, PNR was reached on days 9, 12 and 12, and 12, 15 and 18, respectively. Delaying initial feeding beyond six days post-hatching significantly ($P < 0.05$) reduced the growth of fry.

Overall survival (between 6-20 days post-hatching) was improved by using older females.

The effects of parental breeding behaviour on fry production and quality were investigated. Egg fertilizing capacity of males was inversely related to their number of spawnings in a day. During oral rearing cumulative fry damage increased linearly during the first eight days after spawning and plateaued at 25%-29%. Possible reasons for fry damage are discussed. Naturally reared fry were lighter ($P < 0.05$) than artificially reared 'siblings'.

The implications of broodfish age and size and parental breeding behaviour for mass production of high quality tilapia fry and the need, advantages and feasibility of artificially rearing Oreochromis eggs and fry are discussed.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

This thesis considers the implications of mouth-brooding as a reproductive strategy for the mass production of high quality fry in the tilapia species Oreochromis niloticus and O. mossambicus.

Oreochromis species such as O. niloticus and O. mossambicus are thought to have evolved in unstable riverine environments such as flood plains (Fryer and Iles, 1972; Noakes and Balon, 1982). In these species an elaborate sexual behaviour culminates in the laying and fertilizing of hundreds of large energy rich eggs in shallow nests (Trewavas, 1983). After fertilization, the eggs are picked up by the female and reared in her buccal chamber which is thought to be specially adapted for brooding (Shaw and Aronson, 1954; Varute and Jirge, 1971). Offspring survival and recruitment to the population are maintained by a combination of (a) mouth-brooding and (b) repeated or multiple spawnings at short intervals (Trewavas, 1983). This mode of reproduction, which is reviewed later in greater detail, has profound implications for the production of quality fry in intensive aquaculture systems.

Although several studies on the breeding biology of mouth-brooding species have been published (Aronson, 1949; Shaw and Aronson, 1954; Lowe (McConnell), 1955; Welcomme, 1967; Iles and Holden, 1969; Fryer and Iles, 1972; Marshall, 1979; Babiker and Ibrahim, 1979a; Payne and Collinson, 1983), data regarding reproductive traits such as egg size and egg production have been derived mainly from the examination of 'mature ovaries'. This technique is only of limited value for the accurate prediction of such traits.

In addition, studies (Lowe (McConnell), 1955; Riedel, 1965; Iles and Holden, 1969; MacIntosh, 1985) that relate numbers of fry produced to maternal size also give unreliable estimates of reproductive potential as they assume that there is a close quantitative association between egg and fry production. Welcomme (1967) and Marshall (1979) have shown that the fry to egg production ratio increases and then decreases with maternal size. Consequently, recent attempts under hatchery conditions to relate reproductive potential based on fry release by females to maternal size showed high unexplained variation in the traits (MacIntosh, 1985). Moreover, the use of fry released as a measure of reproductive performance without investigation of the causes of fry loss does not help in understanding the factors contributing to fry quality and survival. This may be very significant for tilapia culture as a fundamental problem facing all tilapia seed producers is the relatively small number of eggs produced per female per spawning. This is usually only up to a thousand (Noakes and Balon, 1982) compared with, for example, hundreds of thousands obtained from carp (Bishai, Ishak and Labib, 1974).

The problems relating to mass production of quality fry investigated in the present study divide conveniently into three broad categories. The first concerns age-specific reproductive traits of tilapia broodstock that directly influence the quantity and quality (size and variability of eggs and survivorship and growth vigour of resultant fry) of eggs produced. The second relates to the response of eggs and fry to various hatchery rearing conditions. And the third concerns aspects of parental breeding behaviour such

as male spawning frequency and the duration of the mouth-brooding period, which may influence the number and quality of fry produced.

One method that may immediately increase egg and fry production from existing broodstock would be to remove the eggs or yolk-sac fry from the buccal cavity of females for artificial rearing. This system would allow the female to feed earlier and produce another batch of eggs sooner (Dadzie, 1970b; Lee, 1979; Siraj, Smitherman, Castillo-Calluser and Dunham, 1983). Artificial rearing of eggs and fry has been attempted in the past (Shaw and Aronson, 1954; Lee, 1979; Rothbard and Pruginin, 1975; Rothbard and Hulata, 1980; Hughes and Behrends, 1983; Snow, Berrios-Hernandez and Ye, 1983) but egg viability and rates of fry survival were inconsistent. Therefore one aspect of the present study, and one with very practical objectives, was to evaluate current methods in artificial rearing of eggs and fry and to develop a rearing system suitable for eggs and fry of mouth-brooding tilapias that would consistently result in a high survival rate of swim-up fry. In particular, the effect of temperature on egg and fry survival and on fry growth and feeding capabilities under artificial incubation conditions were considered in detail.

In addition to the small number of eggs laid by tilapias, hatchery production of young is complicated by the spawning asynchrony between individuals caused by differences in spawning frequency. These factors necessitate the management of very large numbers of broodstock. To stock, for example, 10ha of ponds at common stocking densities would require 30,000 to 60,000 fingerlings

(Hepher and Pruginin, 1982) which is equivalent to a month's fry production from 75 to 150 spawning females.

To minimise the number of broodstock required to meet target production, factors such as broodstock age and size that influence reproductive capabilities (Nikolskii, 1969; Wootton, 1979; Hislop, 1984) need to be fully exploited. For this reason the present study investigated reproductive traits such as number of eggs spawned, egg size, and frequency of spawning based on studies with three age-classes of O. niloticus and O. mossambicus females.

It is well documented for many teleost species that the growth and survival of fry while dependent on their yolk reserves can be significantly improved if they are derived from larger eggs (Dahl, 1918-1919; Blaxter and Hempel, 1963; Bagenal, 1969b; Reagan and Conley, 1977; Pitman, 1979; Theilacker, 1981; Beacham, Withler and Morley, 1984; Thorpe, Miles and Keay, 1984; Rana, 1985). Thus it was considered important to investigate the effects of egg size on the initial survival, growth and feeding capabilities of O. niloticus and O. mossambicus fry. This investigation was extended to show whether any initial advantages of survival and growth gained during the yolk-dependent phase persisted into later life and to determine the effect a delay in the first feeding of fry has on their survival and growth potential.

Further, because the buccal rearing period is variable in mouth-brooding tilapia species, ranging from 10-21 days (Baerends and Baerends-Van Roon, 1950; Hofstede and Botke, 1950; Panikkar and

Tampi, 1954), the feeding opportunity of fry is also variable. A parallel investigation was therefore conducted to establish whether the quality of naturally reared fry, in terms of growth, differed to that of their 'siblings' from the same clutch reared artificially.

In addition, since hatchery fry production at present relies on natural breeding, an examination of parental breeding behaviour was considered essential to identify factors that may contribute to fry loss or reduced fry quality. Firstly, due to the polygynous behaviour of males, the effects of male mating frequency on his 'fertilizing capabilities' were considered (Chapter 4). Secondly, fry loss associated with oral rearing was examined to determine the efficiency of maternal brooding under hatchery conditions.

1.2 Taxonomic Position of the Tilapia Species Studies

The taxonomic classification of the species studied has been revised on several occasions (see Trewavas, 1983). Here only a synopsis is presented.

Most of the species of the Tribe Tilapiini now being used in aquaculture were grouped initially into the genus Tilapia. The species within this genus were later separated according to differences in their mode of reproduction (Lowe (McConnell), 1959). Those species which evolved as substrate spawners but guard their broods were retained in the genus Tilapia while those which orally rear their clutches were grouped into a new genus - Sarotherodon.

In her most recent synthesis, Trewavas (1983) proposes three separate genera, Tilapia, Sarotherodon and Oreochromis, based on differences in their reproduction and feeding habits, structural characteristics and zoogeography.

The species studied here were listed first within the genus Tilapia and later in the genus Sarotherodon. In this thesis the new classification proposed by Trewavas (1983) which places them in the genus Oreochromis is followed. However, in the text the common name 'tilapia' is used for convenience to describe members of all three genera within the Tribe Tilapiini.

1.3 Reproductive Biology of Tilapias

1.3.1 Life history strategies

There can be several different selective pressures on animals determining how their gonadal material is partitioned during their lifespan. Most theories of life history strategies assume that the reproductive effort expended by parents has evolved to reconcile the opposing effects on species fitness of current and future levels of their reproductive effort (Pianka and Parker, 1975; Stearns, 1976; Calow, 1979).

In birds and other homeothermic animals the need to incubate eggs and the limited foraging and feeding ability of parents may in part dictate the optimum clutch or litter size above which chances of survival to the adult stage are decreased (Lack, 1948a, 1948b).

Teleosts, however, are poikilotherms and therefore do not incubate their eggs, and almost without exception do not feed their offspring. Consequently, in fish there is scope for much larger brood sizes than in homeotherms.

Svärdson (1949), incorporating the observations of Lack (1947, 1948a, 1948b), suggested that there are two extreme reproductive strategies to counterbalance low survival of offspring from larval starvation, predation, etc. To ensure reproductive success fish may increase the number of eggs by decreasing their unit reproductive effort (smaller yolked eggs, no parental protection) or decrease the number of eggs and increase their unit reproductive effort (larger yolked eggs, with some degree of parental protection).

Thus pelagic spawners such as gadoid species which adopt the former option shed between $30-1620 \times 10^3$ eggs in a short spawning season (Hislop, 1984). Due to high larval mortality, associated with larval predation and starvation, however, only 0.1% - 0.5% may develop into juveniles (Hewitt, Theilacker and Lo, 1985). Despite these high mortalities most fish adopt this option and scatter numerous free-floating eggs and exhibit no parental care. Clearly, other advantages such as larval dispersal may be traded for high larval mortality (Barlow, 1981).

The other extreme life history strategy suggested by Svärdson (1949) and others of a decrease in egg numbers and increase in egg size with parental care is an option that has evolved in only 20% (84 of 422) of teleost families (Blumer, 1979). This strategy is most

clearly exemplified by the Tribe Tilapiini. The substrate spawners (genus Tilapia) which are nest guarders, may lay thousands of small (1.0 - 1.5mm) eggs per spawning, whereas those species such as O. niloticus and O. mossambicus which protect their eggs and fry from predators in the buccal cavity, may lay only a few hundred larger (up to 5mm) eggs per spawning (Trewavas, 1983).

1.3.2 Age and size at first maturity

As in other teleosts, tilapias attain sexual maturity before their full growth potential is realised. This results in an increase in size during their reproductive phase (Fryer and Iles, 1972). An outstanding feature of their reproductive strategy however, is the ability of these species to change the age and size at which gonadal maturation (and thence spawning) occurs in response to environmental factors. Fryer and Iles (1972) cite examples of many cichlid species which mature and spawn earlier in habitats where water levels fluctuate, e.g. flood-plains, dams, ponds, small lakes and lagoons. For example, O. niloticus from small lakes were found to mature at 17cm compared with 37cm for the same species from Lake Turkana. They attributed these differences to the stable feeding conditions of Lake Turkana.

The evolutionary significance of this reproductive tactic is still unresolved but much debated (Lowe (McConnell), 1955, 1959; Fryer and Iles, 1972; Gwahaba, 1978; Lowe-McConnell, 1979, 1982; Noakes and Balon, 1982; Trewavas, 1983). Noakes and Balon (1982) suggested that since the ancestral tilapia stocks probably evolved in unstable

riverine and fluvial habitats, earlier sexual maturation may be an adaptation by populations to rapidly colonise newly formed temporary water bodies during flooding. They use the same reasoning to explain the familiar pattern of overcrowding and 'stunting' observed in newly stocked tilapia ponds.

The environmental cues involved in the physiological switch from somatic growth to sexual maturation are also unclear, but several factors are implicated. Field studies indicate that poor body condition, i.e. low weight for length (Lowe-McConnell, 1982), overfishing (Gwahaba, 1978), fluctuations in water levels (Fryer and Iles, 1972; Dudley, 1979), or food supply (Lowe-McConnell, 1982) are each associated with precocious maturity.

Of particular interest is the consequence of early sexual maturity on reproductive performance. There is a constant ratio, which is thought to be species specific, between maximum ovary weight and total body weight (Fryer and Iles, 1972). Therefore, since egg size is known to increase with maternal size, fish maturing earlier (and at a smaller size) will produce relatively more but smaller eggs per unit body weight than larger fish. The production and quality of these eggs and subsequent fry is the subject of later discussion.

1.3.3 Oogenesis and pattern and periodicity of ovarian development

The following account of oogenesis in tilapias is drawn largely from Jalabert and Zohar (1982) and Scott (1979). The ovaries of tilapias, like those of other teleosts, contain a stock of undifferentiated oogonia in their germinal epithelia. During ovarian development some of these oogonia may be recruited for further growth ('recrudescence'); they divide meiotically but stop in prophase. These oogonia, known as primary oocytes, then pass through two discrete growth phases, the first pre-vitellogenic and the second vitellogenic. In the first phase, the primary oocytes increase in size (up to 0.6 - 0.9mm in diameter in O. niloticus) through intra-oocyte synthesis (endogenous vitellogenesis). The second phase is completed by exogenous yolk deposition of vitellogenin: a lipophosphoprotein which is synthesised in the liver, released into the blood and added into the oocytes by micropinocytosis. At the end of vitellogenesis, maturation and ovulation can occur in rapid succession. Meiosis is resumed and the first meiotic division is completed with the formation of a secondary oocyte. Oocyte maturation ends after sperm penetration with the completion of second meiotic division. During vitellogenesis some oocytes may not mature but undergo degeneration - a process known as atresia. At ovulation (expulsion of mature secondary oocytes from the follicles) the ova are released into the lumen of the ovary where they await release (spawning). Thus during cyclical changes in the ovary five stages of oocyte development are evident: previtellogenic primary oocytes, primary oocytes in two phases

of vitellogenesis, atretic oocytes and ovulated oocytes. The timing of spawning, however, may culminate at separate times for different individuals and species to provide the best possible reproductive success for the ecological niche they occupy (Fryer and Iles, 1972).

Ovarian development and egg maturation are, for the vast majority of species, based on an annual periodicity (Scott, 1979). A common feature of these species is the temporal synchrony shown by individuals with respect to oocyte maturation within the ovaries and subsequently to the shedding of their eggs. Histological examination of the ripe ovaries of annual spawners generally reveals a unimodal size distribution of oocytes (Bertin, 1958, quoted by Peters, 1983).

For tilapia species such as O. niloticus and O. mossambicus, however, the natural spawning season may be protracted or even absent (Lowe (McConnell), 1955). Consequently, the stages of ovarian development may be asynchronous between individuals and all five stages of oocyte development may be found in individuals throughout the year (Hyder, 1970; Siddiqui, 1977). In addition, under these aseasonal conditions, the short gonadal cycle of these species facilitates spawning at intervals of only a few weeks (Jalabert and Zohar, 1982; Lowe-McConnell, 1982; Trewavas, 1983).

The oocyte stage from which the next spawn is selected is as yet unclear. There is evidence to show that ripening tilapia ovaries contain two or more groups of oocytes in active vitellogenesis

(exogenous vitellogenesis) - a phenomenon known as plurimodal development (Peters, 1957, 1983). Further, some authors (Liebman, 1933; Kraft and Peters, 1963; Hyder, 1970; Peters, 1983) reported the presence of yolk laden oocytes in tilapia ovaries immediately after spawning, while others (Siddiqui, 1977; Silverman, 1978a; Moreau, 1979) suggested that each spawn originates from undifferentiated primary oocytes. Jalabert and Zohar (1982), commenting on this discrepancy, suggested that these differences may lie in the vague definition of exogenous vitellogenesis but they did not rule out interspecific and environmental differences. Therefore the dangers of using 'ripe' and 'maturing' ovaries in the case of O. niloticus and O. mossambicus for accurate prediction of reproductive traits such as egg size and production are clear.

1.3.4 Sexual behaviour and spawning

In the Oreochromis species, the socially dominant males may be more successful than other males in mating with the many females visiting their nests (polygynous behaviour). The females on the other hand may choose to spawn with one or up to several males (polyandrous behaviour) although this behaviour is reported to be rare (Trewavas, 1983).

Breeding behaviour

In Oreochromis species, unlike substrate spawners and biparental brooders, the male is solely responsible for the selection and construction of territories and mating stations or nests (Lowe (McConnell), 1959; Ruwet, 1963).

Under suitable conditions for breeding, sexually mature males accumulate on spawning grounds at water depths of between 0.5m to 1.5m. There they rapidly demarcate a territory which is then defended vigorously against competitors. Within their territory the males excavate a shallow depression or 'nest' which serves as a focal point for sexual activity. Nests are constructed on sandy bottoms in lakes (Fryer and Iles, 1972) and on soft muddy substrata in reservoirs or ponds. In confined conditions however, a soft substratum is not essential for successful spawning although males still require sufficient area to establish and guard a territory into which females can be attracted. Under natural conditions, O. niloticus 'nests' may be up to 1 metre in diameter and 45cm deep (Fryer and Iles, 1972), while those reported for O. mossambicus range from 15 - 140cm in diameter and are 6cm deep (Vaas and Hofstede, 1952; Bruton and Boltt, 1975).

After constructing a nest in a secured territory, the male may court several females. If in the early stages of courtship a female accepts the suitor she follows him to his nest. Both the male and female may leave the nest to ward off intruders, but if fighting develops the female flees. As time progresses her visits are confined to one territory. There she circles the nest with the male, leaving only momentarily to chase away neighbouring fish. The female then lays her eggs in several batches in the nest. The male swims over the site and sheds his sperm on each batch. These eggs are taken up immediately into the oral cavity of the female. Oreochromis mossambicus females have also been known to snap sperm

directly from the male genital papilla. (Bohrer, 1953; personal observation).

The duration of spawning during which several batches of eggs are laid is variable. For O. niloticus Trewavas (1983) mentions that eggs are shed in about 20 batches during a period of 45 minutes to 2 hours, but about half the eggs are laid in the first four batches. Whereas for O. mossambicus between 4-13 batches of eggs are laid during a 30 - 90 minute period, (personal observation). Vaas and Hofstede (1952), however, state that O. mossambicus females shed all the eggs in one batch. After spawning the male may immediately court and mate with other females.

When a female has shed and collected her eggs she leaves the nest to rear her clutch in nursery grounds (Fryer and Iles, 1972) for between 10 - 21 days (Baerends and Baerends-Van Roon, 1950; Hofstede and Botke, 1950; Panikkar and Tampi, 1954). During this period she may release her fry temporarily to allow them to feed. Should danger threaten the fry they retreat rapidly into the buccal cavity of the mother. The parental bond between fry and mother decreases until the fry are independent at approximately 22 to 30 days after spawning (Baerends and Baerends-Van Roon, 1950; Russock and Schein, 1977).

CHAPTER 2

ASPECTS OF HATCHERY REARING OF
OREOCHROMIS EGGS AND FRY

2.1 Artificial Rearing of Eggs and Fry

2.1.1 Introduction

In Oreochromis species of tilapia the survival of eggs and fry is intrinsically related to the mouth-brooding mode of clutch protection. Therefore to develop an efficient artificial rearing system for the mass production of eggs and fry it is beneficial to simulate the natural pattern of parental rearing.

Under natural breeding conditions a temporary pair-bond develops between the ripe female and the nesting male. During spawning, which may last between 45 minutes to 2 hours, the female releases her eggs in batches of 20-50 and the male sheds his sperms over the site (Trewavas, 1983). She takes the eggs into her buccal cavity as they are laid, which may be before, during or after fertilization. On completion of spawning the pair-bond is terminated and the female leaves to incubate her clutch. During the maternal rearing period, which may vary in Oreochromis species from 10 to 21 days (Baerends and Baerends-Van Roon, 1950; Hofstede and Botke, 1950; Panikkar and Tampi, 1954; personal observations), the eggs or developing fry are rolled over in the buccal cavity by the respiratory movements of the brooding female and by periodic back-flushing.

The reasons why the eggs are rolled or churned in the buccal cavity are as yet poorly understood. Shaw and Aronson (1954), who were the first to address this question, demonstrated that the rearing

of stationary eggs in non-sterile or aquarium water reduced egg and embryo viability. They attributed the low viability of extra-orally reared eggs and embryos to bacterial and fungal infections and speculated that special 'pharyngeal glands' located in the buccal cavity may secrete an anti-bacterial agent which enhances embryo survival. Fishelson (1966), provided another view in suggesting that if the yolky eggs remain stationary, the heavy lipids sink to the lower pole. This disrupts the internal organisation and the embryo fails to develop.

The results of these authors therefore suggest that churning of eggs is essential. To simulate this rolling action of naturally reared eggs, eggs have been agitated with air and water by various methods. Investigators have used conical containers (Valenti, 1975; Rothbard and Hulata, 1980) and shaking tables (Rothbard and Pruginin, 1975; Lee, 1979) for the incubation of tilapia eggs. However, the losses reported in these systems were high. For example, Rothbard and Hulata (1980) reported losses of up to 40% and 20%, respectively, using shaking tables and conical 'Zuger-like' upward flow incubators.

Chemotherapy has been successfully used to improve tilapia egg and embryo survival in recirculatory systems. Valenti (1975) obtained over 90% hatchability for O. aureus eggs using penicillin-G and streptomycin sulphate at 50 IU/ml and 0.05 mg/ml, respectively. Aureomycin, however, was found to be toxic to developing T. macrocephalus (= S. melanotheron, Trewavas, 1983) fry (Shaw and Aronson, 1954). Chemical disinfection of tilapia eggs with formalin,

malachite green, acriflavin and Buffodine has also yielded some success (Shaw and Aronson, 1954; Subasinghe and Sommerville, 1985).

Ultra-violet (UV) irradiation of the rearing water has also been investigated for use in reducing bacterial and fungal incidence. Kimura, Yoshimizu, Tajima, Ezura and Sakai (1976) demonstrated that a UV dose of 23,100 $\mu\text{W}\cdot\text{sec}/\text{cm}^2$ can reduce bacterial populations of 10^{5-7} cells per ml by 99.9%.

In addition to the above considerations the age of eggs prior to their artificial incubation may also critically affect hatchability and survival of fry. Shaw and Aronson (1954) and Rothbard and Hulata (1980) reported that if eggs were removed for rearing prior to the third day after spawning they did not survive. Lee (1979), however, found that when O. aureus eggs were removed on the second day after spawning they developed "fairly well" but hatching failed when eggs were removed immediately after spawning.

In view of the variability in the methods used for the mechanical agitation of eggs and fry, the levels of bacterial and fungal incidence and the age of eggs prior to removal from the buccal cavity for artificial incubation reported by the various authors, and the lack of information given on fertility rates and water quality parameters, there is little means of ascertaining the comparative efficiencies of the various systems described.

A series of trials was therefore established to evaluate the relative performance of upwelling conical and round-bottomed hatching jar

rearing systems with various developmental stages of O. niloticus and O. mossambicus eggs, using a closed recirculatory system and a UV sterilization unit to maintain and standardise water quality.

2.1.2 Materials and Methods

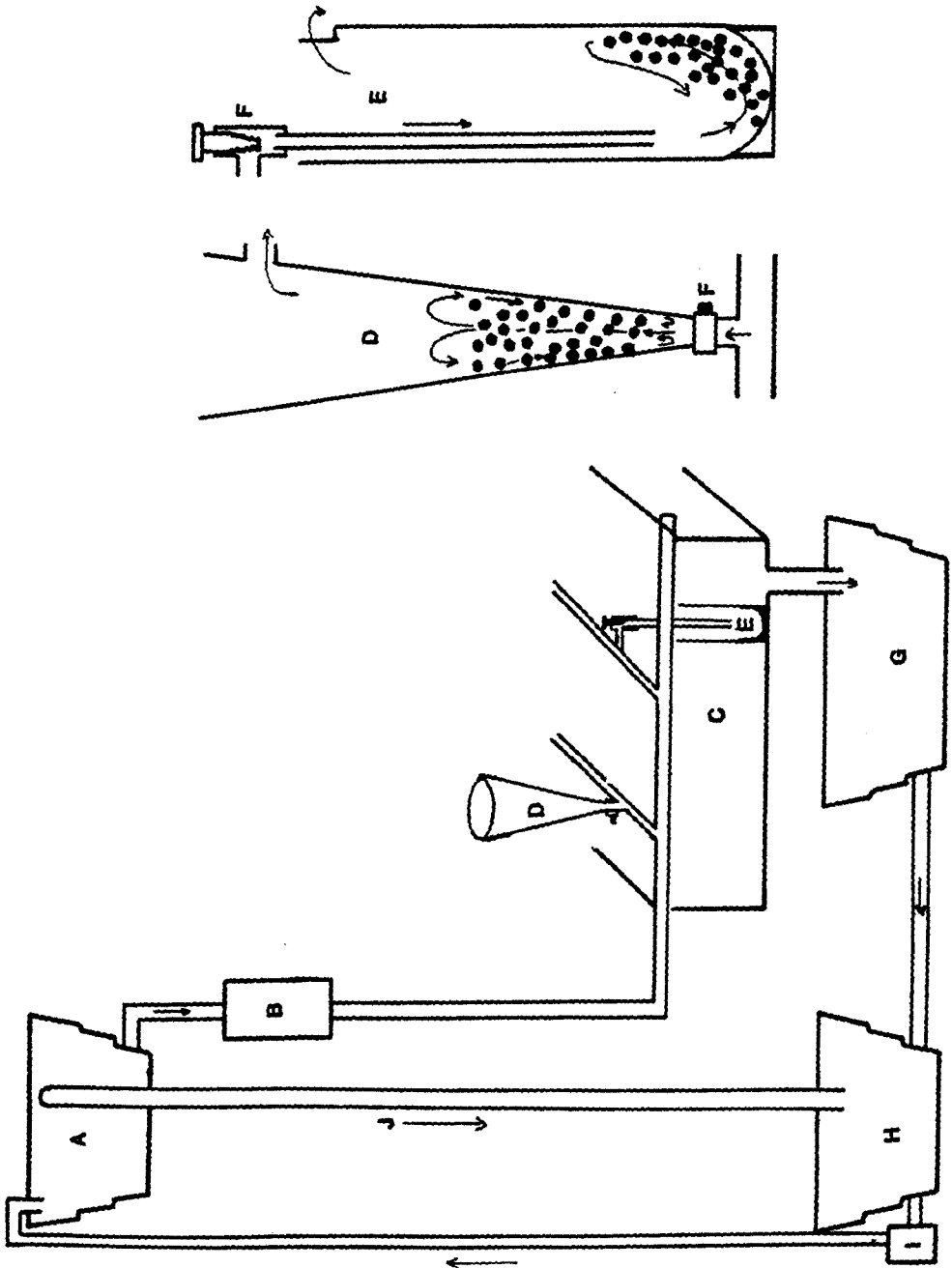
2.1.2.1 Design of incubation system

The recirculating system developed to evaluate the efficiency of conical upwelling and round-bottomed containers for the mass rearing of O. niloticus and O. mossambicus eggs and fry is shown in Fig. 2.1.

Water from a 125 l header tank was fed by gravity through a 30 watt ultra-violet sterilization unit (flow rate 20 l/min, dosage 61,868 $\mu\text{Wsec/cm}^2$) to 16 incubators. The flow rates of water into the conical and round-bottomed jars were controlled by clamps and modified aquarium air valves, respectively. The water flow was adjusted so as to gently agitate the egg clutches and averaged 0.7 l/min (range 0.2 - 1.2 l/min). Overflow water ran into a common trough and drained through a biofilter into a sump tank before being pumped back to the header tank. The temperature of the water was maintained at $28^\circ\text{C} \pm 1^\circ\text{C}$ using five 200 watt thermostatically controlled heaters placed in the sump tank. Aeration was provided in the header tank by three 15 cm air stones connected to a low pressure blower. Oxygen, pH and temperature were measured daily and ammonia and nitrite levels were measured every two weeks (Table 2.1). At least half the water in the system was exchanged every week and replaced with clean pre-warmed water.

FIGURE 2.1 Design of the artificial incubation system used for rearing Oreochromis eggs. Arrows indicate the direction of water flow and pattern of egg movement. Figure not drawn to scale.

- A - 125 l header tank
- B - 30 watt ultra-violet sterilizer
- C - Collecting trough
- D - Upwelling conical jar
- E - Downwelling round-bottomed jar
- F - Clamps and valves
- G - 225 l biofilter
- H - 125 l sump tank
- I - Pump
- J - Overflow



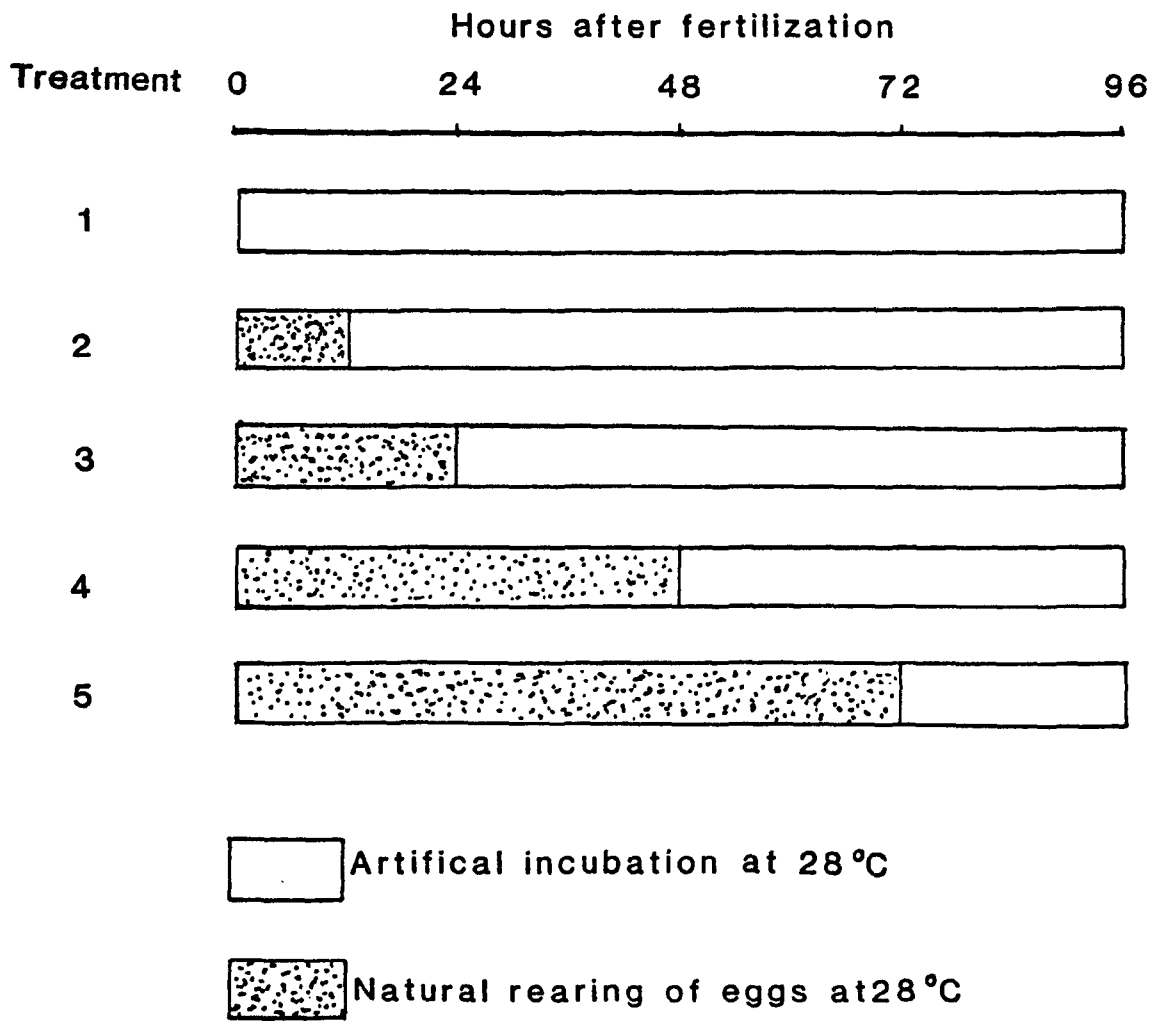


FIGURE 2.2. Experimental procedure followed for the incubation of *Oreochromis niloticus* and *O. mossambicus* eggs removed from females at various times after fertilization

2.1.2.2 Broodstock and incubation of eggs and fry

Pure species of O. niloticus and O. mossambicus (as identified by McAndrew and Majumdar, 1983) were stocked in 1 m² spawning tanks at a sex ratio of 4 females : 1 male.

Figure 2.2 illustrates the five treatments used for the trials. For the artificially obtained egg stocks used in Treatment 1, ovulated eggs were manually stripped from females into clean dry containers and fertilized with milt pooled from three conspecific males. Five minutes were allowed for fertilization. The eggs were then rinsed in a net container with clean, pre-warmed water. For naturally spawned egg supplies, eggs were gently removed from the buccal cavity of tagged brooding females at various times after spawning (Fig. 2.2). Prior to incubation, the number of eggs in each clutch was estimated. Excess moisture was removed on absorbent paper and then 50 randomly sampled eggs and the remainder of the clutch were weighed on a top pan Mettler (PC 4400) balance. The clutches were then transferred to either conical or round-bottomed incubators. In addition, a random sample of 50 eggs from each egg clutch was removed and preserved in Bouin's fixative for the determination of fertility rates.

The eggs were incubated at 28°C and the time of mass hatching noted. The numbers of hatched, swim-up and deformed fry were recorded. To compare egg movements in the incubation jars with the natural 'churning' movements of eggs in the buccal cavity of brooding fish, females of both species were allowed to spawn in 100 l glass aquaria

TABLE 2.1

Physico-chemical parameters of the water in the artificial incubation system used for rearing eggs and fry

Parameter	Mean	Range
Temperature (°C)	28	27 - 29
Dissolved Oxygen (mg/l)	7.2	6.9 - 7.4
pH	7.4	7.01-7.86
Total Ammonia - Nitrogen (mg/l)	0.59	0.22-0.96
Total Nitrite - Nitrogen (mg/l)	0.11	0.06-0.15

and the frequency of 'churning' movements counted during maternal rearing.

2.1.2.3 Efficiency of incubators

The success rate of fry production (Fs) in the two types of incubators was determined using the following relationship:

$$Fs (\%) = Kh \cdot Ks / 100$$

Where Fs = Success rate (%) of fry production in incubators, at 10 days after hatching

Kh = Hatch rate (% of fertile eggs)

Ks = Survival rate of 10 day old swim-up fry (% of hatched fry)

2.1.2.4 Analysis of data

The data obtained were tested statistically using the analysis of variance method described by Duncan (1955).

2.1.3 Results

The physico-chemical characteristics of the water from the incubation system are given in Table 2.1. The level of nitrogenous compounds throughout the trials were low and the rearing water was over 90% saturated with oxygen.

Of the 178 egg clutches used in this study, 11 batches were lost due to low fertility. Deformity rates between treatments and between

TABLE 2.2

Summary of the performance of conical and round-bottomed artificial rearing containers for Oreochromis eggs and fry at 28°C

Type of Incubator	No. of trials	Hatching time (h)	Hatch Rate (%) \bar{x} (SE) (Kh)	Survival Rate (%) \bar{x} (SE) (Ks)	Success rate (%) of fry production to swim-up stage (Fs)
Conical	62	72 - 84 ^a	74.6 (1.61) ^a	80.7 (0.78) ^a	59.7 ^a
Round-bottomed	105	90 -102 ^b	91.6 (1.16) ^b	92.3 (0.96) ^b	84.5 ^b

Figures within each column with different superscripts are significantly different (P < 0.05)

$$1. \text{ Standard Error (SE)} = \frac{\text{Standard deviation}}{\sqrt{\text{No of trials}}}$$

$$2. \text{ Success Rate (FS) \%} = \text{Kh. Ks}/100$$

where FS = Success rate (%) of fry production in incubators at swim-up stage

Kh = Hatch rate (% of fertile eggs)

KS = Survival rate of 10 day old swim-up fry (% of hatched fry)

species were not significantly different ($P > 0.05$) and averaged 1.5% per clutch.

The hatchability and survival rates of fry between treatments (see Fig. 2.2) and between species were not significantly different ($P > 0.05$) and pooled results were therefore calculated (Table 2.2). The time to hatching and survival rates of fry were, however, significantly ($P < 0.05$) higher when incubated in the round-bottomed hatching jars compared with incubation in conical hatching jars (Table 2.2).

The frequency of clutch churning during natural rearing was observed to decrease from 95-105/min after spawning to 20-30/min by day 3, with only occasional churnings by days 5-10.

2.1.4 Discussion

2.1.4.1 An interpretation of the rôle of mouth-brooding in enhancing egg and fry survival

The anti-predator tactic of rearing eggs and fry in the relative safety of the parental buccal chamber is mentioned elsewhere (see Chapters 1 and 4). Here, the suggestion that the buccal chamber may also be morphologically and biochemically adapted to enhance progeny survival during oral rearing is considered.

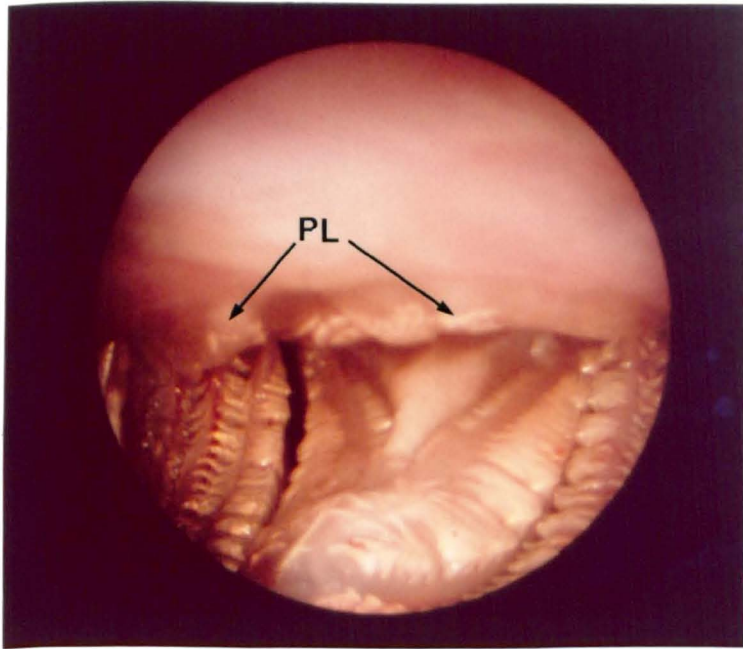
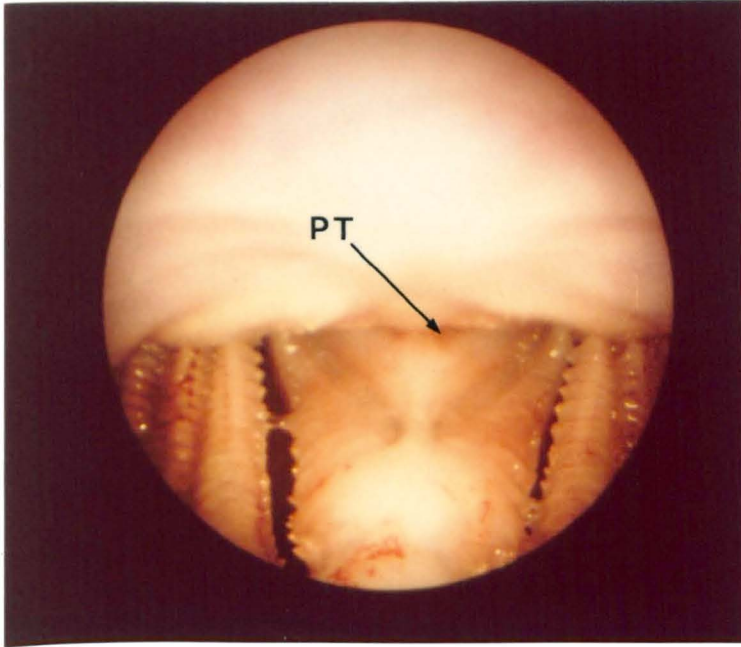
Much of the controversy is centred on the rôle of pharyngeal 'glands' or 'lobes' situated anterior to the pharyngeal teeth pads on the

FIGURE 2.3

Endoscopic view of the buccal chamber of (a) mature non-brooding (weight, 387g) and (b) brooding (weight, 440g) O. niloticus females. Note the proliferation of tissue of and between the pharyngeal 'lobes' of brooding female. This female had been brooding her clutch for five days.

PL - Pharyngeal 'lobe'

PT - Pharyngeal teeth



upper palate (Fig. 2.3). In mouth-brooding tilapias there is a cyclical development of the mucous epithelium of the pharyngeal lobes related to the breeding cycle. During breeding there is a noticeable increase in the degree of folding of the mucous epithelium and in the size of the associated epithelial cells. With the permanent release of fry the folding of the epithelium and the size of the epithelial cells decrease (Varute and Jirge, 1971).

Pellegrin (1903), who first observed these lobes in the mouth-brooding species Pelmatochromis and Geophagus, suggested that they may be related to oral brooding. Shaw and Aronson (1954) first proposed that since these 'glands' are absent or only poorly developed in non-mouth-brooders and immature female mouth-brooders their secretions may have a bacteriocidal function in progeny protection. Reinboth (1956 - quoted by Breder and Rosen, 1966), however, located such glands in T. sparrmanii, a substrate spawner. He also observed no changes in the 'glandular' condition of mouth-brooding Haplochromis multicolor between the breeding and non-breeding phases and concluded that the pharyngeal 'gland' has no antibiotic effect.

Furthermore, attempts to demonstrate anti-bacterial activity of secretions from pharyngeal 'glands' of adult male Sarotherodon melanopleura (Shaw and Aronson, 1954) and from spawning and non-spawning O. niloticus, O. spilurus and O. macrochir females (Nthekeha, 1984) were inconclusive. Subasinghe (1986) repeating the work of these authors with O. mossambicus also obtained similar results.

Nevertheless, in the present study both dead and developing eggs removed from the buccal cavity 3 days after spawning and examined under the scanning microscope showed no evidence of fungal or bacterial contamination (Fig. 2.7). The pathogen and mucus-free chorions of these naturally reared eggs is probably a consequence of the gentle rolling action of eggs in the buccal cavity which may result in the physical cleansing of the eggs.

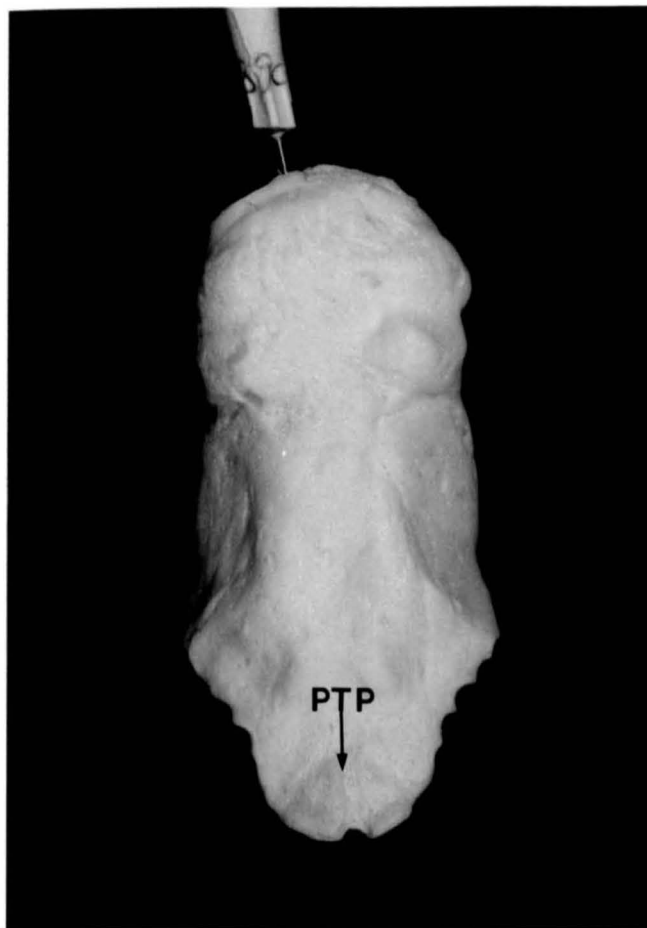
Fish mucus is known to have immunological properties which help immobilize pathogens and parasites (Bly, 1982; Subasinghe, 1986). However, for eggs and fry reared in the buccal cavity, through which water is continuously flushed, the mucus will need to be present in large quantities to provide a non-infective micro-environment. Further, since the fry are not capable of feeding until five to six days after hatching at 28°C (Table 2.9, Fig. 3.8) it is unlikely that the fry will have acquired passive immunity through the ingestion of mucus. The increased mucus secretion during oral rearing, however, may serve to reduce or prevent the irritation of the buccal membrane by the clutch and thus suppress coughing and hence prevent expulsion of the brood (Oppenheimer, 1970).

It is proposed here that, unlike non-spawning females, the prominence of the pharyngeal lobes on the upper palate of spawning females (see Fig. 2.3) may also serve as a mechanical screen or flap which precludes the pharyngeal teeth pads from the oral chamber during brood rearing. Foam casts of the buccal cavity (see Section 4.2.3 for methodology) confirm that the pharyngeal teeth pads of non-brooding mature females are exposed in the oral chamber (Fig. 2.4).

FIGURE 2.4

Foam cast of the buccal cavity of a mature non-brooding O. niloticus (weight, 402g) female. Note the pharyngeal teeth pad exposed in the buccal chamber.

PTP - Pharyngeal teeth pad



The duration and degree of exposure, however, depends on the frequency and magnitude of mouth movements. The teeth on the pharyngeal pads, used for the fragmentation of ingested food, may damage the eggs (and fry) especially during hatching when the mechanical strength of the chorion is weakened by internal digestion (Schoots, Stikkelbroek, Bekhuis and Denucé, 1982). Thus, any partial or complete screening of the pharyngeal teeth pads from the oral chamber may help improve the rearing efficiency by minimising mechanical damage. When the floor of the buccal cavity is lowered during clutch rearing, the eggs are rotated posteriorly in the cavity (personal observation). The pharyngeal lobes will deflect the clutch away from the pharyngeal teeth during this churning movement, minimising egg or fry contact with the teeth on the pharyngeal pads. This hypothesis is investigated further in Chapter 4. From the evolutionary point of view, the posterior rolling action of the clutch may have served as an irritation stimulus giving rise to a proliferation of the epithelium (containing mucous cells) of the upper palate to form a lobe or flap around the pharyngeal teeth (Figs. 2.3 and 4.5).

Fishelson (1966), suggested that the churning or rolling action is essential in maintaining the internal organisation of the heavy lipids of tilapia eggs. However, when eggs were kept stationary in a sterile environment egg survival was high (Shaw and Aronson, 1954; Rana, unpublished data). Further, observations of brooding females in the present trials revealed that the churning frequency decreased from 95-105/min at spawning to 20-30/min by day 3, thereby increasing the stationary period between churning. This reduction

in churning frequency may therefore also help in reducing any mechanical stress on the chorion and embryo.

Clearly then two main conditions would need to be fulfilled to enhance Oreochromis egg and fry survival. The reduction of:

- (i) bacterial and fungal incidence;
- (ii) any mechanical stress that may lead to chorion and embryo damage

2.1.4.2 Feasibility of rearing Oreochromis eggs and fry artificially

To ensure reproductive success teleosts which do not exhibit parental care scatter large numbers of either free-floating, buoyant, sticky or stationary eggs. In species such as O. niloticus and O. mossambicus, where parental care is highly evolved, fewer but larger eggs of high specific gravity are reared in the safety of the parental buccal cavity. Despite the range in egg types and mode of parental care in teleosts, the most generally used technique for experimental and mass rearing of fish eggs and hatchlings involves the principle of continuous movement of eggs and hatchlings by either air or upwelling or circular water flow.

For Oreochromis species the various techniques used for the continuous movement of eggs during artificial rearing have yielded inconsistent results. Shaw and Aronson (1954), Rothbard and Pruginin (1975) and Lee (1979) used shaking tables to agitate eggs of mouth-brooders. In such systems, however, hatchabilities were generally poor. For example, Shaw and Aronson (1954) obtained only a 60%

hatch rate while Rothbard and Pruginin (1975) and Lee (1979) experienced mortality of whole batches of eggs. In some instances up to 40% of egg batches were lost (Rothbard and Hulata, 1980). Upwelling Zuger jars were also used (Mires, 1973; Rothbard and Hulata, 1980) but losses of up to 20% of egg batches were reported (Rothbard and Hulata, 1980). Lee (1979) tested the agitation of eggs with air and with water in conical containers but abandoned these methods because of difficulties in maintaining constant air and water pressure or proper filtration in hatching jars. The results of the above investigations should, however, be interpreted cautiously since factors such as fertility rates, water quality and water volume not given by these authors may vary. Furthermore, hatchling survival was not mentioned. Since newly emergent fry normally remain in the rearing vessel until the swim-up stage, this phase of development should also be considered when comparing the relative performance of various techniques in rearing Oreochromis eggs and fry.

Therefore, in these trials, the number of developing eggs, hatch rates and survival rates to the fry swim-up stage were monitored to assess accurately the performance of rearing vessels. A series of toxicity trials (not reported here) confirmed that the levels of ammonia, nitrite, nitrate and pH common to both types of rearing vessels were below concentrations that affect hatchability, fry growth and survival (Rana, unpublished data).

In these studies the age (and therefore stage of development) of O. niloticus and O. mossambicus eggs prior to artificial rearing

had no significant ($P > 0.05$) effect on the viability of eggs and subsequent fry. In contrast, Shaw and Aronson (1954) and Rothbard and Hulata (1980) reported that eggs of mouth-brooders removed for artificial rearing prior to the third day after natural spawning did not survive. Lee (1979), however, reported that when O. aureus eggs were removed on the second day after spawning for rearing on a shaking table they developed 'fairly well' but hatching failed when eggs were removed immediately after spawning. Shaw and Aronson (1954) and Lee (1979) attributed these losses to bacterial and fungal infection of eggs. In the present studies, however, UV sterilization of the common rearing water at the flow rates and dosage used was likely to reduce bacterial and fungal incidence (Kimura et al., 1976). However, at bacterial loadings of $1-3 \times 10^3$ cells/ml (estimated by spread plate method on Trypton Soya Agar medium - Oxoid Ltd., Basingstoke, Hants., England) in the absence of UV sterilization and chemotherapy, hatch rates of between 56% and 65% have been attained (Rana, unpublished data). Similar results were also obtained by Subasinghe and Sommerville (1985).

The type of incubators used in the present trials, however, significantly ($P < 0.05$) influenced the hatchabilities and subsequent survival rates of fry: being 17% and 25% higher, respectively, for hatchlings reared in round-bottomed vessels compared to those reared in conical containers (Table 2.2). These differences between the two types of vessel suggest that mechanical stress may be the primary cause of poor emergent rates and hatchling survival. In the conical containers, eggs are kept in continuous motion and may therefore be subjected to greater mechanical stress through friction (i.e.

between eggs and between eggs and the container). Furthermore, unlike buoyant eggs, Oreochromis eggs, which have a high specific gravity, require a higher flow rate to maintain them in suspension at high densities in the water column. By comparison, the inflow in the round-bottomed vessel can be positioned to allow the eggs to be gently banked where they rest before being moved again (Fig. 2.1) thus reducing any mechanical stress on the eggs and fry. The resting of eggs is also a feature of the pattern of parental rearing. During mouth-brooding the frequency of churning of eggs was observed to decrease from 95-105/min to 20-30/min by day 3 thereby increasing the stationary period between churning and hence minimising mechanical stress on the developing eggs.

In other teleost species, stress from mechanical forces is known to effect early embryogenesis and chorion integrity (Galkina, 1957; Pommeranz, 1974; Hickey, 1978). Mechanical vibrations and shaking during early development is a common cause of egg mortality in fertilized salmon, herring, cod and plaice eggs (Hempel, 1979) probably resulting from weakening or injury to the vitelline membrane during gastrulation (Gray, 1932; Galkina, 1957; Hickey, 1978). Furthermore, Pommeranz (1974) reported that the 'crushing force' for plaice eggs rises from 1.5 g to 700 g by the end of gastrulation and gradually decreases towards hatching. A similar trend was also observed for herring eggs (Hickey, 1978). Due to a lack of facilities, such measurements were not made during the present studies.

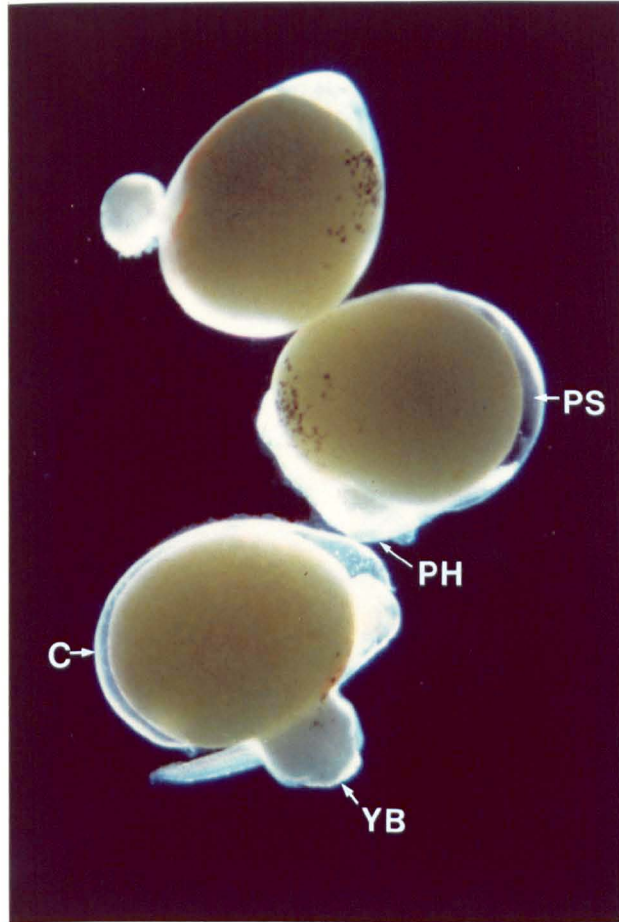
Of interest here though, is the frequent observations of yolk 'blebs' (Fig. 2.5) on O. niloticus and O. mossambicus eggs incubated in

FIGURE 2.5

Examples of eggs (55-60 h old) from upwelling conical incubators showing 'yolk blebs' and premature hatching resulting from chorion damage. Osmotic stress, further frictional damage and colonization of damaged areas by microorganisms may hasten death.

C - Chorion
PH - Premature hatching
PS - Perivitelline space
YB - 'Yolk blebs'

x15



conical and occasionally in round-bottomed containers. During the post-gastrulation stages, when the chorion strength may be diminishing, the continuous mechanical force on the egg may result in different degrees of fracture in the layers making up the chorion. When the thin outer layer (zona radiata externa), which normally protects the embryo against microorganisms (Schoots et al, 1982) fractures, the embryo may be subjected to osmotic stress. This may severely weaken the embryo and may encourage microorganism settlement on the chorion. If these fractures are widened the yolk-sac may be partially extruded as a 'bleb' indicating that death is imminent (Fig. 2.5). Furthermore, the death of the embryo from osmotic imbalance and bacterial toxins may be hastened by external hydrolytic digestion of the chorion by enzymes secreted by microorganisms (Schoots et al, 1982). The failure to consistently rear newly spawned and 1-2 day old eggs by previous workers may therefore be due to chorion damage through prolonged mechanical stress, made worse by subsequent bacterial and fungal invasion. When chorion damage is reduced, for example by removal of the eggs from the brooders after a longer period, chemotherapy may be used to enhance hatchability. For example, Lee (1979) used a 10 minute 1% formalin dip for 48 hr old O. aureus embryos and obtained 88% hatch rate, while Subasinghe and Sommerville (1985) obtained hatch rates of 76% - 87% for 12 hr old O. mossambicus embryos using optimal doses of formalin, malachite green, Acriflavin, and Buffodine.

Further evidence of chorion weakening may be indicated by premature hatching times. Normally, during the hatching period the thick inner porous layers (zona radiata interna) of the chorion are

enzymatically digested (Schoots et al, 1982) in readiness for embryo emergence. In the present studies during natural rearing hatching was observed to occur within 96 - 120 hours of spawning at 28°C. In the round-bottomed containers hatching was complete within 90 - 102 hours of fertilisation compared with 72 - 84 hours in conical containers (Table 2.2). Rothbard and Pruginin (1975) reported hatching times of 50 hours for eggs reared on a shaking table at 25-27°C. Since the water quality in both rearing vessels in the present study was identical, the premature hatching of fry in the conical vessel suggests that the egg shell may be weakened even further nearer the time of hatching. In addition, the skin of these prematurely hatched fry may be easily damaged by constant friction from contact with other eggs, fry or the vessel surfaces. Hickey (1978) calculated that skin damage of approximately 1% of body surface caused 50% mortality in herring, plaice and salmon sac-fry.

In conclusion, since the rearing of eggs and fry in round-bottomed vessels minimised stress on the eggs and fry and yielded a high success rate (Table 2.2) they were used in preference over conical containers in all subsequent incubation studies.

2.2 Effects of Temperature on Rates of Embryonic Development and Fry Performance

2.2.1 Introduction

Temperature is one of the most potent environmental factors influencing the developmental rate of fish eggs and fry (Herzig and Winkler, 1986). Generally, low rearing temperatures retard and high temperatures accelerate development. The habitat temperature and the range over which eggs will develop and hatch normally varies between species, each having an optimal range for maximal developmental success depending on their ecology and life history.

For Oreochromis species an understanding of these thermal tolerance ranges is of considerable ecological as well as aquacultural interest. Especially as the distribution of these species is now much extended outside their natural range by man's accidental and deliberate introduction through fish culture. In open water bodies, which may be at altitude, species such as O. niloticus and O. mossambicus may be subjected to large seasonal and diurnal fluctuations. For example, Denzer (1968) reported a range of 20°C (20-40°C) between winter and summer water temperatures of thermal effluents used to rear O. niloticus in Germany and Caulton (1977) recorded a diurnal range of 10°C (20-30°C) in most rift valley lakes.

Under hatchery conditions, however, it may be possible to control and maintain water temperatures within a narrow thermal range for optimal egg and fry development. This would be of considerable

importance since the various stages of embryonic development may have different thermal tolerance ranges and optimal temperature requirements (Hokanson, McCormick and Jones, 1973; Hokanson and Kleiner, 1974; Irvin, 1974; Gunnes, 1979; Ehrlich and Muszynski, 1981). Furthermore these authors have shown that the earlier morphological stages of eggs are more susceptible to thermal stress than advanced stages. Therefore in the present investigations Oreochromis eggs were exposed to the various test temperatures soon after fertilization to include the most sensitive stages. However, a knowledge of the stages in the development of Oreochromis eggs was necessary to assess the influence of temperature on egg development and survival. Therefore, the embryology of Oreochromis eggs was first investigated.

The shortening of the time to hatching associated with higher rearing temperatures is widely documented for many species (Blaxter and Hempel, 1963; Forrester, 1964; Hokanson and Kleiner, 1974; Stott and Cross, 1973; Alderdice and Forrester, 1974; Gunnes, 1979; Divanach, Kentouri and Paris, 1982). However, early hatching of embryos at low temperatures has also been reported (Kokurewicz, 1969; Hokanson and Kleiner, 1974; Stott and Cross, 1973). These embryos are often morphologically less developed and may die during or soon after hatching. In addition, a large proportion of these embryos may be deformed (Stott and Cross, 1973). Hence, the fragility of the premature and deformed fry reared at low temperatures may result in poor subsequent survival and this is also a point for consideration in the hatchery rearing of O. niloticus and O. mossambicus eggs

and fry. In addition, rearing temperature has been found to affect the size of fry at hatching. Studies with some species (Price, 1940; Kinne and Kinne, 1962; Forrester and Alderdice, 1966) have shown that low incubation temperatures produce larger fry at hatching, whereas studies with other species (Lasker, 1964; Alderdice and Forrester, 1974; Guma'a, 1978; Hassler, 1982) have shown that low incubation temperatures produce smaller hatchlings.

The growth rate of fry developing solely on their yolk reserves and the efficiency with which the yolk is converted into body tissue under different temperature regimes is of particular interest to the fry producer. Many authors (Hayes, Pelluet and Gorham, 1953; Smith, 1957; Hokanson and Kleiner, 1974; Alderdice and Forrester, 1974; Gunnes, 1979; Rombough, 1985) have shown that the maximum body weight of fry developing on their yolk reserves is reached earlier at higher rearing temperatures and the duration of the yolk-sac stage is reduced. In addition, higher rearing temperatures may reduce the age at which free swimming and exogenous feeding commences. Such information for O. niloticus and O. mossambicus would be invaluable to the fry producer. Under conditions of higher rearing temperatures fry may need to be fed earlier. In addition, if opportunities for earlier feeding are not afforded by the brooding mother i.e. fry are retained in the buccal cavity, the growth potential of these fry may be reduced.

The main objectives of these trials were to describe the embryology of O. niloticus and O. mossambicus, to determine the thermal

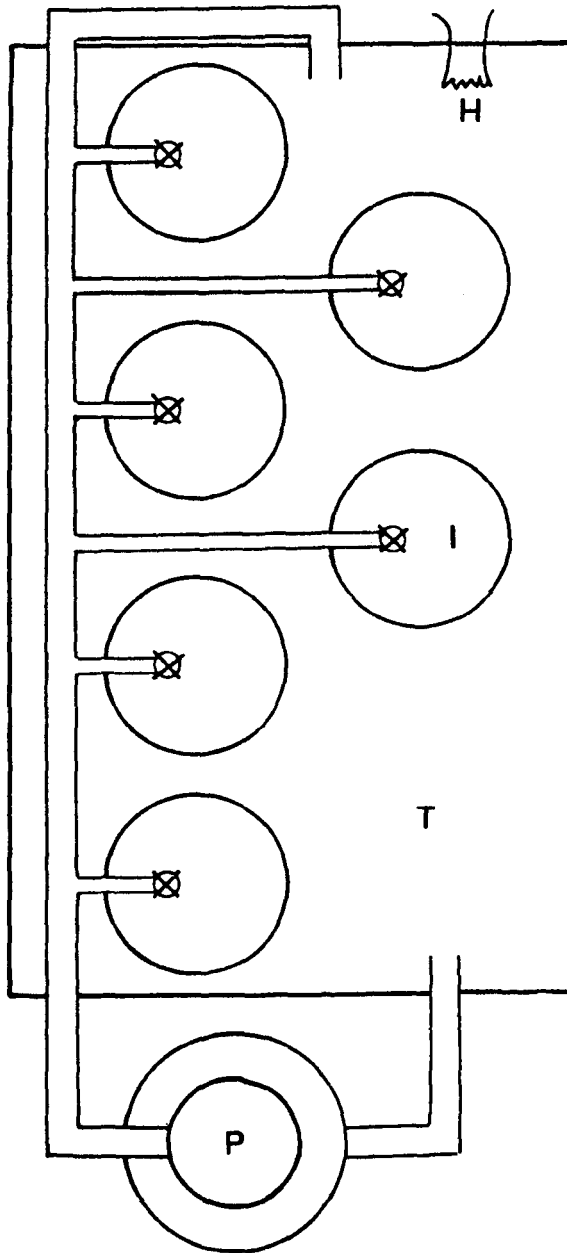


FIGURE 2.6. Layout of rearing unit used in the evaluation of the effects of temperature on egg and fry development. Each unit consisted of a water pump - Eheim 1021 (P), six 0.75 l plastic round-bottomed incubators (I) of the type shown in Figure 2.1, a 200 watt microtonic thermostatically controlled heater (H) and an air-stone in a 20 l plastic tank (T). Figure not drawn to scale.

tolerance ranges of their eggs and fry and to establish how different rearing temperatures influence egg and fry growth (rates of embryonic development, size of emergent fry, growth of fry developing on their yolk reserves), feeding capabilities of fry and susceptibility of fry to starvation.

2.2.2 Materials and Methods

2.2.2.1 Methodology for the artificial incubation of eggs and fry

To rear Oreochromis eggs and fry at various constant temperatures a series of independent rearing units was assembled in a constant temperature room as shown in Fig. 2.6. Six round-bottomed incubation vessels as described in Fig. 2.1 were held in a common 20 l plastic tank. Water was circulated in the vessels to simulate the 'churning' movements created in the buccal cavity of mouth-brooders during maternal rearing.

To ensure uniform water quality in all rearing units synthetic dilution water was prepared with a total hardness of 50 mg/l as CaCO₃ (Ministry of Housing and Local Government, 1969). A comprehensive analysis of the dilution water was carried out with the help of the Forth River Purification Board, Stirling, Scotland (Table 2.3).

Approximately 20 l of dilution water were added to each incubation unit and the final level of water in the holding tanks was noted.

TABLE 2.3

Chemical characteristics* of the dilution water of the rearing units used in the evaluation of the effects of temperature on egg and fry development

	Mean	Range
Total Oxidised Nitrogen	0.3	0.3 - 0.4
Conductivity (µs/cm)	220.0	194 - 245
Suspended Solids	1.0	1.0 - 2.0
Total Ammonia - Nitrogen	0.02	0.01-0.02
Orthophosphate (as P.)	0.01	0.01-0.02
Potassium	0.7	0.5 - 1.0
Sodium	27.4	26.5 - 28.3
Copper	0.01	0.01-0.01
Iron	0.01	<0.01-0.02
Lead	0.005	-
Manganese	<0.005	-
Zinc	<0.006	0.005-0.017

*All values are given in mg/l unless stated otherwise

To compensate for evaporation losses and to maintain the physico-chemical characteristics of the water, deionised water was added daily to each incubation unit. The water temperature of each unit was maintained by 200 watt thermostatically controlled heaters (Armitage Bros., Colwick Industrial Estate, Nottingham). Rearing temperatures were 11°C, 17°C, 20°C, 24°C, 28°C, 30°C, 34.5°C and 39.5°C maintained to within $\pm 0.5^\circ\text{C}$. Aeration in each rearing unit was supplied by aquarium air stones connected to a low pressure air blower.

2.2.2.2 Development of eggs and survival and growth of fry

Unfortunately, due to a lack of O. mossambicus broodstock and the limited space allocated in the constant temperature room only O. niloticus was used in this trial to study the effects of temperature on egg and fry development.

To standardize paternal effects on the performance of developing eggs and fry, eggs were stripped manually and fertilized at 28°C with the milt from a single conspecific male. The motility of the sperms was first confirmed by microscopic examination. Two acclimation conditions of eggs were considered: eggs fertilized at 28°C and then subjected to test temperatures - Group A eggs, and eggs fertilized and reared for 48 hours at 28°C before subjection to test temperatures - Group B eggs. Group A eggs were used to include the most sensitive developmental stages of the embryo and Group B eggs were used to simulate the common practice in hatchery rearing of removal of eggs from the brooding parent after two to three days.

Due to asynchronous spawning and the low fecundity of these fish, however, the study had to be conducted as two trials:

- (a) Embryonic development and egg viability
- (b) Fry performance

(a) Embryonic development and egg viability

In this trial a clutch of Group A eggs was divided into eight batches and incubated at 11°C, 17°C, 20°C, 24°C, 28°C, 30°C, 34.5°C and 39.5°C. Random samples of 20 eggs were removed from each incubation temperature at 0, 2, 4, 6, 8, 10, 12, 24, 30 and 48 hours after fertilization and thereafter at 24 hour intervals until hatching and fixed in Bouin's fluid. The preserved egg samples were examined later under a binocular dissecting microscope (Olympus CO11) to identify the morphological stages of egg development and to establish the effect of rearing temperature on the rate of embryonic development. To determine egg survival the following definitions for a living egg were used:

- (i) in embryos prior to the heart beat stage - the presence of a well defined germ ring in preserved samples
- (ii) in older embryos - the presence of a heart beat in samples taken after 24 hours before fixation in Bouin's fluid

In addition, to determine the influence of temperature on hatching, duplicate samples each of 30 eggs were incubated at each temperature and the numbers of hatched fry recorded.

To record the morphological stages of egg and fry development a series of photographs was taken. Selected eggs were serially dehydrated for 1 hour in each of 30%, 50%, 70% and 100% glycerine. The contracted chorions were then removed under a dissecting binocular microscope with a drawn out glass rod and forceps and the dechorionated eggs stored in 100% glycerine. Eggs and fry were then photographed using a Tessovar apparatus (Carl Zeiss).

(b) Fry performance (Fry survival and growth, and feeding capabilities of fry)

Eggs from a large 450 g gravid O. niloticus female were stripped manually and fertilized at 28°C with milt from the same male as used in trial (a). These eggs were first incubated at 28°C for 48 hours before transferring them to the various test temperatures (Group B eggs). Results from trial (a) showed that all eggs incubated at 11°C and 39.5°C died within 24 hours. Therefore in this trial eggs were incubated only at 17, 20, 24°C, 28, 30 and 34.5°C. Resultant hatchlings served as a supply for the following investigations on fry performance. In addition, duplicate samples each of 30 eggs were incubated at each of these temperatures and their hatching rates recorded.

Fry survival and growth

Random samples of 30 hatchlings were transferred in duplicate to containers held in the same rearing unit as the eggs; the numbers of fry surviving were monitored daily at each test temperature.

To determine the influence of temperature on the growth of fry developing solely on their yolk reserves, random samples of 20 fry were removed with a pipette from the supply vessel of each test temperature at hatching and then at 3 day intervals. These fry were killed in benzocaine (1:10,000 solution in water) and immediately rinsed in deionised water to minimise shrinkage.

Each sample was then divided into two groups of equal numbers and the yolk-sacs (if present) of one group were removed from the body under a binocular microscope. Mean dry body weight and mean dry fry (body + yolk, if present) weight were determined. After removing excess surface moisture with absorbant paper, the samples were weighed and then oven dried overnight at 50°C. The dried samples were then cooled in a desiccator to room temperature and reweighed to an accuracy of 0.1 mg on a pan balance (Mettler H80).

Feeding capabilities

Feeding capability was defined as the percentage of fry in a sample that were capable of exogenous feeding. At three day intervals after hatching a random sample of 10 fry incubated at each temperature was removed and transferred into a 500 l glass container held in the same incubation unit. An excess quantity of finely ground (<300µm) broodstock diet, dyed with carmosine, E122, was provided for two to three hours. The fry were then killed in benzocaine and examined under a microscope for the presence of dyed food in the gastrointestinal tract. The time to

onset of feeding was recorded as the time when 50% of the fry had started feeding. The point-of-no-return (Blaxter and Hempel, 1963) was identified as the time when 50% of the fry were incapable of ingesting the supplied diet.

2.2.3 Results

2.2.3.1 Description of *O. niloticus* eggs

Ovulated eggs are ovoid in shape (Fig. 2.7a). The major axis is 1.2 to 1.5 times the length of the minor axis and egg size ranged from 1.65 x 2.00mm (2.85mm³) to 2.60 x 3.15mm (11.15mm³). They are generally pale yellow to orange brown in colour.

The egg is surrounded by a multi-layered protective envelope, the chorion, which is flaccid and at this stage is opposed to the plasma-lemma surrounding the yolk mass. The micropyle, which appears as a funnel-shaped indentation of the chorion, is located at the animal pole, along the line of the major axis (Fig. 2.7a). The egg consists largely of a superficially homogenous yolk mass of fluid consistency. Under a scanning microscope, however, the yolk can be seen to consist of numerous yolk granules of various sizes (Fig. 2.7b).

2.2.3.2 Outline of the embryonic stages in *O. niloticus*

Time periods given here are for eggs and fry reared at 28°C. Fourteen arbitrary stages have been defined to illustrate the course

FIGURE 2.7(a)

Photomicrograph of an egg of O. niloticus. Note the funnel-shaped micropyle (M) at the animal pole.

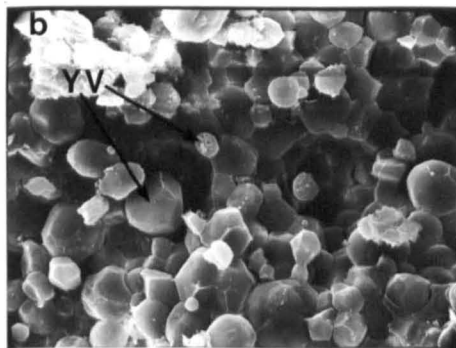
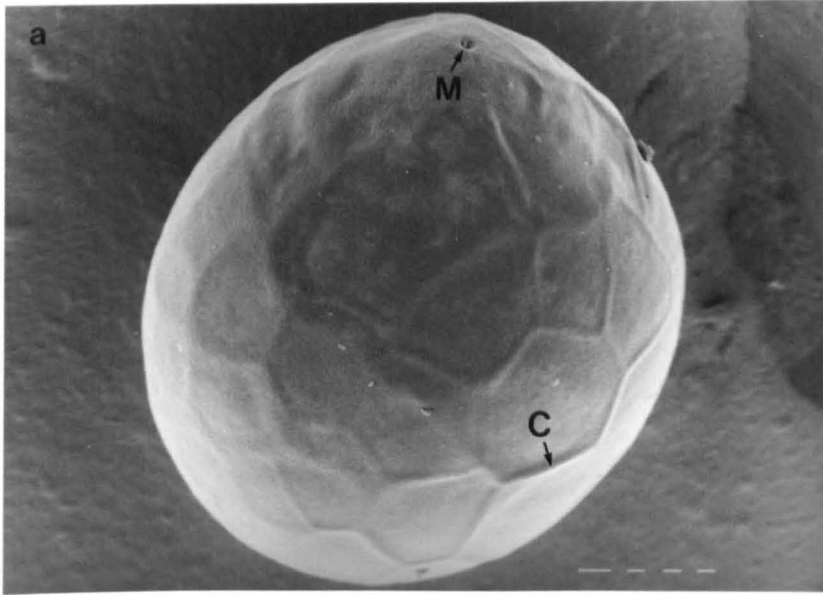
C - Chorion

x30

FIGURE 2.7(b)

Photomicrograph of egg yolk showing the yolk granules (YV).

x960



of embryonic development from fertilization to complete yolk exhaustion.

Stage 1 (Fig. 2.8a-b)

This is defined as the period from fertilization upto the first cleavage.

Once the sperm has penetrated the egg (unfertilized egg - Fig. 2.8a) via the micropyle, the cytoplasm gradually accumulates at the periphery of the yolk mass in the polar region nearest the micropyle (animal pole) to form a distinct raised protoplasmic cap (Fig. 2.8b) which develops into a single cell. Concomitantly, the perivitelline space forms between the chorion and the yolk mass (see Fig. 2.5) to occupy approximately 10-15% of egg volume. The moisture content of the egg rises from 51% to 54-55% within an hour of contact with water. The chorion hardens and the egg becomes turgid.

Stages 2-6 (Fig. 2.8c-g)

The first five rounds of cleavage are shown in Fig. 2.8. Within two to three hours of fertilization the cell cleaves first along the meridional plane to produce 2 cells (Stage 2, Fig. 2.8c) then synchronously at right angles to the first to yield 4 cells (Stage 3, Fig. 2.8d). By four hours after fertilization a vertical division of the blastomeres of the 4 cell stage, parallel to the first cleavage plane, results in 8 cells (Stage 4, Fig. 2.8e). An hour later another vertical cleavage produces 16 cells in

FIGURE 2.8

Examples of cellular cleavages during early embryogenesis of O. niloticus eggs.

- (a) Unfertilized egg
- (b) Stage 1
- (c) Stage 2 } (2-3h, 28°C)
- (d) Stage 3 }
- (e) Stage 4 (4h, 28°C)
- (f) Stage 5 (5h, 28°C)
- (g) Stage 6 (6h, 28°C)

x20

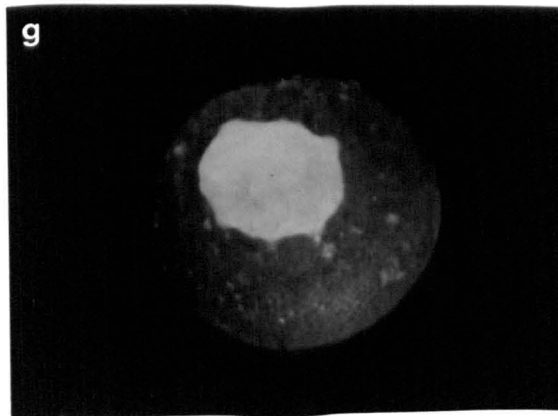
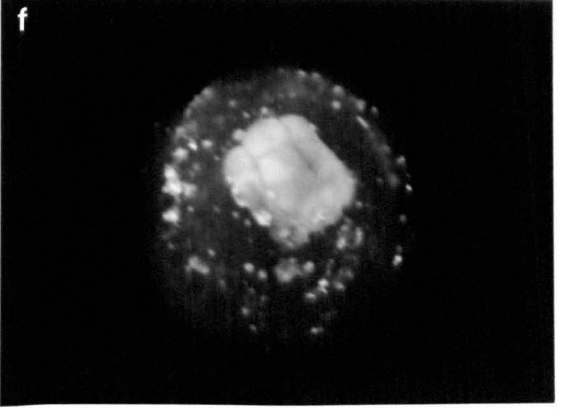
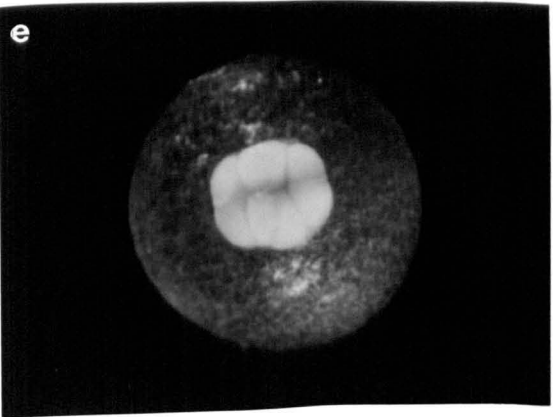
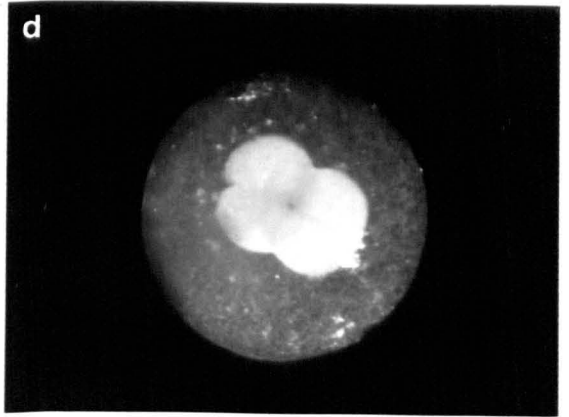
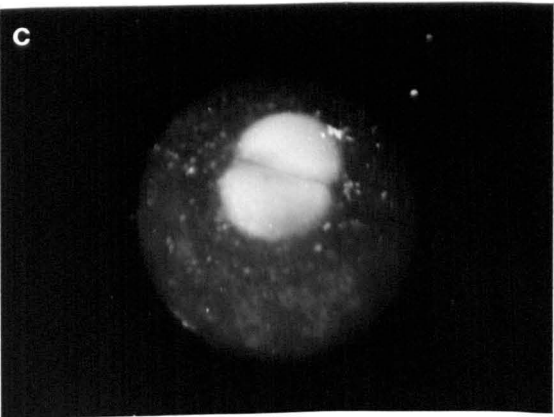
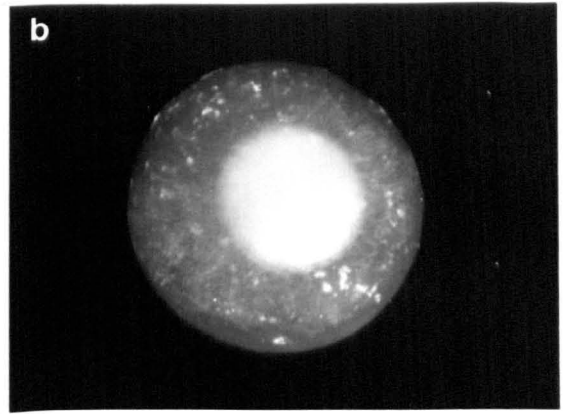
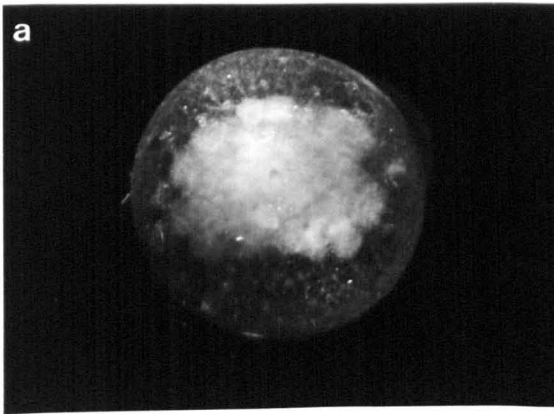


FIGURE 2.9

Embryonic development of O. niloticus eggs during
blastulation and gastrulation

- (a) Stage 7 (10h, 28°C)
- (b) Stage 8 (10-12h, 28°C)
- (c) - (f) Stage 9 (14-30h, 28°C)

- BD - Blastoderm
- BP - Blastopore
- EA - Embryonic axis
- ES - Embryonic shield
- GR - Germ ring
- HF - Head fold
- K - Keel
- OC - Optic cup

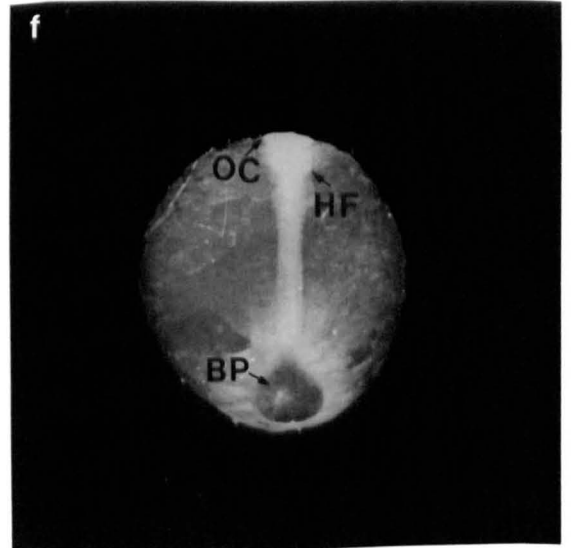
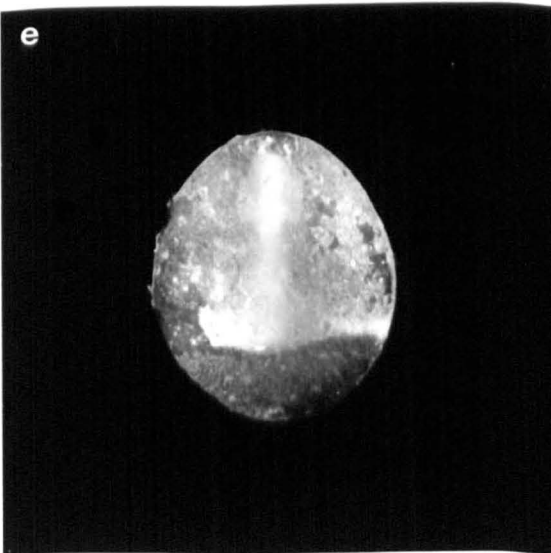
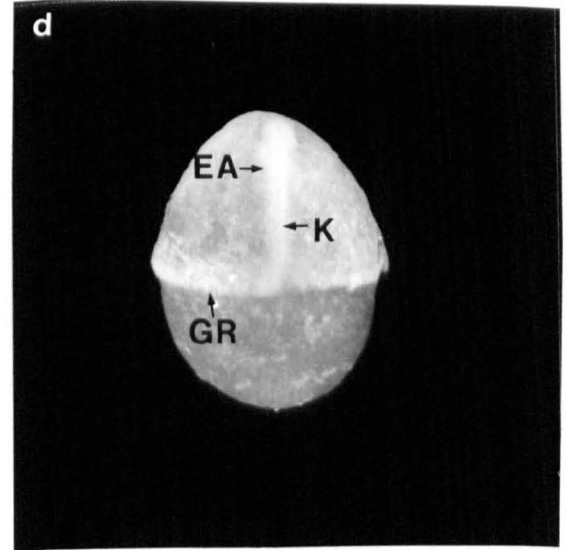
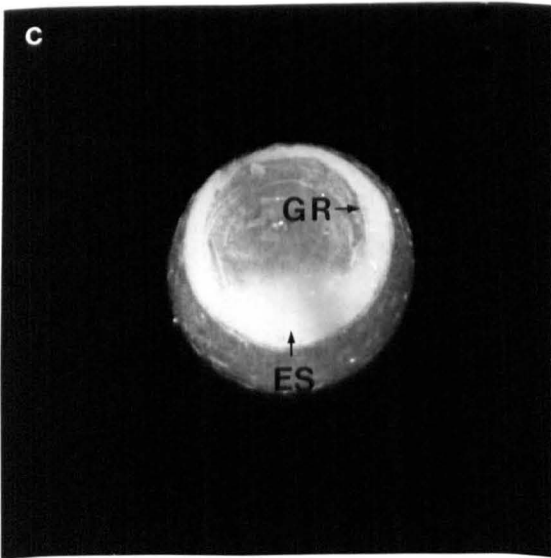
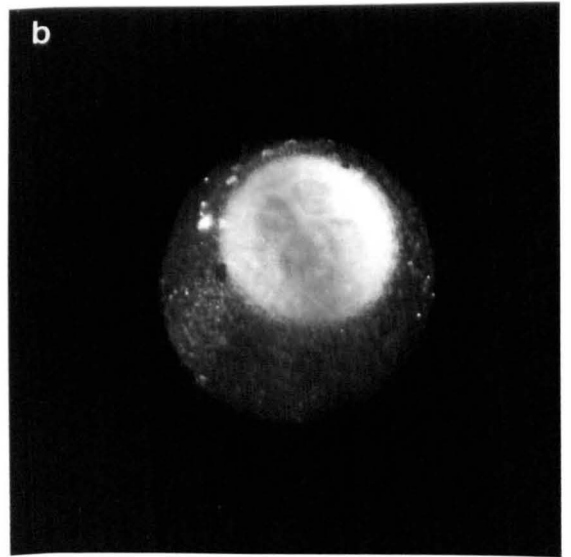
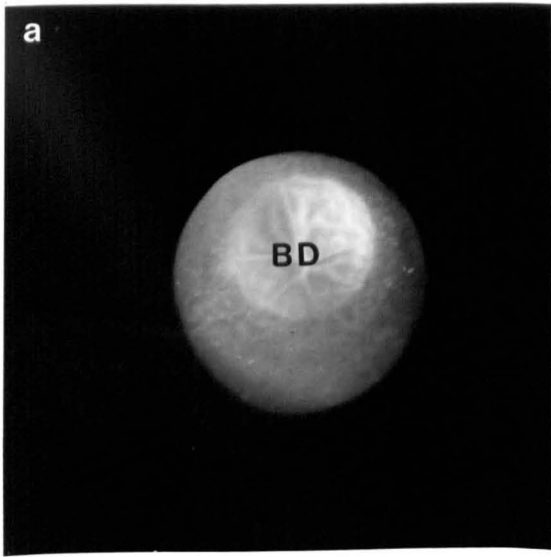


FIGURE 2.10

Progression of embryonic development from closure of blastopore to hatching in O. niloticus.

(a)-(c) Stage 11 ((a) and (b), 30-48h, 28°C);
(c) 72, 28°C)
(d) Stage 12 (90-102h, 28°C)

HE - Hatching embryo
L - Lens
M - Melanophores
PB - Pectoral bud
S - Somites

x20

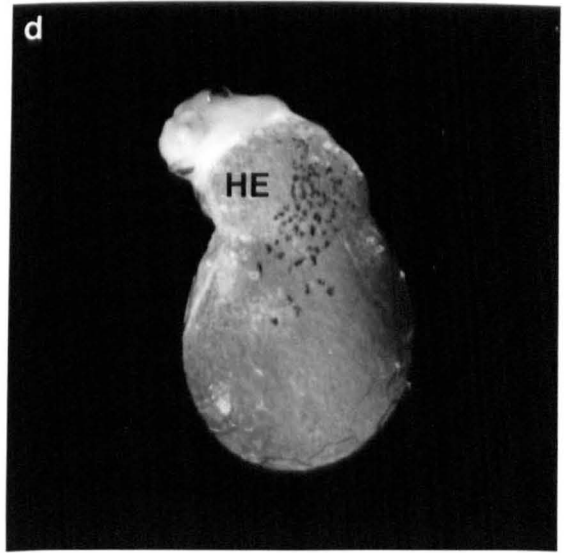
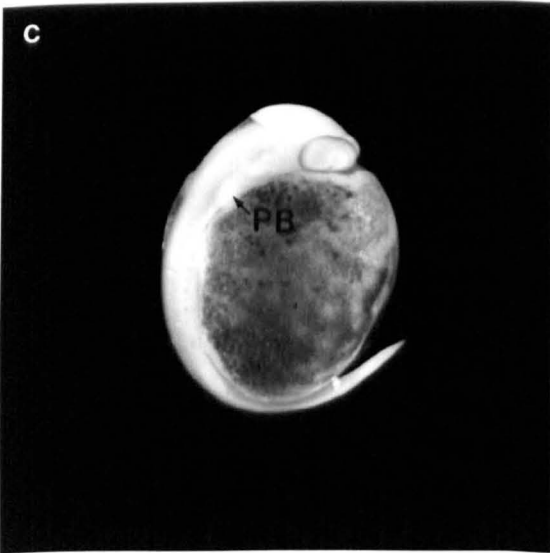
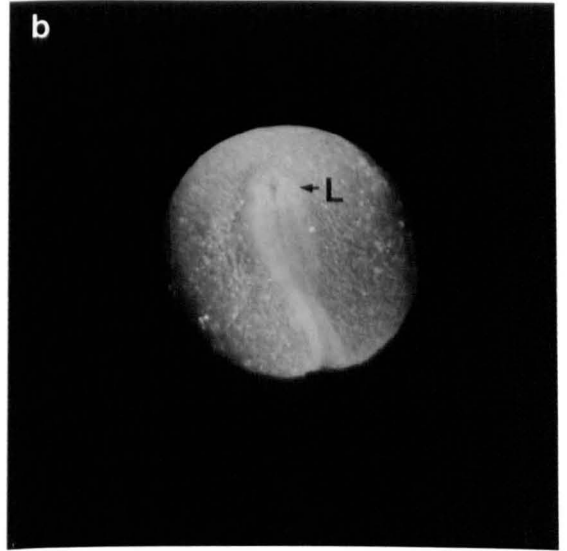
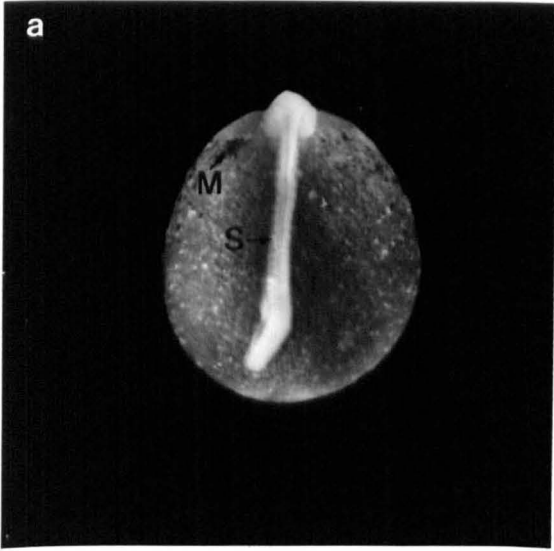


FIGURE 2.11

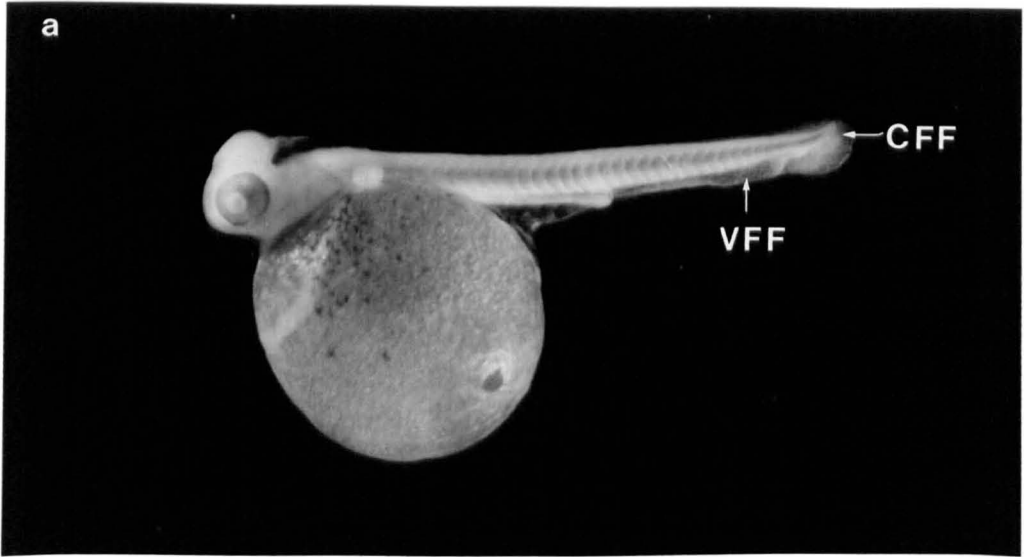
Development of hatched O. niloticus fry.

- (a) Stage 12 (90-102h, 28°C) x20
- (b) Stage 13 (5-6 days post-hatch, 28°C) x15
- (c) Stage 14 (9-12 days post-hatch, 28°C) x12.5

CFF - Caudal fin fold

SB - Swim bladder

VFF - Ventral fin fold



a single layer of four parallel rows of four cells (Stage 5, Fig. 2.8f). Beyond the 16 cell stage divisions are less synchronous. By six hours after fertilization a horizontal cleavage plane results in 32 cells (Stage 6, Fig. 2.8g) resembling a cobbled surface of rounded cells.

Stage 7 (Fig. 2.9a)

During this stage the blastula develops (Fig. 2.9a). Within 10 hours after fertilization the blastoderm becomes flattened and forms a distinct cap over the yolk at the animal pole.

Stage 8 (Fig. 2.9b)

By ten to twelve hours after fertilization gastrulation commences. The blastoderm continues to expand over the yolk and a thin area, which will form the yolk sac epithelium, becomes apparent. A thickened rim, known as the germ ring, forms the leading edge of the blastoderm. The thickening of the germ ring is visibly greater in one region forming the embryonic shield (Fig. 2.9b). This is the region from which the future embryo develops.

Stage 9 (Fig. 2.9c-f)

Epiboly commences. The embryonic shield enlarges and is clearly defined with a broad thick base. The anteroposterior embryonic axis becomes evident. The gastrula extends over one quarter of the yolk mass (Fig. 2.9c). The embryonic shield extends further and the gastrula extends to the equator. Rudimentary

optic vesicles and head folds develop (Fig. 2.9d). As epiboly progresses the gastrula extends further to reach past the equator, the embryonic axis elongates and the embryonic head folds begin to lift the cephalic end of the embryo from the yolk (Fig. 2.9e). With further development the keel of the central nervous system becomes apparent. Microscopic melanic pigments appear laterally to the head folds. The optic cups can be seen and the brain divisions develop. The embryonic keel becomes prominent and somites develop. The germ ring encloses the yolk leaving only a small opening - the blastopore (Fig. 2.9f). In live specimens, the heart, which is located laterally to the head, can be seen to contract rhythmically on the yolk mass at 24 hours after fertilization, but no red blood corpuscles can be seen as yet.

Stage 10

The germ ring completely encloses the yolk mass; the completion of epiboly is characterised by this closure of the blastopore or yolk plug. The time for the yolk plug to be closed over is proportional to the yolk mass (Shaw and Aronson, 1954). In live embryos red blood corpuscles are visible in the heart and macroscopic melanophores are numerous on the yolk sac. The three main divisions of the brain, the forebrain, midbrain and hindbrain are distinguishable and the neural keel is well defined.

The entire process of epiboly occurs between 14 and 30 hours after fertilization.

Stage 11 (Fig. 2.10a-c)

The somites increase in number. By 30 to 48 hours after fertilization the tail of the embryo which has lifted off the yolk sac increases in length (Fig. 2.10a) and spontaneous trunk movements occur. The heart beats rapidly and red blood corpuscles can be seen circulating through the heart. The lens develops but as yet the eye is not pigmented (Fig. 2.10b). Later the eye becomes well developed, the lens is conspicuous and eye pigmentation increases in intensity. Melanophores appear on the yolk sac along the trunk region of the embryo and the pectoral buds appear. By 72 hours after fertilization the embryo encompasses approximately 270° of the yolk circumference (Fig. 2.10c). Flexing and rotation of the entire embryo is common.

Stage 12 (Fig. 2.10d and 2.11a)

The spontaneous flexing of the embryo within the turgid chorion increases. The chorion weakens through internal digestion and within 90 to 102 hours the embryo emerges, usually head first (Fig. 2.10d). Emergent fry have a large yolk sac and are unable to swim. The mouth develops but the lower jaw displays only feeble movements. The operculum covers the four gill arches which bear blunt gill filaments. The ventral and caudal fin folds are visible but fin rays are not yet present (Fig. 2.11a).

Stage 13 (Fig. 2.11b)

During this stage the yolk is consumed rapidly. The digestive system differentiates and the swim bladder inflates within six days of hatching. The gills and pelvic, dorsal, ventral and caudal fins are well formed (Fig. 2.11b). When the swim bladder is inflated (approximately six days after hatching) the fry achieve neutral buoyancy and become free-swimming.

Stage 14 (Fig. 2.11c)

Within 9-12 days after hatching the yolk reserves are exhausted and the fry resembles an adult (Fig. 2.11c).

2.2.3.3 Survival and hatching success of eggs at various incubation temperatures

The survival pattern of selected morphological stages of embryonic development of O. niloticus eggs reared at different incubation temperatures is presented in Table 2.4 and Fig. 2.12. These curves are of eggs fertilized at 28°C and reared at the test temperatures (Group A eggs). When reared at 17°C and below or at 39.5°C eggs failed to develop to the 2 cell stage. Within the range of 17°C - 34.5°C the thermal tolerance of embryos decreased progressively with development. At 20°C even though 100% of eggs sampled reached the 2 cell stage (Stage 2) only 45% progressed to the blastula stage (Stage 7), whereas at 34.5°C 100% of eggs sampled reached the blastula stage but only 55% completed epiboly

TABLE 2.4

Survival of artificially incubated O. niloticus eggs¹, in relation to temperature

Incubation Temperature (°C)	Developmental stages of eggs (%)					
	Two cell	Blastula	Embryonic shield	Epiboly	Closure of germ ring	Hatch ² rate
11.0	0	0	0	0	0	0
17.0	0	0	0	0	0	0
20.0	100	45	35	30	25	25.5 (9.8)
24.0	100	100	100	95	90	84 (6.9)
28.0	100	100	100	95	95	94.5 (3.9)
30.0	100	100	95	80	95	90 (5.9)
34.5	100	100	85	65	60	48.5 (2.9)

¹ Eggs were manually stripped and fertilized at 28°C then subjected to test temperatures - Group A eggs

² Mean values based on duplicate treatments are given with 1 standard deviation

TABLE 2.5

Summary of the effect of temperature on the survival of O. niloticus eggs¹ and swim-up fry³

	<u>Median Tolerance Limit</u> - TL ₅₀ ²		Hatching Range (°C)	Optimum Range (>90%) (°C)
	Upper (°C)	Lower (°C)		
Group A eggs	34.0 (33.3-34.7)	21.8 (21.1-22.5)	20.0 - 34.5	25.0 - 30.0
Group B eggs	35.1 (34.3-35.8)	14.8 (13.9-15.8)	17.0 - 34.5	23.5 - 32.0
Swim-up fry	32.1 (31.9-32.2)	21.8 (21.4-22.3)	-	28.0 - 30.0

¹Group A eggs - fertilized at 28°C and then reared at test temperatures in duplicate
 Group B eggs - fertilized and reared at 28°C for 48 hours and then reared at test temperatures in duplicate

²TL₅₀ given with 95% confidence limit were calculated using the Trimmed Spearman-Kärber Method
 (Hamilton, Russo and Thurston, 1977)

³Fry from Group B eggs

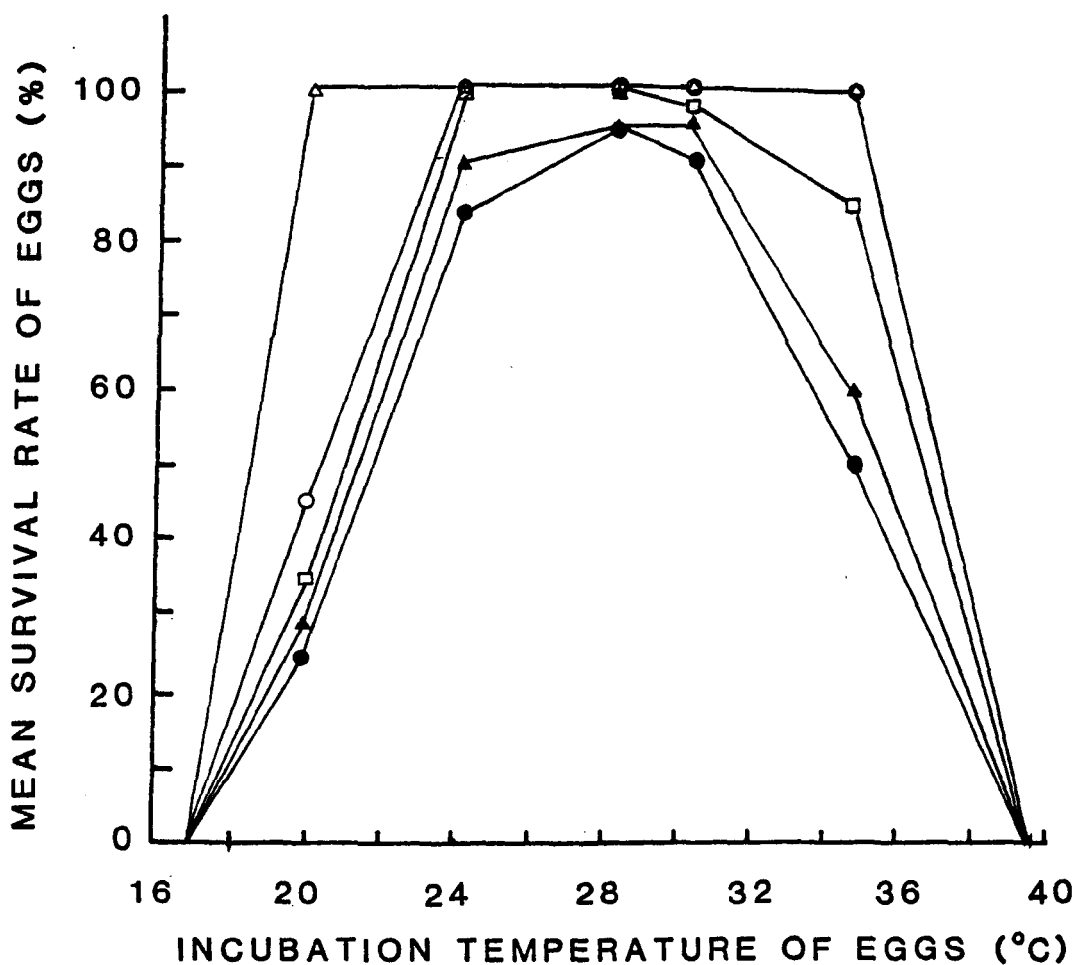


FIGURE 2.12. Influence of incubation temperature on the pattern of embryo survival in *O. niloticus*. Eggs were artificially fertilized at 28°C and incubated at test temperatures shown. Embryonic stages shown are: Δ 2 cell stage; \circ blastula stage; \square embryonic shield stage; \blacktriangle closure of the germ ring; and \bullet hatching.

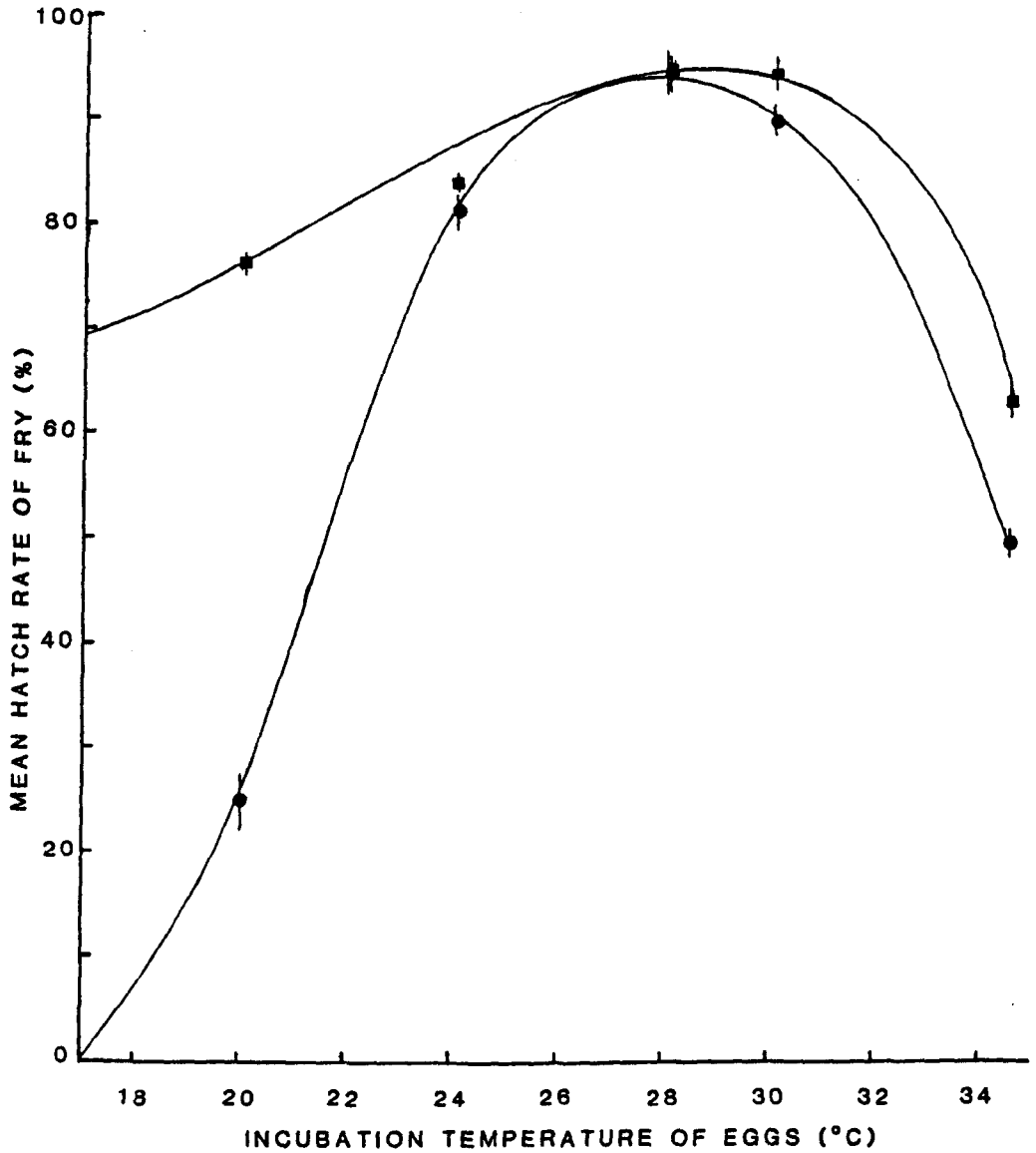


FIGURE 2.13. Comparison of hatching rates of *O. niloticus* eggs from two acclimation conditions. Group A eggs (●), eggs fertilized at 28°C then reared at test temperatures; Group B eggs (■), eggs fertilized and reared for 48h at 28°C before rearing at test temperatures. Mean hatch rates of duplicate treatments given with ± 1 SD.

(Stage 10). Optimal development (i.e. > 90%) of all stages (Stages 1-12) occurred over a narrow range of 25°C-30°C (Fig. 2.12).

The viability of eggs (measured as hatching success) reared at the various temperatures are presented in Table 2.5 and Fig. 2.13. For eggs fertilized and reared at 28°C for 48 hours and then reared at test temperatures (Group B eggs) hatching (Stage 12) occurred between 17°C to 34.5°C compared with 20°C to 34.5°C for Group A eggs. These differences were also reflected in their median lethal tolerance limits (TL_{50}); Group B eggs having a lower lower TL_{50} and higher upper TL_{50} than Group A eggs (Table 2.5).

Maximal hatch rates for Group A and Group B eggs occurred at 28°C and were not significantly different ($P > 0.05$). Outwith this temperature, however, the viability of Group B eggs was significantly higher ($P < 0.05$) than Group A eggs (Fig. 2.13).

2.2.3.4 Relationship between embryonic development and incubation temperature

The length of time required to reach selected embryonic stages in O. niloticus eggs fertilized at 28°C and then reared at various constant test temperatures (Group A eggs) are shown in Fig. 2.14. The time taken for morphological differentiation decreased exponentially with temperature. This exponential decrease however, was most marked for the closure of the germ ring stage (Stage 10).

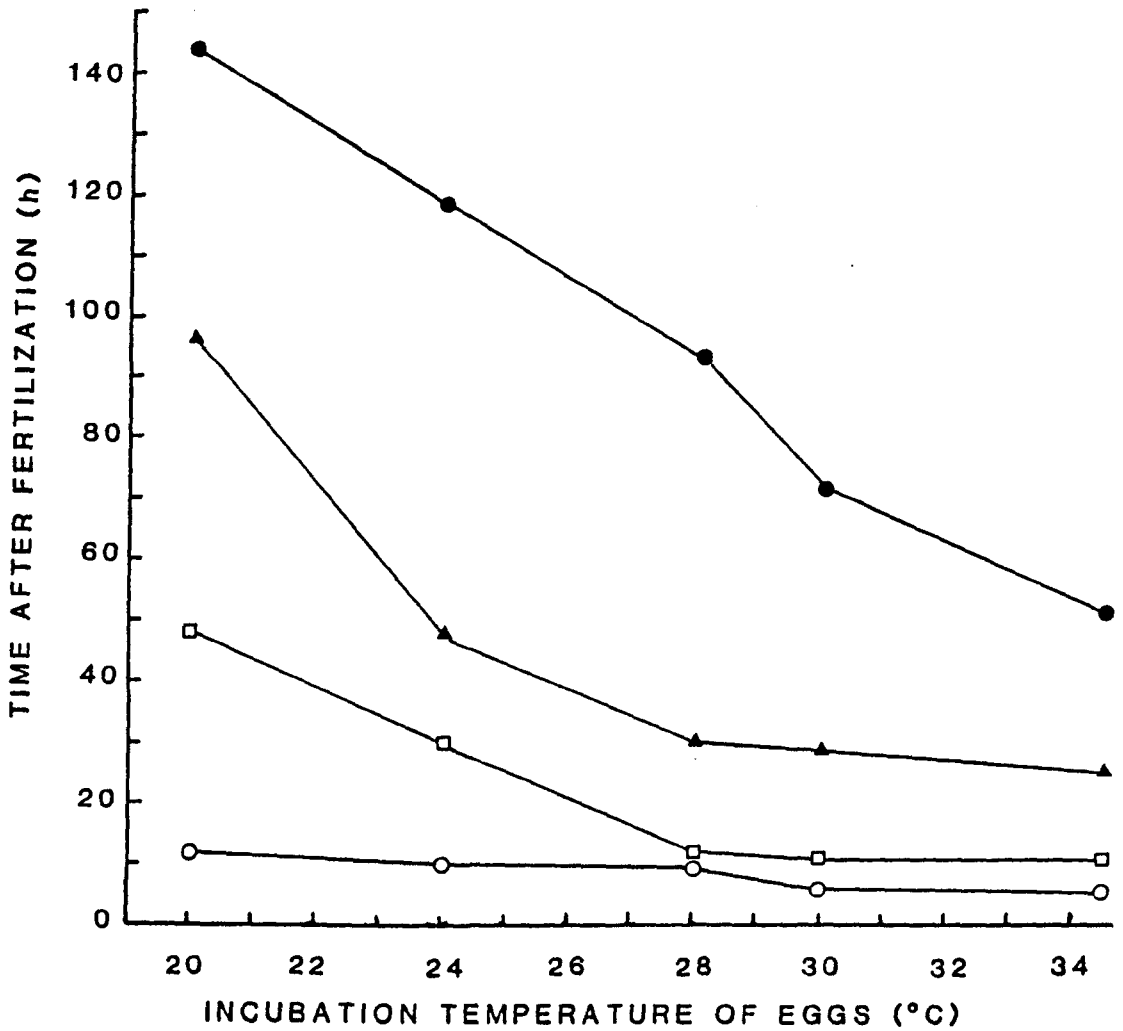


FIGURE 2.14. Temperature-dependence of the development of *O. niloticus* eggs fertilized at 28°C and then reared at the test temperatures shown. Embryonic stages: ○ blastula; □ embryonic shield; ▲ closure of germ ring; and ● hatching

FIGURE 2.15

Influence of incubation temperature (T) on mass hatching times (HT) of O. niloticus fry. (a) Group A eggs, and (B) Group B eggs. The equation best describing the relationship was:

(a) $HT = 11.4 - 0.27T$; $r^2 = 0.978$ with $df = 3$, $P < 0.01$

(b) $HT = 12.8 - 0.32T$; $r^2 = 0.962$ with $df = 4$, $P < 0.01$.

Broken lines indicate the 95% confidence limits. Hatching times based on mean values of duplicate treatments, each containing 30 eggs.

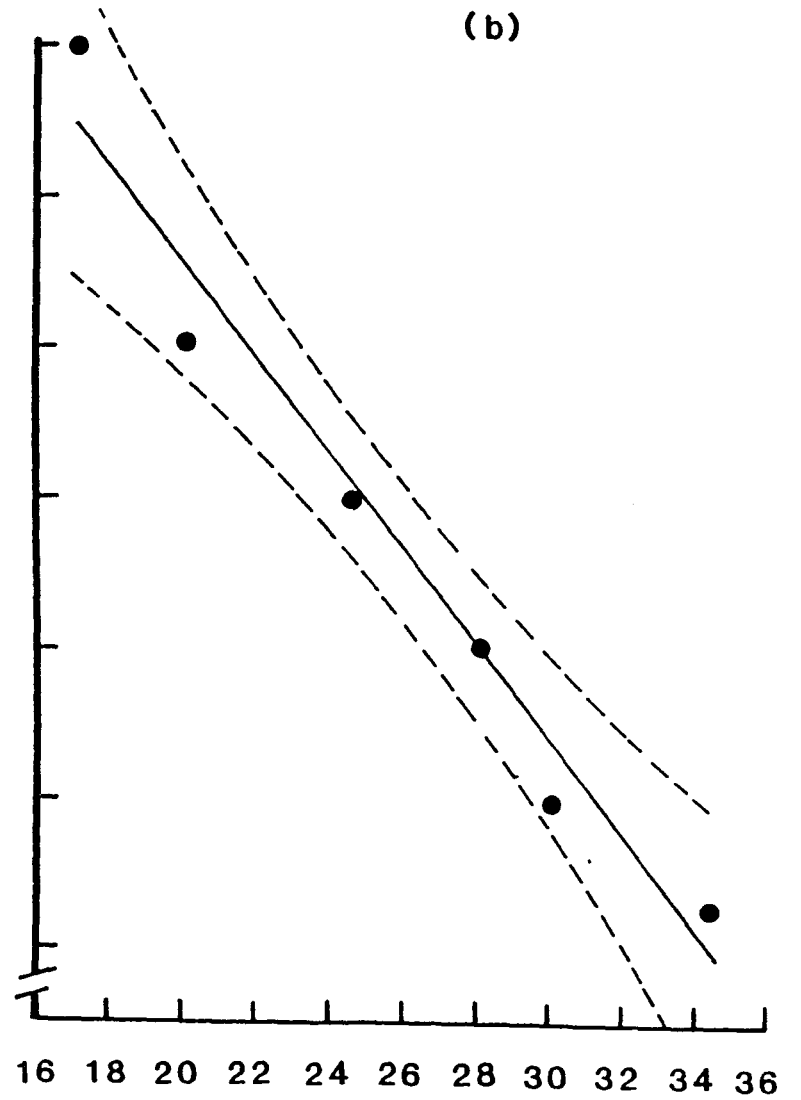
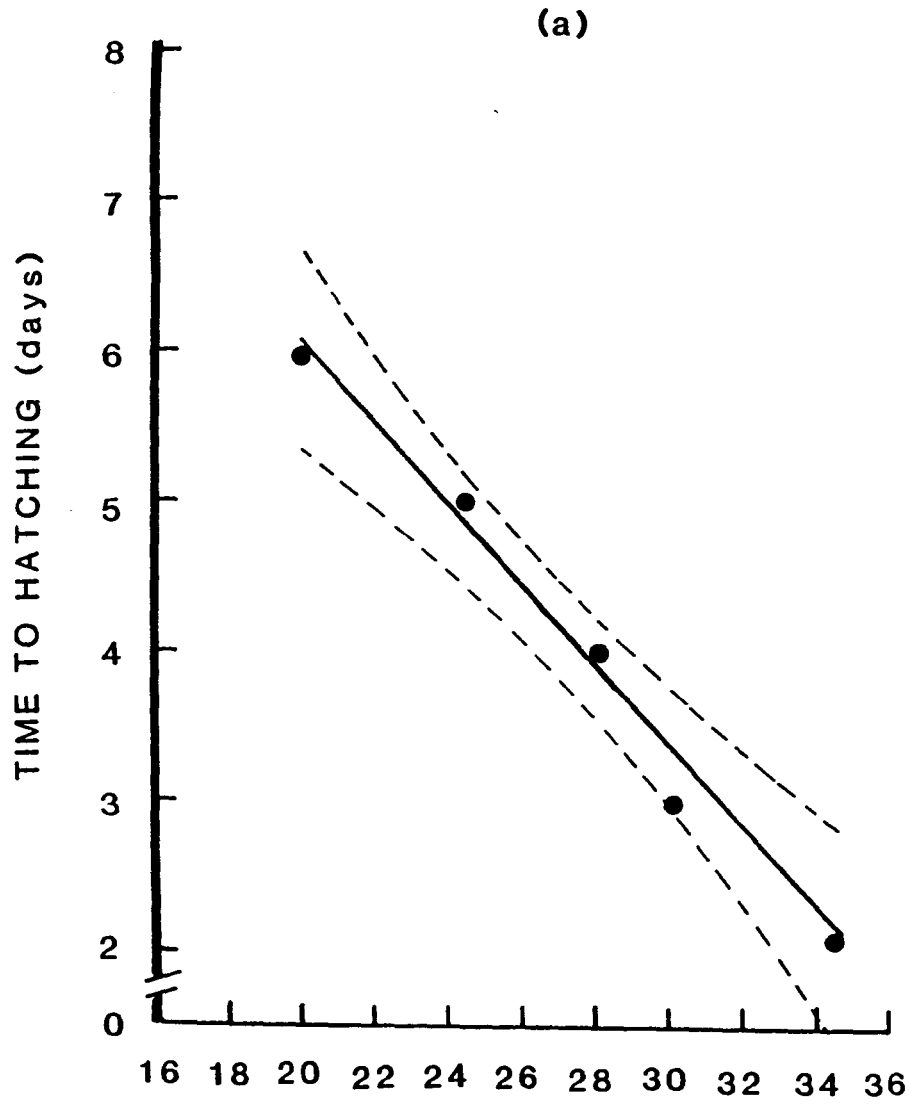


FIGURE 2.16

Influence of incubation temperature (T) on the rate of development ($1/D$) of O. niloticus embryos to hatching.

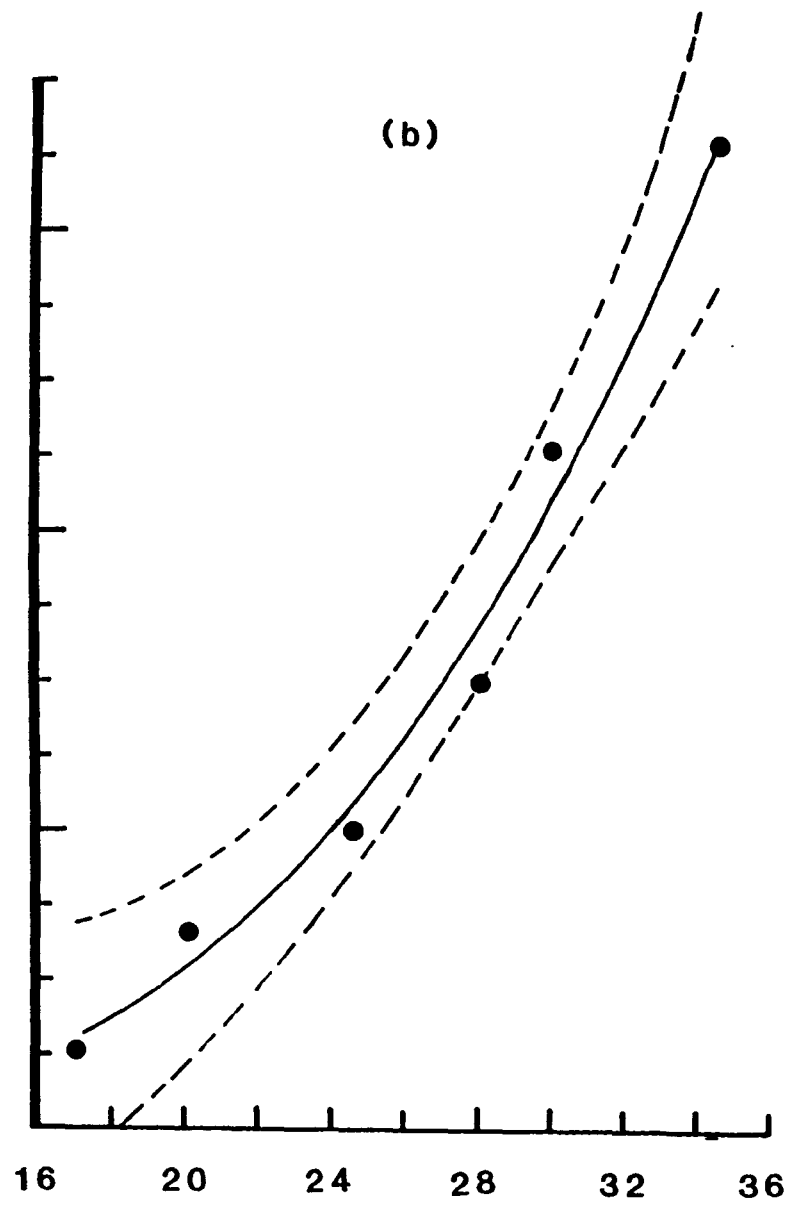
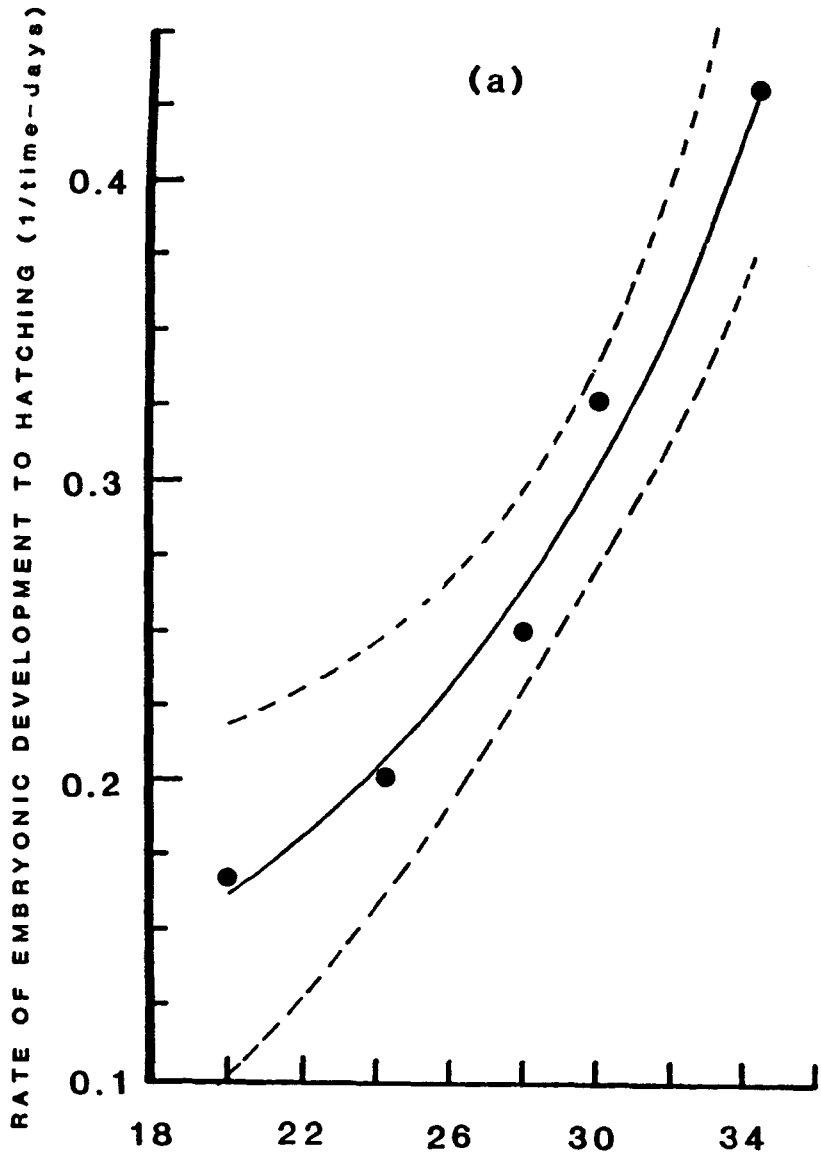
(a) Group A eggs, and (b) Group B eggs

The equation best describing the relationship was a second order polynormal:

$$(a) \quad \begin{aligned} 1/D &= 0.445 - 0.033T + 0.010T^2; \\ r^2 &= 0.984 \text{ with } df = 3, P < 0.01 \end{aligned}$$

$$(b) \quad \begin{aligned} 1/D &= 0.273 - 0.201T + 0.0007T^2; \\ r^2 &= 0.985 \text{ with } df = 4, P < 0.001 \end{aligned}$$

Broken lines indicate the 95% confidence limits. Rates of developments based on mean values of duplicate treatment, each containing 30 eggs.



INCUBATION TEMPERATURE OF EGGS (°C)

In this study the time to mass hatch was inversely related to incubation temperature. For Group A eggs hatching times ranged from 2.3 days at 34.5°C to 6 days at 20°C. Times to mass hatch for eggs fertilized and reared at 28°C for 48 hours before being subjected to test temperatures (Group B eggs) were similar. In Group A and B eggs, 97.8% and 96.2%, respectively, of the total variation in hatching times was accounted for by incubation temperature (Fig. 2.15).

A computer evaluation of the empirical data revealed that the relationship between the rate of development to hatching ($1/t$ -days) and incubation temperature of Group A and B eggs was best represented by a curvilinear relationship as shown in Fig. 2.16. This relationship accounted for 98.4% and 98.5% of the total variation in rates of development to hatching in Group A and B eggs, respectively.

2.2.3.5 Survival of hatchlings

The temporal survival pattern for O. niloticus fry reared at various temperatures is shown in Fig. 2.17 and times to 50% fry survival (ST_{50}), estimated from Fig. 2.17, are given in Table 2.6.

The numbers of yolk-sac fry surviving at 34.5°C declined sharply immediately after hatching and were reduced by 50% within 2.5 days of hatching (Fig. 2.17). Mortalities of fry reared at 24°C, 28°C and 30°C commenced at about 18, 14 and 13 days after hatching, respectively, and reached 50% by days 21, 16.5 and 14 (Fig. 2.17, Table 2.6). With the exception of fry incubated at 34.5°C and 20°C

FIGURE 2.17

Temporal pattern of survival of O. niloticus fry reared at the various temperatures shown. Incubation temperatures:

○ , 20°C; △ , 24°C; ▲ , 28°C; ■ , 30°C; and ● , 34.5°C.

Note rapid rate of mortality at yolk exhaustion occurred only at 24°C, 28°C and 30°C (see arrows). At 20°C and 34.5°C dead fry still had substantial yolk reserves. Mean values based on duplicate treatments.

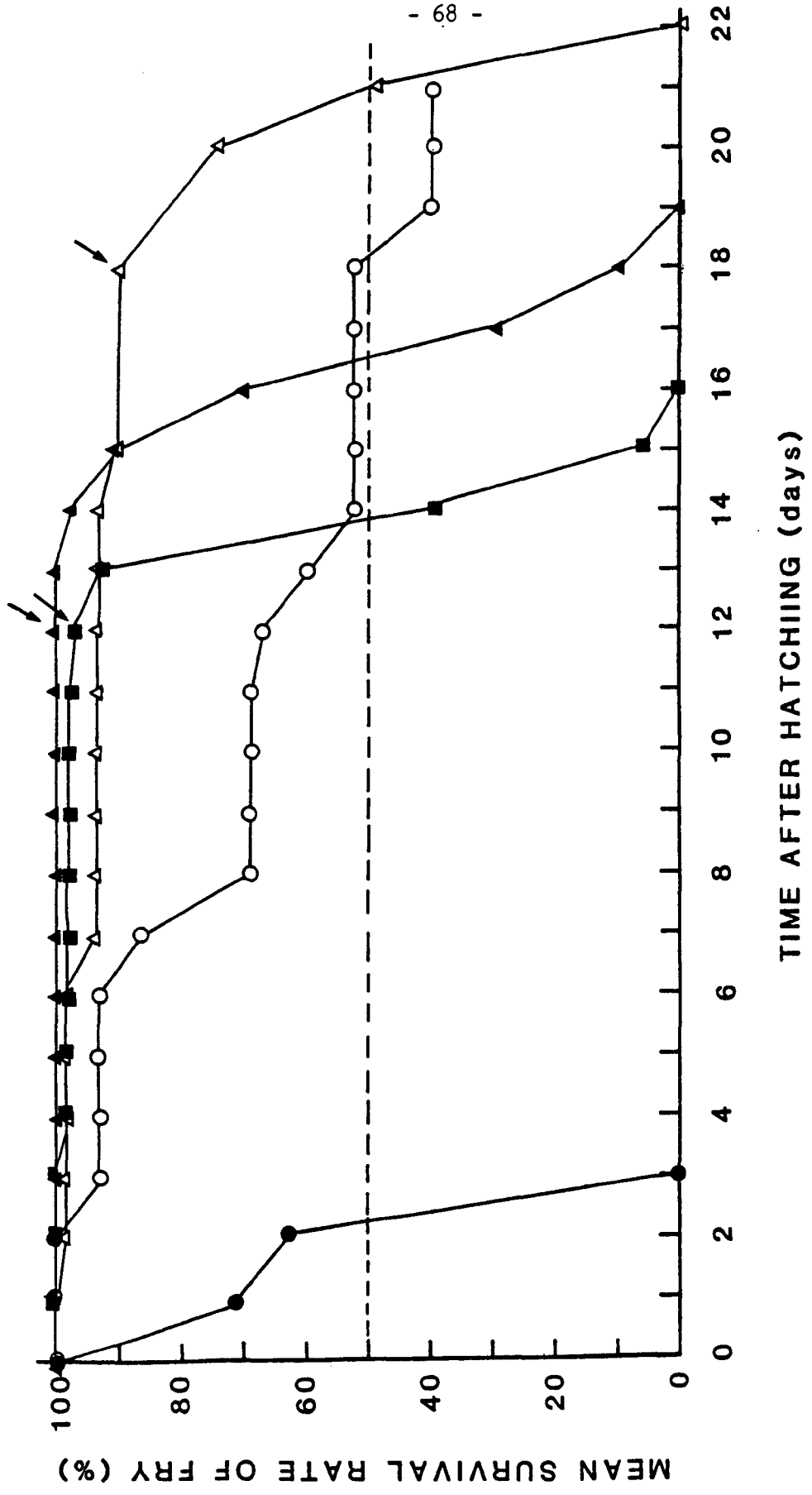


TABLE 2.6

Estimated time to 50% survival (ST_{50}) of O. niloticus fry. Values extrapolated from Fig. 2.17

Incubation Temperature (°C)	Survival time (ST_{50}) (days after hatching)
20.0	18.5
24.0	21.0
28.0	16.5
30.0	14.0
34.5	2.5

the highest rate of mortality coincided with the end of the yolk-sac stage (Stage 14) (Fig. 2.17).

The upper and lower TL_{50} s from hatching (Stage 12) to swim-up (Stage 13) were 32.1°C and 21.8°C , respectively (Table 2.5). Optimal survival (>90%) to swim-up stage occurred at 28°C - 30°C .

2.2.3.6 Growth of fry developing solely on their yolk reserves

The mean dry body (fry minus yolk) and yolk weight of emergent fry reared at various temperatures are shown in Fig. 2.18. Fry body weight and yolk weight were inversely related, that is a fry hatching with a lower body weight contained greater yolk reserves. An increase in the rearing temperature from 20°C to 30°C resulted in an increase in the body weights of fry at hatching. Raising incubation temperatures beyond 30°C however, decreased the body weight of the emergent fry.

Temporal changes in mean body and yolk weights of fry reared at 24°C , 28°C and 30°C are shown in Fig. 2.19 and data relating to their growth characteristics are given in Tables 2.7 and 2.8.

The growth rate (SGR %/day) of fry (Table 2.7) and the time at which maximum body weight was reached were temperature-dependent (Table 2.8, Fig. 2.19). For the first three days after hatching the growth rate of fry reared at 24°C averaged 10.8%/day resulting in the fry attaining only 14.4% of their maximal body weight. Whereas fry reared at 30°C grew at a rate of 22.6%/day and attained 31% of their

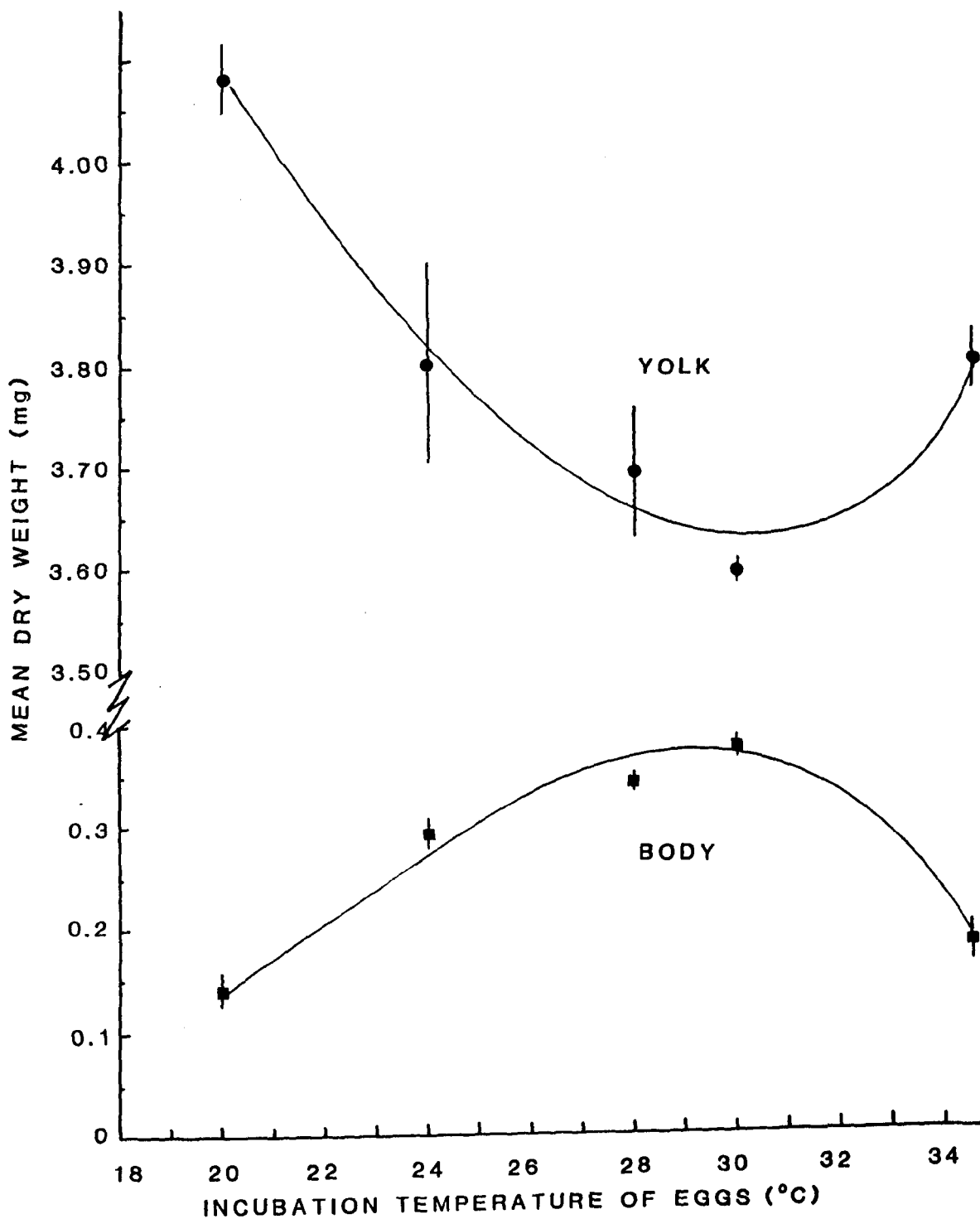


FIGURE 2.18. Effect of incubation temperature on the mean dry body (fry less yolk) and yolk weight of emergent *Q. niloticus* fry. Eggs were first incubated for 48h at 28°C. Means based on values of duplicated treatment.

FIGURE 2.19

Temporal change of body (fry less yolk) and yolk weights of O. niloticus fry reared at 24°C (●); 28°C (■); and 30°C (▲). Upper lines represent total fry weights (body + yolk, if present). Mean values based on duplicate treatments.

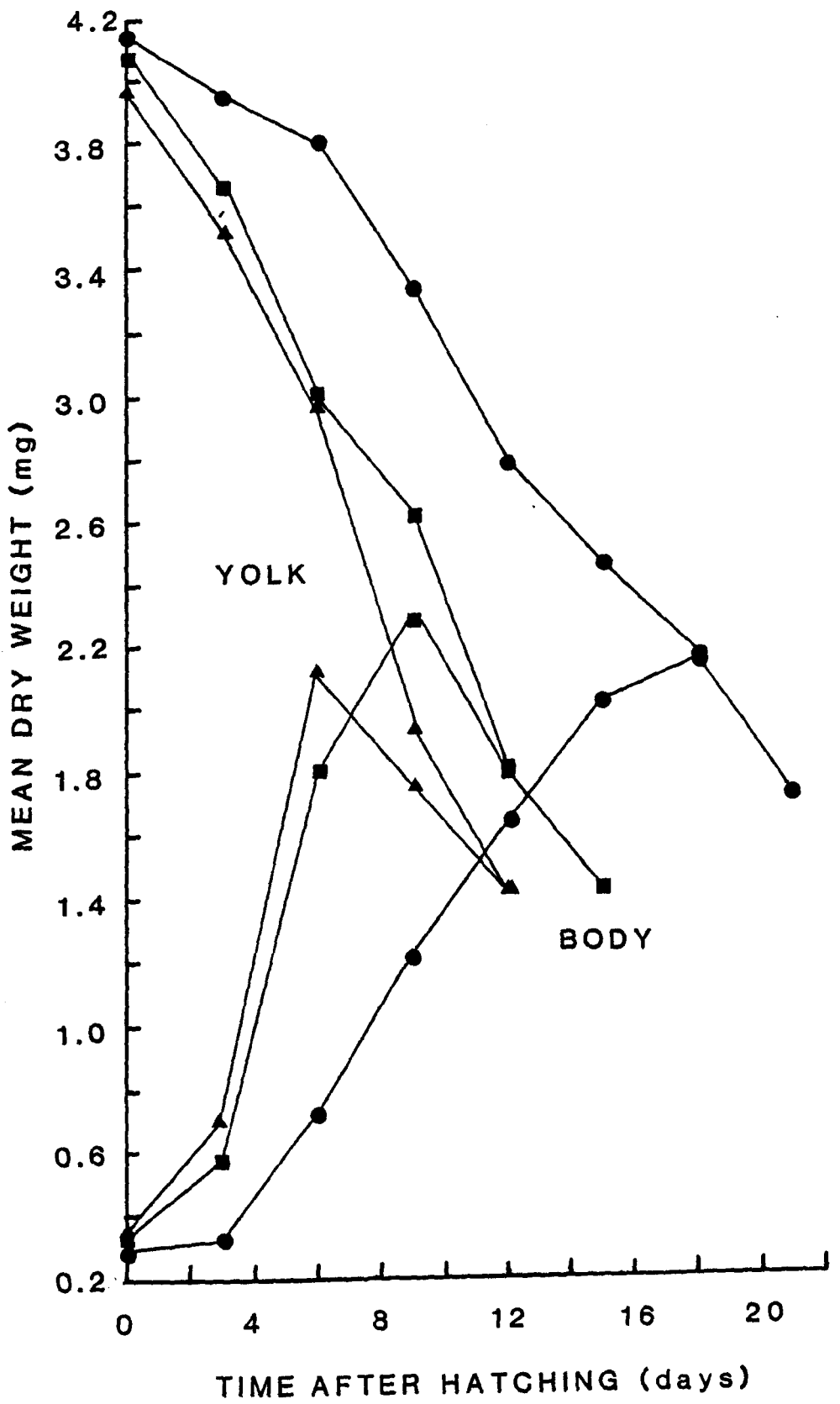


TABLE 2.7

Influence of temperature on the average specific growth rate (%/day)¹ of unfed O. niloticus fry² developing solely on their yolk reserves

Incubation Temperature (°C)	Age of fry (days after hatching)						
	0-3	3-6	6-9	9-12	12-15	15-18	18-21
17.0	-	8.4	8.6	-	-	-	-
20.0	6.5	14.2	16.8	-	-	-	-
24.0	10.8	15.3	17.6	9.9	8.1	0.94	- 7.6
28.0	20.9	37.8	8.6	- 8.6	- 7.4	-	-
30.0	22.6	39.1	- 6.1	- 7.2	-	-	-

$$^1 \text{ Specific growth rate (\%/day)} = \frac{\text{Log}_e W_{tx} - \text{Log}_e W_{to}}{tx - to}$$

where W_{tx} = final mean dry body weight at tx

W_{to} = initial mean dry body weight at to

tx = final time

to = initial time

² Fry from Group B eggs

TABLE 2.8

Influence of temperature on the growth characteristics of unfed O. niloticus fry¹ developing solely on their yolk reserves.

	Rearing temperature (°C)				
	20.0	24.0	28.0	30.0	34.5
Body weight of fry at 3 days (% of maximum)	-	14.4	24.9	31.0	-
<u>Yolk reserves</u>					
Gross yolk utilization efficiency (%)					
- of one day old fry ²	63.4	53.1	56.7	60.0	38.3
- at maximum body weight ³	-	55.4	57.2	61.7	-
End of yolk-sac stage (days)	-	18	12	12	-
Age at maximal weight (days)	-	18	9	6	-

¹ Fry from Group B eggs

² Gross yolk utilization efficiency (%) =

$$\frac{\text{dry body weight (mg)}}{\text{dry egg weight (mg)}} \times 100$$

³ Gross yolk utilization efficiency (%) =

$$\frac{\text{body gain (dry weight, mg)}}{\text{yolk consumed (dry weight, mg)}} \times 100$$

maximum body weight over the same period (Tables 2.7 and 2.8). Consequently, maximal growth was reached at days 18, 9 and 6 for fry reared at 24°C, 28°C and 30°C, respectively (Fig. 2.19). At rearing temperatures of 24°C and 28°C fry maintained a growth rate of 17.6% and 8.6%, respectively, between days 6-9, whereas over the same period fry incubated at 30°C stopped growing and began to lose weight (Table 2.7) even though yolk reserves were still present (Fig. 2.19).

The gross yolk utilization efficiency (GYUE) of one-day old fry

$$\text{measured as } \frac{\text{dry body weight (mg)}}{\text{dry egg weight (mg)}} \times 100$$

showed no clear trend with respect to temperature. Gross yolk utilization efficiency of fry from hatching up to maximal body weight

$$\text{measured as } \frac{\text{body gain (mg dry weight)}}{\text{yolk consumed (mg dry weight)}} \times 100$$

was temperature-dependent between 24°C and 30°C, being 55.4% at 24°C compared with 61.7% for fry reared at 30°C. The lower temperature prolonged the yolk-sac period; yolk reserves were exhausted by days 18, 12 and 12 at 24°C, 28°C and 30°C, respectively, (Table 2.8).

2.2.3.7 Feeding capabilities of fry developing solely on their yolk reserves

The feeding capabilities of fry reared at 24°C, 28°C and 30°C were temperature-dependent as shown in Fig. 2.20 and Table 2.19.

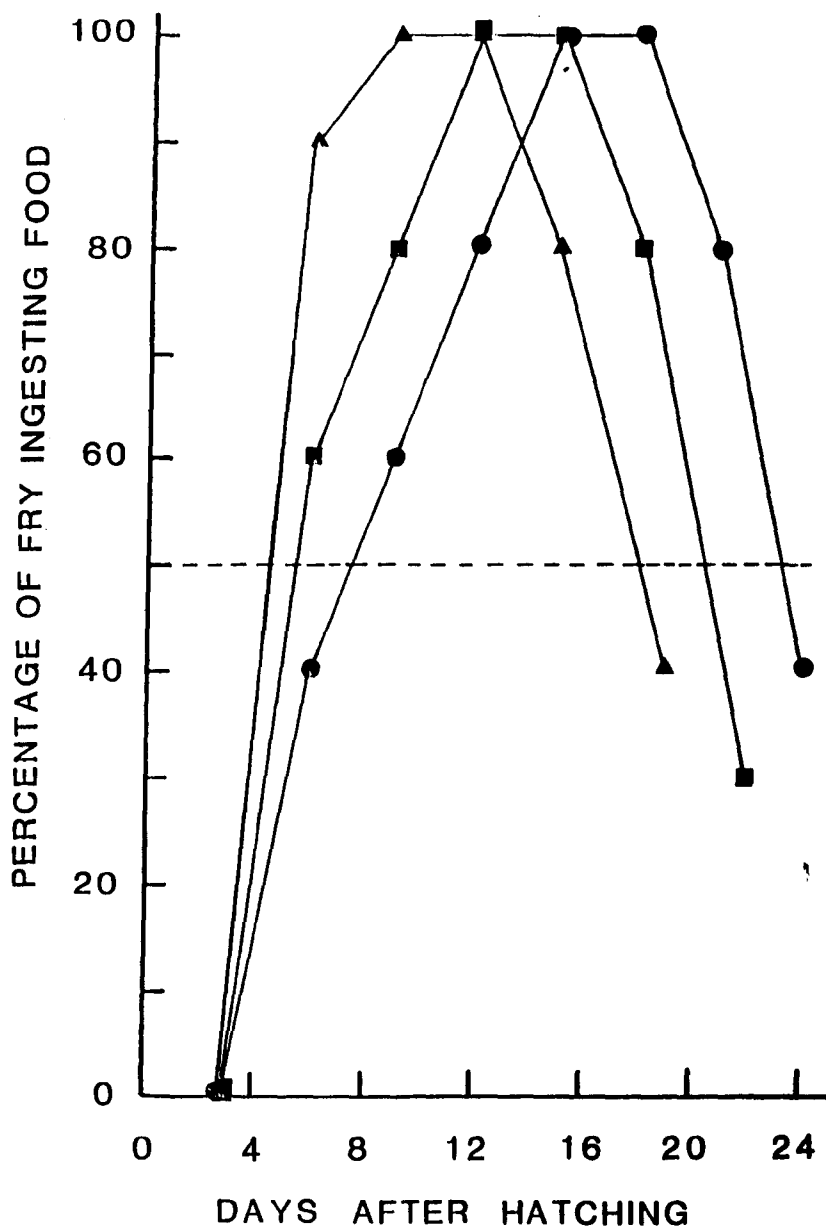


FIGURE 2.20. Effect of rearing temperature on the feeding capabilities of *O. niloticus* fry developing solely on their yolk reserves. ● , 24°C; ■ , 28°C; ▲ , 30°C.

TABLE 2.9

Feeding capabilities of unfed O. niloticus fry¹ developing solely on their yolk reserves at three constant temperatures

	Incubation Temperature (°C)		
	24.0	28.0	30.0
Onset of exogenous feeding ² (days post hatching)	8	5	4
Age at maximal feeding ³ (days post hatching)	15 - 18	12 - 15	9 - 12
Number of fry at maximal feeding ³ (days post hatching)	100	100	100
Point-of-no-return (PNR)	23	20	18

¹Fry originating from Group B eggs

²50% of fry ingesting exogenous food

³Maximum number of fry in sample that are capable of ingesting food

In fry reared at 24°C onset of feeding (50% of fry feeding) occurred at eight days after hatching, double the length of time taken by fry reared at 30°C. Similarly, the period of maximal feeding (maximum number of fry capable of exogenous feeding) of fry reared at 24°C occurred between 15-18 days after hatching compared with 9-12 days for fry reared at 30°C. During maximal feeding 100% of the fry reared at 24°C, 28°C and 30°C were capable of ingesting food. The lowest rearing temperature of 24°C, however, prolonged the point-of-no-return to 23 days compared with 18 days for fry reared at 30°C.

2.2.4 Discussion

2.2.4.1 Influence of rearing temperature on egg survival

The results obtained for O. niloticus support the view of many authors (Lewis, 1965; De Sylva, 1969; Hokanson et al., 1973; Hokanson and Kleiner, 1974; Irvin, 1974; Hassler, 1982) that the thermal tolerance range of fish eggs decreases as their developmental stage progresses.

Eggs fertilized at 28°C and reared at 39.5°C suffered almost immediate heat death, while at 17°C inhibition of complete cytoplasmic migration to the animal pole occurred which also eventually resulted in death. Within this range 100% of eggs sampled at temperatures between 20°C and 34.5°C developed to the two cell stage. Therefore the extreme thermal tolerance limits (TL_0) for zygote development in O. niloticus may lie between 17°C-20°C and 34.5°C-39.5°C. The

subsequent morphological stages, however, showed marked differences in their susceptibility to rearing temperatures (Fig. 2.12). Optimal development (>90%) for all morphological stages took place over a narrower range of between 25°C and 30°C (Table 2.5). Outwith this range fewer eggs progressed from the blastula stage through to the closure of the germ ring.

The sensitivity of eggs to thermal stress (measured as hatching success) was also influenced by the length of time for which they were reared at normal temperatures prior to their incubation at the various test temperatures. When eggs were reared at 28°C for 48 hours before subjection to test temperatures (Group B eggs) hatching occurred over a wider temperature range than in Group A eggs (Fig. 2.13; Table 2.5). In addition, the optimal hatch rate (>90%) for Group B eggs also occurred over a wider temperature range than in Group A eggs (Table 2.5). Maximal hatching was, however, similar in both groups of eggs, occurring at 28°C. Moreover, outwith this temperature the hatch rate of Group B eggs was significantly ($P < 0.05$) higher than Group A eggs (Fig. 2.13), resulting in higher, upper median tolerance limits and lower, lower median tolerance limits (Table 2.5).

These trends are in general agreement with those reported for some temperate freshwater and marine fishes. Hokanson et al. (1973) and Hassler (1982) demonstrated that for northern pike the age, and therefore the development stage, of the embryo before subjection to test temperatures influenced hatching success. When embryos

were allowed to develop for at least five hours at normal temperatures (9°C-12°C) before subjection to test temperatures these authors found hatching success to be higher than in embryos reared for a shorter period at normal temperatures. Similarly, in yellow perch, embryos reared for a longer period at normal temperature (12°C) before subjection to test temperatures, hatching occurred over a wider temperature range (Hokanson and Kleiner, 1974). In contrast, however, Hassler (1982) reported that the age of northern pike prior to rearing at various temperatures did not affect the temperature range of hatching, although higher upper TL₅₀s were found.

The reasons for differential egg mortality at various morphological stages are uncertain. The results obtained for O. niloticus here and those for yellow perch (Hokanson and Kleiner, 1974) and northern pike (Hokanson et al., 1973; Hassler, 1982) suggest that thermal tolerance and hatch rate can be improved by using older embryos reared initially at normal temperatures. Moreover, Hokanson and Kleiner (1974) observed that hatch rate was not significantly affected by gradually (0.5°C-3.0°C/day) raising rearing temperatures.

These differential embryo mortalities may be related to the pattern of gene activity and its temporal expression. There is now firm evidence that in teleosts the mature ovum contains a stockpile of maternal template RNA which is activated and mobilized after fertilization. If this template-directed protein synthesis is blocked, inhibited or disrupted, blastomere cleavage halts abruptly (Davidson, 1968) and the embryo fails to develop. This may have

occurred at extreme rearing temperatures. Furthermore, there is also considerable evidence (see Davidson, 1968) to show that informational RNA synthesised by the embryonic genome during blastula and gastrula stages are largely stored for subsequent utilization. Therefore, it is plausible to suggest that if newly fertilized eggs are subjected immediately to various constant temperatures, successive morphological development will occur for as long as the temperature is within the functional thermal threshold range for RNA and protein synthesis and enzymatic systems. When embryos are first incubated at normal temperatures and then subjected to test temperatures, however, they may acquire a stockpile of RNA, essential enzymes and proteins during the normal rearing phase for later use and therefore may proceed to develop further until the test temperature becomes critical for new biochemical events. This may explain why for the Group B eggs hatching occurred over a wider temperature range.

2.2.4.2 Rates of development and time to hatching

The main objective of this aspect of the study was to establish a working relationship between the rate of embryonic development to hatching and temperature and to identify the optimum temperature range for the incubation of O. niloticus eggs.

The time required for O. niloticus embryos to develop from one stage to another decreased with increasing rearing temperature, a trend also observed in perch, Perca fluviatilis, embryos (Guma'a, 1978). For example, in the present studies closure of the germ ring occurred at 48h, 30h and 26h in eggs reared at 24°C, 28°C and 34.5°C

respectively. For O. mossambicus eggs reared under similar conditions as in the present studies, the stage was attained between 30-36h at 28°C (Rana, unpublished data). Shaw and Aronson (1954), however, rearing S. melanotheron eggs in 40% seawater reported longer times to germ ring closure of 72h and 48-72h for eggs reared at 25°C and 29°C, respectively. However, S. melanotheron is a brackish water fish and can tolerate full-strength seawater, and low salinities have been reported to prolong embryo development (Hempel, 1979). Nussbaum and Chervinsky (1968) also reported longer incubation times to closure of the germ ring of 60 hours in O. niloticus eggs removed from the buccal cavity of the female and reared at 27°C. However, eggs were not removed from the mother before 60 hours and rearing temperature of the brooders was not given.

It is generally accepted that the time to hatching in teleost species decreases exponentially with temperature (Herzig and Winkler, 1986), that is the rate of embryonic development to hatching increases at higher temperatures. Over the temperature range of the present trial (which included the extreme upper and lower thermal limits), however, time to hatching was best described by a linear relationship of negative slope (Fig. 2.15). The rate of development of eggs to hatching, on the other hand, was found to be retarded at lower temperatures and accelerated at higher temperatures (Fig. 2.16). The anomaly of these results may be due in part to the hatching of fry during the night which were not observed and recorded until the next day and to the large temperature interval of 5°C which may have obscured any curvilinear relationship between temperature and hatching time. In view of the lack of sufficient data no

accurate calculation of the minimum theoretical temperature at which development would cease (Biological Zero) and the time to hatching at infinite temperature (D_0) could be made.

The rate of development to hatching based on hatching times however, cannot be assumed to be synonymous with the 'rate of embryonic development' since this assumes that morphological differentiation and the hatching event are inextricably related. There is evidence to suggest that in fish species hatching may not occur at a fixed ontogenic stage and therefore at high and low temperatures the emergent embryos may be at different levels of morphological development (Penaz, 1974; Braum, 1978; Gulidov and Popova, 1981; Herzig and Winkler, 1986). Hatching is, however, thought to be caused by the action of temperature on the secretion of hatching enzymes and on embryonic movement (Schoots et al., 1982). The rate of development based on time to hatching reflects more accurately on the rate at which hatching is approached rather than on the rate of embryonic development.

Over the common hatching temperature range (20°C-34.5°C) of Group A and B eggs, the age of eggs prior to incubation did not influence times to hatching; these being 6 and 2.3 days at 20°C and 34.5°C, respectively. At 17°C the incubation period of eggs fertilized and reared at 28°C for 48 hours before incubation (Group B eggs) was prolonged by 48 hours compared to eggs reared at 20°C. There is a paucity of information on times to hatching for tilapia eggs. Shaw and Aronson (1954) rearing S. melanotheron eggs at 25°C, 29°C and 33°C in 40% seawater reported hatching times of 10, 7 and 6 days, respectively. Lee (1979) rearing 48h old O. aureus eggs on

a shaking table at 18°C reported hatching times of 8 days which is in close agreement with the present study and Rana (1985) rearing O. mossambicus eggs in round-bottomed containers at 28°C reported hatching times of four days. Watanabe, Kuo and Huang (1984), on the other hand, reported hatching times of approximately three days for O. niloticus eggs reared in upwelling containers at 27.5°C-31.5°C.

Hatching times may also be influenced by other factors such as oxygen levels, salinity and pH (Blaxter, 1969; Braum, 1978). In addition, mechanical stress on the eggs may also influence hatching times (Section 2.1.4.2). The time to hatching may also be related to the amount of yolk invested by the parent in eggs. For example, in warm water species such as the Indian major carp, Cirrhinus mrigala, Chinese carps and common carps which produce eggs with a large perivitelline space and a small volume of yolk, hatching occurs within 11-16h and 43h, respectively, at 29°C (Jana, Sarkar and Kundu, 1985). Whereas in O. niloticus eggs which contain a small perivitelline space and a large volume of yolk, hatching occurs within 90-102h at 28°C. Similarly, in temperate water species, the incubatory period of plaice, Pleuronectes platessa, eggs, which have a small perivitelline space and a large yolk, is 10 days at 14°C compared with three to four days at the same temperature for the eggs of the pilchard, Sardina pilchardus, which have a large perivitelline space and only a small volume of yolk (Russell, 1976).

2.2.4.3 Influence of rearing temperature on size of emergent fry

The mean body and yolk weights of emergent fry reared at each test temperature were inversely related (Fig. 2.18).

The trends reported in the literature for the effects of rearing temperature on the size of emergent fry show wide differences between species. In the present study the body weight of emergent fry increased at higher incubation temperatures and reached its optimum at 30°C, then declined. Studies by Alderdice and Forrester (1974) on flathead sole, Hippoglossides elassodon; Hassler (1982) on northern pike, Esox lucius; Lasker (1964) on Pacific sardine, Sardinops caerulea; and Guma'a (1978) on perch, Perca fluviatilis, showed a similar trend in body size of emergent larvae when reared at different temperatures. In contrast, studies on whitefish, Coregonus clupeaformis, (Price, 1940), desert minnow, Cyprinodon macularis, (Kinne and Kinne, 1962) and Pacific cod, Gadus macrocephalus, (Forrester and Alderdice, 1966) have shown that low incubation temperatures produce longer larvae at hatching. It has been suggested that longer bodies in larvae increase swimming speeds and give more economical movement (Braum, 1978).

Additionally, it has been found that oxygen concentration affects the size of emergent larvae. For example, in salmonid eggs, low oxygen levels retard fry development and fry hatch at a smaller size (Silver, Warren and Doudoroff, 1963; Mason, 1969).

The trend observed in the present studies may reflect the embryos' increasing ability to translocate and utilize their yolk reserves more efficiently at higher temperatures. Estimates of gross yolk utilization efficiencies (GYUE %) of emergent fry (Table 2.8) suggest that there may be an optimum temperature range within that normally encountered (24°C-35°C). The high GYUE observed at 20°C may signify a reduced rate of yolk translocation in conjunction with low metabolism but may also be indicative of inactive or moribund fry (Blaxter and Hempel, 1966). At temperatures above 30°C, however, the lower body weight and GYUE observed may be due in part to higher metabolic activity and a relative inability of the embryo to convert yolk into body tissues despite its rapid translocation from within the yolk sac. It should be noted, however, that the GYUE of emergent fry was calculated using egg weight inclusive of chorion as the measure of initial yolk reserves and is therefore an overestimation resulting in an underestimation of GYUE. Nevertheless, since the chorion weight would be expected to be similar for all eggs the relative trend of GYUE of emergent fry at various temperatures will be similar.

2.2.4.4 Survival of fry developing solely on their yolk reserves at various temperatures

Optimal survival (>90%) to the swim-up stage of fry depending on their yolk reserves occurred between 28°C and 30°C. The onset of mortality for fry reared at 24°C, 28°C and 30°C coincided with their suffering complete yolk exhaustion. Due to the higher metabolic demand and accelerated growth rate of these poikilotherms, survival

times (ST_{50}) of fry reared at 28°C and 30°C were approached 4.5 and 7 days earlier, respectively, than for fry reared at 24°C.

Outwith this range mortality was independent of yolk reserves. Mortality of fry reared at 20°C occurred gradually over a prolonged period of 18 days. An examination of moribund fry revealed distended gall bladders, an observation commonly associated with starvation (Talbot and Higgins, 1982). This may imply that yolk reserves in these fry were not mobilized or translocated in sufficient quantities to sustain and maintain the intrinsic rates of various metabolic processes during development. Since the yolk is transported exclusively from within the yolk-sac through a network of blood capillaries and sinuses (Bachop and Schwartz, 1974) the lower heart beat rate at this low temperature may critically limit the quantities of nutrients reaching the developing fry.

At 34.5°C, however, the rapid rate of fry mortality observed was probably due to heat death caused by factors such as protein denaturation, inactivation of enzyme systems and disruption of membrane integrity (Schmidt-Neilson, 1970).

The results obtained in the present study may be compared with those of other authors. Blaxter and Hempel (1966) also observed survival times to be reduced at higher temperatures in herring larvae; being 40 and 20 days at 5°C and 15°C, respectively. Similarly, Ishibashi (1974), rearing T. sparrmanii at 24°C, 27°C and 30°C reported survival times of 12, 10 and 7 days after hatching, respectively.

Survival, however, may also be affected by the interaction of temperature with other environmental conditions such as salinity (Hempel and Blaxter, 1963; Holliday, 1969; Watanabe et al., 1984), ammonia (Rice and Stokes, 1975; Calamari, Marchetti and Vailati, 1981) and pH (Craig and Baksi, 1977; Trojnar, 1977) and also with egg size (see Section 3.2).

2.2.4.5 The effects of rearing temperature on the growth of fry developing solely on their yolk reserves

In order to interpret the effects of temperature on the growth of O. niloticus fry, the utilization of yolk for efficient tissue construction and metabolism should be considered. Metabolism consists of three components: growth metabolism, being the energy required by the fry for chemical transformation of yolk into body tissues; basal metabolism, or the energy required for tissue activity and physio-chemical changes of the resting fry such as respiration; and active metabolism, which provides the energy for movement. For thermal conformers, like O. niloticus these components will increase with both rearing temperature (within thermal tolerance limits) and the size of fry. Higher rearing temperatures, which increase the rates of chemical reactions and increase the growth rate, impose a higher metabolic demand on the yolk reserves. These effects of temperature on growth may be monitored by two methods. The rate of tissue construction (i.e. specific growth rate, %/day) and gross yolk utilization efficiency, both of which are considered below.

By monitoring SGR of fry developing solely on their yolk reserves it may be possible to determine in detail the temporal effects of rearing temperature on growth efficiency. Also, the time at which yolk reserves are first unable to meet the growth and metabolic demands can be accurately determined from SGRs. Gross yolk utilization efficiency (GYUE), however, only measures the efficiency with which the yolk consumed is converted to body tissue but can be misleading in that high values may be due to inactive and moribund fry rather than high efficiency (Blaxter and Hempel, 1966).

The growth rate of O. niloticus fry developing solely on their yolk reserves was accelerated at higher rearing temperatures (Table 2.7). At rearing temperatures below 24°C their growth rate was greatly retarded. Fry reared at 17°C failed to grow during the first three days, while those at 20°C attained a growth rate of only 6.5%/day. At 34.5°C rapid heat death resulted in total fry mortality by day 3 and due to high accumulative mortalities at 17°C and 20°C, growth trials at these temperatures were terminated at 3 and 9 days, respectively.

As the rearing temperature was increased from 24°C to 30°C the total metabolic demand on yolk reserves during the period from hatching to maximum body weight increased more than two-fold from an average of 0.11mg of yolk/day to 0.27mg of yolk/day, respectively, thus drawing more heavily on the finite yolk reserves at higher temperatures. This higher metabolic rate, however, was more than compensated for by the greater efficiency with which body tissues were

assimilated. At 30°C the rate of body growth was more than twice that for fry reared at 24°C (Table 2.7). In addition, since the heart beat and therefore the circulatory rate is also increased at higher temperatures the high growth rate may have also been due in part to the accelerated rate of yolk mobilization and translocation to the embryo. Thus as the rearing temperature was changed from 24°C-30°C the intrinsic growth rate of O. niloticus fry was approached.

Since growth of the fry was solely dependent upon yolk reserves there would come a point when yolk reserves would only be able to meet the basal metabolic demands of the fry, growth would cease and the maximum body weight would have been attained. Due to the accelerated rate of development at 30°C this stage was reached 3 and 12 days earlier in fry reared at this temperature than in fry reared at 28°C and 24°C, respectively. Although the SGRs of fry during the 3-6 day period at 28°C and 30°C were similar, the greater metabolic demands at 30°C exceeded that which could be provided for by the remaining yolk reserves. Hence the metabolic deficit during further development was met by tissue resorption which resulted in concomitant weight loss (Table 2.7). Despite the positive growth rate of the fry body between the 6-9 day period at 28°C the decline in SGR from the 3-6 to the 6-9 day period (Table 2.7) suggests that the yolk reserves were inadequate to meet all the nutritional and metabolic needs for the maintenance of optimal growth rates. Moreover, since the nutritional profile of the yolk constituents may change during development (Monroy, Ishida and Nakano, 1961;

Terner, Kumar and Choe, 1968; Hayes, Tinsley and Lowry, 1973; Atchison, 1975) there may be a shortfall in specific proteins and lipids which may be met by tissue resorption.

The effect of rearing temperature on the efficiency of fry development on their yolk reserves may also be compared by determining the ratio between dry body weight and the dry weight of yolk consumed - GYUE (Blaxter and Hempel, 1966). Using this ratio it was found that the efficiency of development for O. niloticus fry from hatching to the stage of maximal body weight increased with rearing temperature, rising from 55.4% at 24°C to 61.7% at 30°C (Table 2.8). Experiments on the effects of temperature on GYUE in other teleosts indicate an optimum temperature range for efficient development (Blaxter, 1969), a trend not observed here. However, since GYUE was only determined for fry reared at 24°C, 28°C and 30°C and fry reared at 34.5°C suffered total mortality, the optimum efficiency may lie between 30°C and 34.5°C. The GYUE values for O. niloticus in the present study compare favourably with those reported for temperate freshwater and marine species of between 40%-70% (see Blaxter, 1969).

Direct comparisons between O. niloticus and other species, however, should be made with caution since GYUE values may be greatly affected by environmental factors, differences in the developmental stages of fry used, methods of determination and egg size. In trout, Gray (1928) obtained a GYUE of 56% to the end of yolk-sac stage at 15°C using dry weights, while Smith (1957) reported 60% at 10°C. Hayes and Pelluet (1945) rearing salmon, Salmo salar, to the same stage

reported efficiencies to increase from 42% to 59% when reared between 0°C-16°C. While Hollett and Hayes (1946) converting chemical data into calorific values of yolkless fry obtained a GYUE of 41% at 10°C. In herring, the efficiency of development of larvae on their yolk reserves to their maximum dry weight stage varied between 50%-60% at 8°C and was 57% at 12°C (Blaxter and Hempel, 1966). The range at 8°C being due to inter-racial differences.

The development efficiency values given by most of these authors are based on dry weight changes up to the end-of-yolk-sac stage. This may however, lead to an underestimation since body weight may begin to decrease before the end-of-yolk-sac stage. Therefore it may be more accurate to measure GYUE of fry up to their maximum body weight stage.

2.2.4.6 Effects of temperature on the feeding capabilities of previously unfed fry

The feeding capabilities (% of sample capable of exogenous feeding) of O. niloticus fry closely followed their growth pattern. Observations indicated that, in addition to accelerated growth rate at higher rearing temperatures, swim-up stage and neutral buoyancy were attained earlier, an observation also reported for other species (Hayes et al., 1953; Gunnes, 1979). Inflation of the swimbladder to achieve neutral buoyancy is an important prerequisite for successful feeding (Aronovich, Doroshev, Spectorova and Makhotin, 1975; Doroshev, Cornacchia and Hogan, 1981), reducing the energetic costs of swimming, while also improving feeding efficiency. Failure or

delay in inflating the swimbladder can delay exogenous feeding (Aronovich et al., 1975; Doroshev et al., 1981) and depress growth (Von Ledebur and Wunder, 1938, cited by Doroshev et al., 1981). The onset of feeding (50% of sample ingesting food), however, may also be affected by environmental conditions. Studies by Brownell (1980a, 1980b) on larvae of 10 marine species have shown that onset of feeding was inhibited by high pH, low concentrations of dissolved oxygen and unionised ammonia.

In the present study, onset of feeding coincided with the swim-up stage and occurred earlier at higher temperatures (Table 2.9). Onset of feeding occurred at 4, 5 and 8 days after hatching when reared at 30°C, 28°C and 24°C, respectively (Fig. 2.20, Table 2.9). Further, the steeper slopes of the feeding capability curves of fry reared at higher temperatures shown in Fig. 2.20 indicate that maximal feeding (maximum number in sample ingesting food) was attained at a higher rate at these temperatures. Consequently, maximal feeding occurred between 9-12 days after hatching at 30°C compared with 15-18 days at 24°C (Table 2.9). These values may be compared with results of other authors. Ishibashi (1974) rearing T. sparrmanii hatchlings at 30°C, 27°C and 24°C reported 'first-feeding' times of 2, 3 and 4 days, respectively, while Bogdanova (1970) and Rana (1985) rearing O. mossambicus at 24°C-26°C and 28°C, respectively, observed feeding on day 7 and day 6. Hallerman, Smitherman and Dunham (1983) however, rearing O. aureus and O. niloticus fry at 29°C reported "swim-up stage and exogenous feeding" at 9 days after hatching, i.e. twice the length of time

observed in the present study for O. niloticus, although the swim-bladder was reported to be developed 5-6 days after hatching. However, Hallerman et al. (1983) do not define their use of the term 'exogenous feeding' and may therefore be different to that used in the present study. The lower ages of fry at first-feeding reported by Ishibashi (1974) for T. sparrmanii were probably related to the smaller egg sizes and earlier developmental times of the Tilapias (Philippart and Ruwet, 1982).

Exogenous feeding generally commences before the yolk is fully absorbed (Blaxter, 1969; Bogdanova, 1970; Aronovich et al., 1975; MacCrimmon and Twongo, 1980; Doroshev et al., 1981; Rana, 1985) as also found in the present study. For O. niloticus here, onset of feeding occurred at 10, 4 and 2 days before their maximal body weight was reached when reared at 24°C, 28°C and 30°C, respectively, while maximal feeding was established 3 days before maximal body weight was achieved at 24°C and 3 days after at 28°C and 30°C. These results may be explained by considering the SGR, which was determined at three day intervals, rather than fry body weight. At 24°C, 28°C and 30°C onset of feeding by fry coincided with their maximal growth rate (Table 2.7 and 2.9). Feeding by fry from this point onwards, at the higher temperatures, may help to meet the immediate nutritional deficit of the waning yolk reserves. Maximal feeding, however, coincides with the period of starvation (weight loss) probably reflecting the pressing need to feed in order to avoid irreversible starvation.

In the present studies, the point of irreversible starvation or point-of-no-return (PNR) was reached within 18 days of hatching in fry reared at 30°C compared with 23 days at 24°C. This shortening in the period to PNR with increasing rearing temperature is a trend also reported by Ishibashi (1974) for T. sparrmanii and by Blaxter and Hempel (1963) for herring, C. harengus. The period to irreversible starvation may, however, also be influenced by factors such as salinity (Blaxter, 1969) and egg size (Blaxter and Hempel, 1963; Rana, 1985). Under some conditions the feeding capabilities and PNR of the fry may be prolonged by behavioural changes. The reduction in swimming activity observed in fry reared at 24°C, for example, may help to reduce the rate of tissue resorption, allowing the fry to maintain their physiological integrity for a longer period. Furthermore, under starvation conditions, fry may actively move to areas of lower temperature which may help to conserve energy by reducing their metabolic demands (Ehrlich and Muszynski, 1981).

CHAPTER 3

REPRODUCTIVE TRAITS OF THREE AGE-CLASSES OF HATCHERY
REARED OREOCHROMIS BROODFISH AND THE QUALITY OF THEIR
EGGS AND FRY

3.1 The Influence of Broodfish Age and Size on their Reproductive Performance

3.1.1 Introduction

In tilapias the evolution of parental care has led to an increase in egg size and a corresponding reduction in the number of eggs per clutch (Noakes and Balon, 1982). The low number of eggs per spawn together with the asynchronous spawning behaviour of O. niloticus and O. mossambicus under hatchery conditions (Mires, 1982; personal observation) would necessitate the maintenance of large numbers of broodstock which may incur high feed, space and labour costs. These requirements, however, may be reduced by selecting for and using broodstock showing optimal reproductive traits such as egg size, fecundity, clutch weight and egg to body weight ratio.

One objective of the hatchery fry producer is to produce fry of uniform size. The degree of uniformity, however, will depend in part, on the variability in egg size within ovaries, and between spawners and with the genetic stocks and strains used (Bagenal, 1971). Information for tilapias on the degree of variability in egg size within spawns of individual females is meagre; an extensive literature search only revealed such information for O. mossambicus (Peters, 1983).

The reproductive productivity of broodstock, however, will depend on their nutritional status (Wootton, 1979; Townshend and Wootton, 1984; Watanabe, Itoh, Kitajima and Fujita, 1984), spawning frequency and the numbers of eggs shed per spawning.

In addition, the number of eggs produced from a given ovarian biomass will be inversely related to egg size. In marine and freshwater fish species the increase in egg numbers and size with maternal age and size is well documented (see Mann and Mills, 1979; Wootton, 1979; Hislop, 1984; Thorpe et al., 1984). For the tilapias this increase in egg size and number with female size has been reported by many authors (Lowe (McConnell), 1955; Peters, 1959; Riedel, 1965; Welcomme, 1967; Botros, 1969; Dadzie, 1970b; Marshall, 1979; Siraj et al., 1983). Much of this information, however, is based on ovarian egg counts, and where spawned eggs were used most authors relied on seine and gill-netted specimens where partial ejection of the clutch during capture of the brooder could easily occur.

In practice, success in determining the number of Oreochromis broodstock required to meet a target production of fry will at least in part, be due to a knowledge of their reproductive traits.

Fecundity is usually defined as the number of maturing oocytes present in the ovaries of females prior to spawning (Bagenal, 1978; Bagenal and Braum, 1978). This classical definition, however, is only of value in determining the reproductive potential of fish species which have a well-defined annual spawning season. The mature ovaries of such species contain mature oocytes for the current year's spawn and undifferentiated oocytes for the next spawning season. In multiple-spawners such as the tilapias, however, the recruitment of oocytes for maturation appears to be more complex. Histological examination of 'ripening tilapia ovaries' reveals that the size of the maturing intra-ovarian oocytes exhibits a multimodal

distribution, which results in plurimodal ovarian development (Peters, 1983). That is, an ovary which has two or more groups of oocytes maturing at the same time, the largest of which finally ovulates. Siddiqui (1977) and Peters (1983) provided histological evidence to show that only in 'ripe tilapia ovaries' is the size distribution of oocytes distinctly bimodal. On this basis they suggested that the potential number of eggs in a clutch can be determined well in advance of spawning. However, the difficulty in separating the current spawn from the next group of developing oocytes in tilapia ovaries and the presence of atretic and degenerating eggs in the ovary (Peters, 1983) may lead to inaccurate measurements (often overestimates) of fecundity. Hence the classical definition of fecundity is inappropriate for this study on the reproductive potential of O. niloticus and O. mossambicus and for any such studies in the tilapias generally. Nevertheless, many authors (Riedel, 1965; Welcomme, 1967; Botros, 1969; Siddiqui, 1977; Marshall, 1979; Payne and Collinson, 1983) used intra-ovarian egg counts to determine fecundity without first (a) establishing the bi-modality of oocyte size distribution, or (b) providing a consistent criterion for distinguishing 'ripe eggs'.

Various alternative definitions of fecundity for tilapia species have been proposed. Lowe (McConnell) (1955) suggested that fecundity should represent the total number of fry produced during the lifetime of the parent. Under culture conditions, however, tilapia broodstock ideally would be used only during their optimal reproductive period and therefore this definition would be unsatisfactory. Consequently, Mires (1982) suggested that fecundity should be

restricted to a period of 12 months. Due to the parental care exhibited by mouth-brooders, Bagenal (1978) suggested that both 'ovarian fecundity' and 'brooding fecundity' (number of fry in buccal cavity) should be considered and suggested that under non-seasonal spawning conditions fecundity should be related to one egg clutch.

The reliability of using brooding fecundity as a measure of reproductive potential is also questionable. Estimates of fecundity based on fry counts rely heavily on seine and gill-netted specimens (Lowe (McConnell), 1955; Welcomme, 1967; Marshall, 1979). In these conditions, especially in the final phase of buccal rearing, the clutch may be partially expelled by the parent, although Welcomme (1967) assumed that "fry were not ejected during catching". Furthermore, the numbers of fry reared in the oral cavity, even under controlled conditions, are considerably below values from ovarian and direct egg counts (Lowe (McConnell), 1955; Riedel, 1965; Welcomme, 1967). Consequently, the reproductive potential may be underestimated. Moreover, even though fry counts may be a more useful criterion for the hatchery operator it may be presumptuous to assume that the relationship between egg number and numbers of fry released is linear. Welcomme (1967) showed that the relationship between the ratio of fry brooded to eggs produced (i.e. brooding efficiency) and parent size in T. leucosticta (= O. leucostictus, Trewavas, 1983) was not linear. Also, there may be behavioural and environmental factors which may alter this relationship (see Chapter 4).

Ideally fecundity should be estimated by egg counts from freshly spawned clutches. The fecundity of individual females can then

be expressed either as the total number of eggs, i.e. as absolute or total fecundity, or as the number of eggs per unit body weight, i.e. as relative fecundity. These fecundity traits together with clutch weight and egg to body weight ratio can then be related to maternal age, weight and length to establish which of these growth parameters exerts the most influence on the predictability and variation in their reproductive traits.

The aims of this part of the study were, for O. niloticus and O. mossambicus, to:

- (1) obtain a measure of the variability of egg size within individual spawns;
- (2) determine the spawning frequency of females;
- (3) estimate the variation in the reproductive traits between females from different age-classes, individual females and consecutive spawnings of the same individual;
- (4) investigate the association between reproductive traits with length, and weight in females of a similar age and in the mixed age structure;
- (5) establish a simple model to show any interacting influence of growth parameters on the predictability of the reproductive traits.

3.1.2 Materials and Methods

3.1.2.1 The origin and rearing of broodstock

Oreochromis niloticus and O. mossambicus species were obtained from genetically pure stocks, held at the Institute of Aquaculture, University of Stirling. Each species was originally obtained from single strains, O. niloticus from Egypt and O. mossambicus from Singapore (McAndrew, personal communication).

To obtain broodstock representative of a heterogenous gene-pool and of a known reproductive history, fry from many females were pooled and reared to sexual maturity. Juveniles (5-10g) of known age were stocked at 5/l in 1m² grey glass-fibre tanks. They were fed a commercial trout diet (No. 3 and No. 4 pellets, Edward Baker, Bathgate, Scotland) three times daily at 5% body weight/day. Food ration was gradually decreased to 2% body weight/day at sexual maturity.

To maintain females of known spawning history the broodstock were hand sexed fortnightly to remove males. To exclude the possibility of sib-matings in later trials these males were discarded. To discourage spawning in the absence of males, females were stocked at 0.5/l (60 fish/tank) as spawning is inhibited by high stocking density (Balarin and Haller, 1982). Males and females used in these studies were reared from fry originating from different parents.

Three age groups of females were reared: 0+, 1+ and 2+ age-classes.

Females from these age-classes were used throughout the remainder of the study.

3.1.2.2 Spawning of broodfish

Oreochromis niloticus and O. mossambicus females were selected randomly from their respective holding tanks and stocked at a ratio of 3 females : 1 male in 1m² and 2m diameter spawning tanks. The age of females during this trial, representing the 0+, 1+ and 2+ age-classes, ranged from 5-7 months, 11-14 months and 20-26 months for O. niloticus, and 5-8 months, 14-16 months and 18-23 months for O. mossambicus, respectively.

To facilitate recognition of fish individually, females were injected subcutaneously with alcian blue dye and identified with numbered floy tags. The fish were fed ad libitum three times daily on a commercial trout diet containing 40% crude protein (No. 4 pellet, Edward Baker, Bathgate, Scotland).

The spawning tanks were checked twice daily, i.e. early morning and late afternoon, for the presence of spawning or brooding females.

3.1.2.3 Sampling procedure for eggs

Brooding females were caught gently with a net from the spawning tanks. The eggs were flushed from the mouth into a clean container and the mouth re-opened to inspect the oral cavity for remaining eggs. The abdomen was then gently squeezed in the anteroposterior

direction to check for residual eggs in the ovary. The identification number of the fish was recorded and its standard length and weight was measured before returning it to the spawning tank. This procedure was repeated until at least five females from each age-class had spawned at least three times each.

The lengths (L) and heights (H) of 30 randomly sampled eggs from freshly spawned egg clutches were measured under a calibrated binocular microscope. Egg volumes were then calculated from each clutch examined using the formula, $V = \frac{\pi}{6} LH^2$, which describes the volume of an ellipse.

To estimate the number of eggs in a clutch a preliminary trial was conducted to compare the accuracy of direct enumeration with an indirect method using egg biomass. Eggs from four clutches were pooled and then divided into five groups. The number of eggs in each group was then counted three times. Fifty randomly sampled eggs from each group and the remainder of the eggs in each group were dried on absorbant paper, weighed and oven-dried overnight at 50°C. The eggs were then cooled to room temperature in a desiccator and re-weighed three times to an accuracy of ± 0.1 mg on a top pan balance (Mettler H80). The number of eggs in each group was re-calculated by dividing the total egg weight of each group by the mean egg weight of each group. Statistical comparisons between the direct and indirect egg counts were made using Student's 't' test and showed that indirect egg counts were as accurate ($P > 0.95$) as direct counts. The quicker indirect method was therefore used for egg enumeration.

3.1.2.4 Biometrics

To estimate the variation of egg size within spawns, the coefficient of variation (CV%) was calculated using the following relationship:

$$CV(\%) = \frac{\text{standard deviation}}{\text{mean egg volume}} \times 100$$

To obtain the best linear relationship between the growth parameters and the reproductive traits various transformation models were tested. Transformations using natural logarithms of both variates gave the best predictability and were therefore used in all correlation and regression analyses. A correlation matrix was constructed using a computerised statistical package (Minitab, Pennsylvania State University) in order to investigate the degree of association between female age, length and weight with mean egg size, absolute and relative fecundity, total clutch weight and egg to body weight ratio. In addition, stepwise regression analyses were carried out on each reproductive trait (using natural logarithmic transformation of data) to ascertain if the predictability of the reproductive traits could be improved by considering more than one growth parameter in the predictability model. The significance of the improvement was compared using an 'F' test.

To elucidate the influence of female age on their reproductive traits, a two-way nested analysis of variance was carried out on the data. Since the age-class was predetermined and therefore non-random, the least significant difference method (Steel and Torrie, 1980) was used to test for differences in reproductive traits between the three age-classes. Since the females representing each age-

class were chosen at random, Duncan's (1955) multiple range test was selected to test for the differences in the reproductive traits between females of the same age-class. Further, the relative expected magnitudes of the variance components were calculated according to Sokal and Rohlf (1969 - p263).

3.1.3 Results

3.1.3.1 Variation in egg size within individual egg clutches

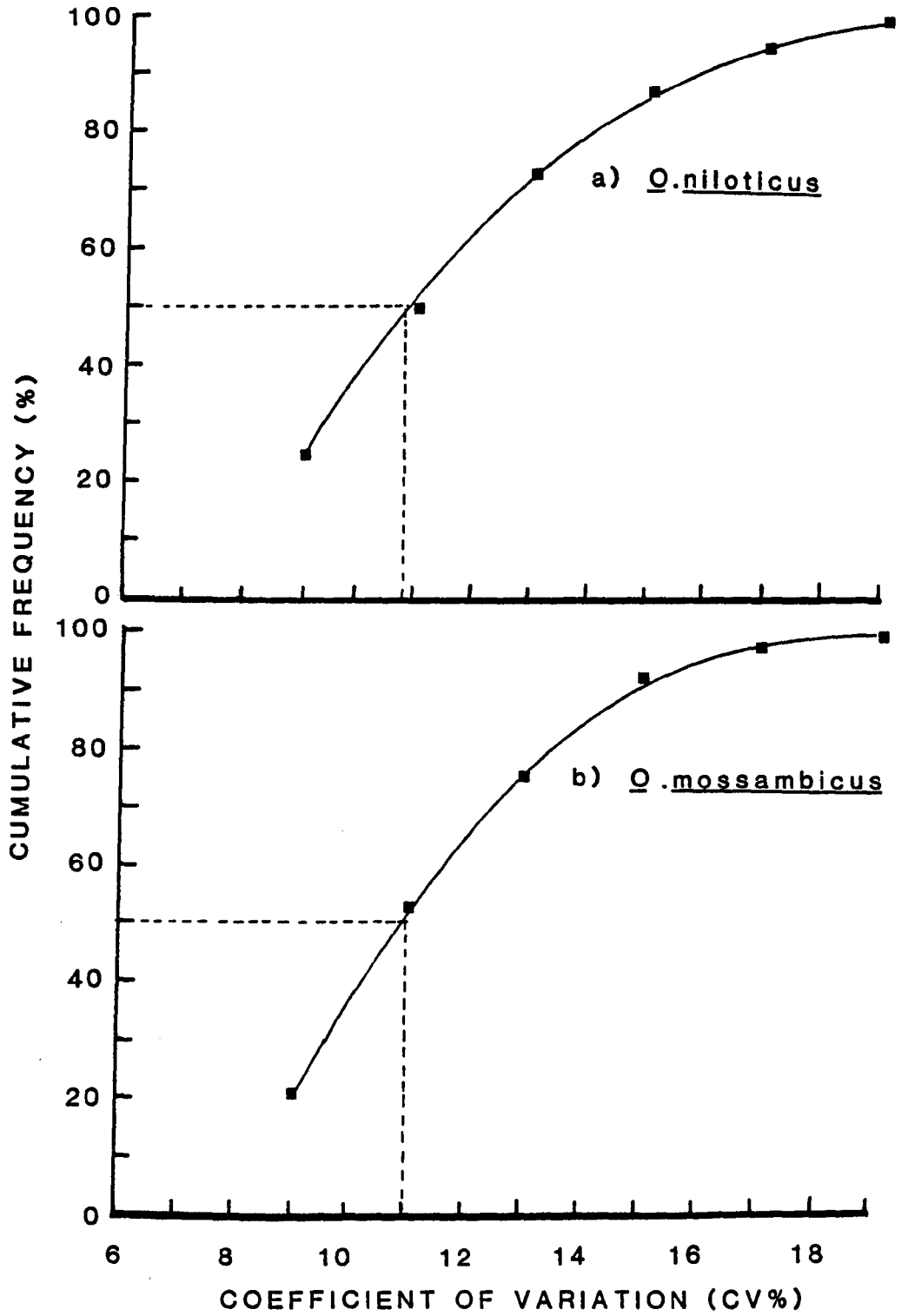
The cumulative frequency distribution of the average variation in egg size (measured as egg volume) within individual spawns, expressed as a percentage of the mean (CV%) is presented in Fig. 3.1. In both species, individual egg sizes within clutches were normally distributed about the mean. The coefficient of variation of egg size between egg clutches, however, was asymmetrically distributed. From Fig. 3.1 it can be seen that in 75% of the O. niloticus and O. mossambicus clutches examined the average intra-clutch variation in egg size expressed as a percentage of their mean was less than 13.5% and 13%, respectively; the median and mean being approximately 11% for both species.

3.1.3.2 Variation in egg size between females

Mean dry egg weights between all three age-classes of O. niloticus females and between 0+, and 1+ and 2+ O. mossambicus females were significantly different ($P < 0.05$); the yearling females produced the smallest eggs (Table 3.1).

FIGURE 3.1

Cumulative frequency distribution of the variation in egg size within egg clutches from (a) Q. niloticus, and (b) Q. mossambicus females. Dotted lines show the median values. Frequency distributions are based on (a) 43 clutches from 23 females, and (b) 38 clutches from 15 females.



For O. niloticus and O. mossambicus the expected variation in mean egg size between consecutive spawnings of individual females was low and accounted for only 7% and 15% of the total variation in egg size, respectively (Table 3.3). In O. niloticus this low variation together with the small variation in egg size between females resulted in age-class accounting for 80% of the total variation in egg size. This value, however, was 53% for O. mossambicus due to the higher egg-size variation between females of this species.

In both species, the degree of association between length (Table 3.4) or weight (Table 3.5) of females of similar age and their mean egg size was insignificant ($P > 0.05$). When the data for females from all three age-classes were combined however, mean dry egg weight was highly correlated with maternal age, weight and length (Table 3.6). Nevertheless, as can be seen from the regression analysis data in Table 3.6, age explained the highest proportion of the total variation in egg size while maternal length was the poorest predictor.

The predominant effect of maternal age on egg size was also borne out when more than one growth parameter was used to improve the predictability of egg size. The addition of either weight or length to age decreased the residual variance by only 1-3% (Tables 3.6 and 3.7).

3.1.3.3 Total fecundity and relative fecundity

In O. niloticus, total fecundity (number of eggs per spawn) and relative fecundity (number of eggs/Kg ϕ) between 0+, and 1+ and 2+ females showed significant ($P < 0.05$) differences (Table 3.1), whereas in O. mossambicus even though mean total fecundity increased with age-class the rise was insignificant ($P > 0.05$) (Table 3.1). The differences between species were largely due to the pattern of distribution of the total expected variation (see Table 3.3). For O. niloticus the expected variation in total and relative fecundity attributable to differences between consecutive spawnings of individual females, and between females within the same age-classes, was considerably lower than for O. mossambicus. In O. niloticus 64% and 68% of the expected variation in total and relatively fecundity, respectively, was accounted for by age-class. In O. mossambicus, however, the expected variation in total fecundity between females within the same age-class accounted for 75.5% of the total variation, resulting in insignificant differences ($P > 0.05$) between age-classes.

The increase in the number of eggs shed per spawning by O. niloticus and O. mossambicus females of similar age, was significantly correlated with body length (Table 3.4) and weight (Table 3.5). In both species, no such closeness of association was observed for relative fecundity.

Overall, total and relative fecundity of both species increased significantly ($P < 0.01$) and decreased significantly ($P < 0.01$), respectively, with either maternal age, weight or length (Table 3.6).

TABLE 3.1

Summary of mean reproductive traits of O. niloticus and O. mossambicus females from 0+, 1+ and 2+ age-classes

Species	Age-class (months)	Female weight range (g)	Reproductive traits ^{1,2,3}				
			Mean dry egg weight (mg)	Total fecundity (no.egg/spawn)	Relative fecundity (no.egg/Kg♀)	Clutch wet weight (g)	Egg to body weight ratio (%)
<u>O. niloticus</u>							
	0+ (5-7)	28-90	1.75 ^a	309 ^a	8606 ^a	1.18 ^a	3.25 ^a
	1+ (11-13)	162-206	2.72 ^b	915 ^b	4920 ^b	5.21 ^b	2.75 ^a
	2+ (23-26)	180-498	3.61 ^c	1158 ^b	3339 ^b	9.15 ^c	2.71 ^a
	Overall range (n=93) ⁴	16-498	1.39-4.93	121-2030	1146-13518	0.51-17.52	1.10-5.50
	Overall mean (n=93) ⁴		2.78	762	5200	5.00	2.80
<u>O. mossambicus</u>							
	0+ (5-8)	25-98	1.44 ^a	603 ^a	10216 ^a	2.00 ^a	3.39 ^a
	1+ (15-16)	110-162	2.16 ^b	646 ^a	5566 ^b	3.16 ^b	2.74 ^a
	2+ (18-23)	167-266	2.37 ^b	856 ^a	4720 ^b	4.30 ^b	2.39 ^a
	Overall range (n=74) ⁴	25-271	0.71-3.20	123-1460	1080-15655	0.42-6.73	0.6-6.5
	Overall mean (n=74) ⁴		1.89	642	7665	2.80	3.1

¹ Means of 3 consecutive spawnings.

² Figures in same column with different superscripts are significantly different (P < 0.05)

³ For mean traits of individual females see Appendices 1 and 2

⁴ Overall range based on total number of observations in the study

TABLE 3.2

Analysis of variance showing the effects of variability within and between age-classes on the mean reproductive traits of O. niloticus and O. mossambicus females

Species	Source of Variation	Degrees of freedom	Reproductive trait (mean sums of squares) ^{1,2}				
			Mean dry egg weight (mg)	Total fecundity (no.egg/spawn)	Relative fecundity (no.eggs/Kg♀)	Clutch wet weight (g)	Egg to body weight ratio (%)
<u>O. niloticus</u>							
	Age-class	2	15.47**	3.45 x 10 ⁶ ***	1.32 ⁸ x 10 ⁸ ***	286***	1.67
	Age x female	15	0.499***	1.97 x 10 ⁵ ***	5.50 x 10 ⁶ **	7.67**	1.15*
	Age x female x spawning	36	0.069	5.13 x 10 ⁴	2.20 x 10 ⁶	3.13	4.99
<u>O. mossambicus</u>							
	Age-class	2	3.05**	2.7 x 10 ⁵	1.31 x 10 ⁸ ***	19.8*	3.90
	Age x female	12	0.310***	2.28 x 10 ⁵ ***	1.10 x 10 ⁷ ***	4.19***	2.70***
	Age x female x spawning	30	0.044	2.03 x 10 ⁴	3.10 x 10 ⁶	0.34	3.45

1

- * P < 0.05
- ** P < 0.01
- *** P < 0.001

2

For differences between age-classes and individual females see Appendices 1 and 2

TABLE 3.3

Expected variation in the mean reproductive traits within and between 0+, 1+ and 2+ age-classes of O. niloticus and O. mossambicus females

Species	Reproductive trait	Expected variance components (% of total) ¹		
		Among age-classes	Among females within age-classes	Between 3 consecutive spawnings by individuals
<u>O. niloticus</u>				
	Mean dry egg weight	79.6	13.7	6.7
	Total fecundity	64.4	17.3	18.3
	Relative fecundity	68.1	10.6	21.3
	Clutch wet weight	66.9	19.6	13.5
	Egg:Body weight	3.9	29.0	67.1
<u>O. mossambicus</u>				
	Mean dry egg weight	53.3	31.3	15.4
	Total fecundity	2.8	75.5	22.1
	Relative fecundity	54.1	21.6	24.3
	Clutch weight	34.8	51.4	13.8
	Egg:Body weight	5.6	65.6	28.8

¹ Calculations of expected variances based on Sokal and Rohlf (1969) - Page 260-263

TABLE 3.4

The degree of association (r)¹ between mean reproductive traits and body length for O. niloticus and O. mossambicus females of similar age

Age ²	Reproductive trait ³					
	Female length (cm)	Mean dry egg weight	Total fecundity	Relative fecundity	Clutch weight	Egg to body weight ratio
0+						
<u>O. niloticus</u> (24)	7-12	-0.068	0.746***	-0.69	0.819***	0.158
<u>O. mossambicus</u> (32)	9-14	-0.038	0.644***	0.119	0.594***	0.105
1+						
<u>O. niloticus</u> (24)	16-19	0.007	0.448*	0.114	0.653***	0.208
<u>O. mossambicus</u> (20)	14-17	-0.189	0.443*	-0.141	0.656***	-0.154
2+						
<u>O. niloticus</u> (14)	17-25	-0.121	0.519*	0.007	0.499*	-0.103
<u>O. mossambicus</u> (14)	17-20	-0.112	0.645**	0.101	0.559*	0.010

¹ Correlation analysis based on natural logarithmic transformation of both variates

² Values in parentheses indicate number of data points used

³ Level of significance of associations

* P < 0.05 *** P < 0.001
 ** P < 0.01

TABLE 3.5

The degree of association (r)¹ between mean reproductive traits and body weight for O. niloticus and O. mossambicus females of similar age

Age ²	Reproductive trait						
	Female weight (g)	Mean dry egg weight	Total fecundity	Relative fecundity	Clutch wet weight	Egg to body weight ratio	
0+							
<u>O. niloticus</u> (24)	26-70	0.061	0.690***	-0.164	0.800***	0.105	
<u>O. mossambicus</u> (32)	25-103	-0.029	0.674***	0.134	0.622***	0.118	
1+							
<u>O. niloticus</u> (24)	158-212	0.031	0.386*	-0.027	0.585**	0.041	
<u>O. mossambicus</u> (20)	107-172	0.128	0.393*	-0.020	0.689***	0.134	
2+							
<u>O. niloticus</u> (14)	180-498	0.091	0.519**	0.007	0.431*	-0.093	
<u>O. mossambicus</u> (14)	155-270	-0.047	0.512**	-0.112	0.437*	-0.158	

¹ Correlation analysis based on natural logarithmic transformation of both variates

² Values in parentheses indicate number of data points used

³ Level of significance of associations

* P < 0.05

** P < 0.01

*** P < 0.001

TABLE 3.6

Intercepts (a), regression coefficients (b) and coefficients of determination (r^2) of the regression analysis¹ for reproductive traits in *O. niloticus* and *O. mossambicus*

Species	Reproductive trait (Y)	Intercept (a) ²			Regression coefficient (b) ²			Coeffs. of determin. (r^2) ³		
		Broodstock		Standard length(cm)	Broodstock (X)		Standard length(cm)	Age (months)	Broodstock Weight (g)	Standard length(cm)
Age (months)	Weight (g)	Age (months)	Weight (g)							
<i>O. niloticus</i> ⁴	Mean dry egg weight (mg)	-0.305±0.161	-0.511±0.201	-1.481±0.344	0.509±0.064	0.299±0.040	0.888±0.123	73.9	70.9	68.6
	Total fecundity (no. eggs/spawn)	4.460±0.430	3.674±0.400	1.674±0.633	0.801±0.167	0.564±0.080	1.737±0.227	49.4	68.4	71.4
	Relative fecundity (no. eggs/Kg ♀)	10.344±0.313	10.582±0.399	11.926±0.692	-0.766±0.122	-0.436±0.079	-1.268±0.249	62.7	56.3	52.4
	Clutch wet weight (g)	-2.013±0.435	-2.987±0.362	-5.974±0.573	1.332±0.170	0.871±0.072	2.645±0.206	72.5	86.3	87.6
	Egg:Body weight ratio (%)	1.570±0.302	1.618±0.362	1.975±0.607	-0.235±0.117	-0.129±0.072	-0.361±0.219	13.8	11.3	9.6
<i>O. mossambicus</i> ⁴	Mean dry egg weight (mg)	-0.441±0.025	-0.668±0.040	-1.628±0.067	0.416±0.009	0.287±0.009	0.858±0.026	50.4	35.0	37.6
	Total fecundity (no. eggs/spawn)	5.490±0.613	4.603±0.083	3.504±0.142	0.348±0.239	0.397±0.019	1.100±0.054	19.2	39.0	39.6
	Relative fecundity (no. eggs/Kg ♀)	10.724±0.057	11.511±0.834	13.420±0.140	-0.752±0.022	-0.603±0.189	-1.75 ±0.053	37.9	35.9	36.7
	Clutch wet weight (g)	-0.987±0.063	-2.133±0.085	-4.177±0.145	0.744±0.025	0.680±0.019	1.945±0.056	32.7	40.6	39.7
	Egg:Body weight ratio (%)	1.944±0.060	2.472±0.085	3.438±0.144	-0.356±0.023	-0.320±0.019	-0.917±0.055	10.1	12.4	12.1

¹ Regression analysis based on natural logarithmic transformed data: $\text{Log } Y = a + b \text{ Log } X$. $n = 93$ and 74 for *O. niloticus* and *O. mossambicus*, respectively, using a total of 36 and 29 individual females

² 't' ratio for all 'a' and 'b' values were significant at $P < 0.05$. 'a' and 'b' values given with their 95% confidence limits

³ r^2 values for all regression equations were significant at $P < 0.01$. Correlation coefficient (r) = $\sqrt{\frac{r^2(\%)}{100}}$

⁴ For the correlation matrix of growth and reproductive traits see Appendices 3 and 4

TABLE 3.7

The influence of combinations of growth parameters on the coefficient of determination (r^2) for O. niloticus and O. mossambicus reproductive traits

Species	Reproductive trait	n	COEF. OF DETERMINATION ($r^2\%$) ¹			
			Age & Weight	Age & Length	Length & Wt.	Age, Wt. & Length
<u>O. niloticus</u>						
	Mean dry egg weight	93	75.1	72.9	74.9	75.6
	Total fecundity	93	73.4	75.1	76.2	77.0
	Relative fecundity	93	63.2	65.6	63.2	68.1
	Clutch wet weight	93	86.9	87.9	87.9	88.0
	Egg to body wt. ratio	93	14.8	21.7	15.4	22.0
<u>O. mossambicus</u>						
	Mean dry egg weight	74	52.3	39.7	52.5	52.6
	Total fecundity	74	23.9	41.4	42.8	43.9
	Relative fecundity	74	39.8	37.6	39.6	39.8
	Clutch wet weight	74	41.4	43.3	40.7	41.4
	Egg to body wt. ratio	74	13.6	13.6	13.3	13.6

$$^1 \text{ Correlation coefficient (r)} = \frac{\sqrt{r^2 (\%)}}{100}$$

For total fecundity length explained the highest proportion of the total variation and age the lowest proportion of the total variation, whilst for relative fecundity age explained the highest and length the lowest proportion of total variation (Table 3.6). The greater influence of length on total fecundity and of age on relative fecundity was also observed when all three growth parameters were considered together (Table 3.7). For example, in O. niloticus the addition of age and weight to length in the regression model helped to explain only a further 5.6% of the residual variance of total fecundity, whilst the addition of weight and length to age decreased the unexplained variance of relative fecundity by only 5.4% (Tables 3.6 and 3.7).

3.1.3.4 Clutch weight

The trends between clutch weight (wet weight) and the growth parameters are given in Table 3.6. In both species they were similar to the trends in fecundity. In O. niloticus the low expected variation in clutch weight between consecutive spawnings of individual females and between females within each age-class resulted in age-class accounting for 67% of the total variation (Table 3.3). This resulted in clutch weight being significantly different ($P < 0.05$) between all three age-groups of fish (Table 3.1). In O. mossambicus the higher variation between females within age-classes resulted in only 0+, and 1+ and 2+ females being significantly ($P < 0.05$) different (Table 3.1).

In both species the increase in clutch weight with body length (Table 3.4) and weight (Table 3.5) in females of similar age was highly

correlated. Overall, clutch weight increased most closely with female length and weight (Table 3.6). The addition of either length or weight to age in the regression model, however, significantly ($P < 0.05$) increased the predictability of clutch weight.

3.1.3.5 Egg to body weight ratio (EW:BW)

Due to the large expected variations between consecutive spawnings by individuals and between females within the same age-class (Table 3.3), there was no significant ($P > 0.05$) age effect on EW:BW (Table 3.1). In addition, the body weight and length of females of similar age had no effect on EW:BW. For the combined data of the mixed age structure, however, there was a small but significant ($P < 0.01$) decrease in EW:BW with age, weight and length in both species (Table 3.6). For O. niloticus the predictability of EW:BW was improved significantly ($P < 0.05$) by considering age and length or age, weight and length. This, however, was not the case for O. mossambicus (see Tables 3.6 and 3.7).

3.1.4 Discussion

3.1.4.1 Egg size

In species which lay eggs containing small volumes of yolk and show no parental care the period from fertilization to exogenous feeding is short in comparison to those species which produce large eggs and exhibit parental care. For example, the physiological age ($-^{\circ}\text{C}$ days) at exogenous feeding in grass carp, Ctenopharyngodon idella, (reared at 28° - 31°C) (Shireman and Smith, 1983), in grouper, Epinephelus tauvina, (reared at 27°C) (Chen, show, Chao and Lim, 1977) and in red sea bream, Chrysophrys major, (reared at 15.5°C) (Kittaka, 1977) which produce 0.90 - 1.15mm^3 (unhydrated), 0.52mm^3 and 0.52 - 0.70mm^3 eggs, respectively, was 78° - 84°C days, 108°C days and 99°C days. In the mouth-brooder O. niloticus (reared at 28°C) which produce 2.85 - 11.15mm^3 eggs, however, feeding was found to commence at 249° - 260°C days. These older physiological ages at exogenous feeding of fry in mouth-brooders together with the variability in release times of fry will increase the dependence of fry on their yolk reserves. Therefore egg size and egg-size variation may be of importance to the quality of the subsequent fry under natural and hatchery conditions.

Information on the degree of variation in egg size within individual fish spawns is meagre. Blaxter and Hempel (1963) reported a range of 33-200% for herring, Clupea harengus, spawns and De Ciechomski (1966) reported a range of 50-60% for Argentine anchovy, Engraulis anchoita, spawns. This compares favourably with the observed range of 34-95% for O. mossambicus spawns (Rana, 1985).

Variation in egg size has been expressed as the range (Blaxter and Hempel, 1963; De Ciechowski, 1966; Bagenal, 1971), but since the range is greatly influenced by a few extreme egg sizes in a clutch it is not a good measure of egg-size variability. This may be better represented by the average variation expressed as a percentage of the mean egg size of the clutch (coefficient of variation, CV%). In the present study the median and mean coefficient of variation of egg size within spawns was approximately 11% for both species (Fig. 3.1). Due to this low CV mean egg size of clutches was considered to be representative of eggs within individual clutches.

The reasons for intra-ovarian variation in egg size is as yet unclear. Meyen (1940, quoted by Kirpichnikov, 1981) suggested that the size range of eggs increases when food supply is limiting. He proposed that only those oocytes developing in the proximity of the main ovarian blood vessels receive optimal amounts of nutrients. Thus oocytes developing along minor blood vessels receive less nutrients and are therefore smaller. But when food is abundant all maturing oocytes receive sufficient nutrients and are therefore more similar in size at ovulation. Evidence for this is given by Anokhina (1960, quoted by Mann and Mills, 1979) who found a greater variability in egg size in C. harengus in the Baltic Sea in years when food was scarce.

There is, however, conflicting evidence on the influence of maternal food intake on egg size. For rainbow trout (Scott, 1962), the viviparous guppy, Lebistes reticulatus (= Poecilia reticulata), (Hester, 1964) and common carp, Cyprinus carpio, (Hulata, Moav and

Wohlfarth, 1974) a reduced food intake lowered fecundity but did not significantly reduce egg or fry size, whereas Bagenal (1969a) observed that poorly fed brown trout produced larger but fewer eggs. In contrast, Townshend and Wootton (1984) reported that a low ration significantly reduced egg diameter in the convict cichlid, Cichlasoma nigrofasciatum. Under the controlled hatchery conditions of the present study, however, it is unlikely that the higher variation in egg size found within some clutches was due to food shortage.

In the present study, both species used were known to originate from single genetic strains (B. McAndrew, personal communication) and since females were reared under similar conditions in one recirculated water system the only variables likely to have affected their reproductive traits, including egg size, were the growth indices of maternal age, weight and length and the individual genotype variability of females.

Superimposed on the variation in egg size within spawns, however, fish may show intraspecific variation due to seasonality changes, different genetic stocks and age and size composition of breeding stocks. Consequently, the range in egg size within species in marine and freshwater fish may vary between 4% and 560% (Bagenal, 1971).

In teleosts, large fish produce more eggs and larger eggs than small fish (Wootton, 1984). The present study confirms this trend for O. niloticus and O. mossambicus (Table 3.6). However, when mean egg size between spawnings from females of similar age were considered, maternal length (Table 3.4) and weight (Table 3.5) showed no significant ($P > 0.05$) influence on mean egg weight. This,

together with the highly significant ($P < 0.001$) differences in mean egg size between individual O. niloticus females and between individual O. mossambicus females within age-classes (Table 3.2) suggests that egg-size variation within an age-class may be primarily determined by the genotype of the parent fish. Given this constraint on egg size it may be concluded that 80% and 53% of the total variation in egg size is a function of female O. niloticus and O. mossambicus age-class, respectively (Table 3.3). Since maternal age was auto-correlated with weight and length egg size shows a highly significant ($P < 0.01$) causal relationship with all three growth indices (Table 3.6). Nevertheless, the stronger influence of age on egg size was reflected in the higher degree of association compared with weight or length and explained a greater proportion of the total variation in egg size than either weight or length (Table 3.6). The predictability of egg size from maternal size, however, can be improved significantly ($P < 0.05$) by monitoring more than one growth parameter. For example, considering length and age together in the regression model improved the predictability of egg size by 6% and 13% for O. niloticus and O. mossambicus, respectively (Tables 3.6 and 3.7).

Evidence for maternal age having a predominant influence on egg size has also been shown for other species. Hulata *et al.* (1974) noted that even though the body weight of yearling and 23 month old common carp were similar there were large differences in egg size. Data reported by Dadzie (1970b) revealed that O. aureus of similar age (length 13.5-27.0 cm) showed no causal relationship between egg and body size. An examination of data given by Peters (1983 - Fig. 3)

also indicated that there is no relationship between 10-80g T. tholloni, T. zillii and T. guineensis, and between 10-40g S. melanotheron females and their egg size. Over a larger female size range (10-400g) which probably reflects a larger age structure, however, Peters (1983 - Fig. 4) found mean egg size in S. melanotheron, S. galilaeus and O. niloticus to be strongly related to maternal body weight.

In addition, the present study supports the view that egg size in the tilapias may also be species-specific (Lowe (McConnell), 1955; Trewavas, 1983). When females of a similar age of both species were reared and spawned under similar conditions the mean egg size of O. niloticus was larger than that of O. mossambicus (Table 3.1). A similar comparison with the published data for other Oreochromis species is limited. The majority of the earlier studies on egg size were based on ovarian eggs from wild specimens whose age structure could not be defined accurately. Furthermore, egg dimensions vary depending on the stage of ovarian development. Consequently, the sizes of eggs reported for O. niloticus (Daget, 1954; Lowe (McConnell), 1955; Bauer, 1968; Peters, 1983), O. aureus¹ (McBay, 1961), O. esculentus (Lowe (McConnell), 1955), O. leucostictus (Welcomme, 1967), O. spilurus (Cridland, 1962), O. hornorum (Hickling, 1960) and O. mossambicus (Vaas and Hofstede, 1952; Fishelson, 1966; Peters, 1983) show no inter-species variation.

Footnote: ¹ studied under the name T. nilotica (Trewavas, 1983)

3.1.4.2 Egg production

Fecundity

Unlike egg size, the number of eggs shed per spawning (total fecundity) and total weight of eggs spawned in O. niloticus and O. mossambicus of similar age increased significantly with maternal length (Table 3.4) or weight (Table 3.5). This, together with the significant difference in total fecundity and clutch weight between individual females within the same age-class (Table 3.2) suggests that, in contrast to egg size, maternal weight and length rather than age had a greater influence on total fecundity and egg production. Since female weight and length are auto-correlated with age, total fecundity and clutch weight were also significantly related to female age (Table 3.6). Nevertheless, body weight and length explained a higher proportion of the total variation in total fecundity and clutch weight than age (Table 3.6). A similar association for haddock was shown by Hodder (1963) who reported a correlation coefficient (r) of 0.66 between age and fecundity compared with 0.83 between weight and fecundity.

In addition, the degree of association between fecundity and maternal age, length and weight may vary between species. Raitt and Hall (1967) showed that in the redfish, Sebastes marinus, neither age, weight nor length affected fecundity. In plaice, Pleuronectes platessa (Simpson, 1951), in Hippoglossoides platessoides (Bagenal, 1957; Pitt, 1964) and in pike, Esox lucius (Terleckie, 1973) found that length and weight, but not age, were correlated with total fecundity. Whereas for whitefish, Coregonus spp. (Berg and Grimaldi,

1965), flounders (Kandler and Pirwitz, 1957), haddock (Raitt, 1932; Hodder, 1963), roach (Mackay and Mann, 1969) and capelin (Winters, 1970, 1971) there was found to be a real effect of age even after the influence of length had been taken into account.

In addition to information on total fecundity, information on the reproductive capabilities of the broodstock biomass would be of value to the fry producer, especially since food and space costs would be based on these considerations. In the present study it was found that the relative fecundity of broodfish within any given age-class was not significantly influenced by maternal length (Table 3.4) and weight (Table 3.5), while for the mixed age structure relative fecundity decreased significantly with broodstock age and size (Table 3.6). Within age-classes, however, there were significant ($P < 0.01$) differences between individual females (Table 3.2). The variability in relative fecundity, however, was considerably higher for O. mossambicus compared with O. niloticus (Table 3.3) therefore one method to improve broodstock productivity might be to use younger broodstock.

Since the present study suggests that larger fish, even of the same age, may lay more eggs, maternal growth rate and the factors that influence their growth will be of crucial importance in increasing egg yields for fry production. One such factor affecting growth is food supply. Whilst the influence of food ration on egg size is unclear, there is a general consensus that there is a marked effect on total fecundity and clutch weight (Wootton, 1979). Scott (1962) reared rainbow trout, Salmo gairdneri, under various feeding

regimes and showed that food restriction increased the proportion of atretic eggs in the ovaries and decreased the proportion of fish that reached sexual maturity. Similar findings were also reported for the guppy, P. reticulata (Hester, 1964). In addition, studies on winter flounder, Pseudopleuronectes americanus, showed that food limitation lowers fecundity by restricting the recruitment of oocytes for maturation (Tyler and Dunn, 1976). In contrast, Mironova (1977) reported an increase in egg production in O. mossambicus females reared on low rations. In the present study since broodstock were well fed, and were reared under similar environmental conditions, differences in total fecundity and clutch weight probably reflected the weight, length and genotypic variation among individual fish. The effect on growth rate of hierarchical interaction between females and their access to food in holding tanks cannot, however, be excluded.

The rate (b) at which mean total fecundity and mean clutch weight increased with female age, weight and length showed interspecific differences, being higher for O. niloticus than O. mossambicus. In O. niloticus total fecundity increased at a rate proportional to length (L) to the power 1.74 compared with $L^{1.10}$ for O. mossambicus (Table 3.6). These values are considerably lower than those previously reported by other workers. Welcomme (1967) and Babiker and Ibrahim (1979a) observed that the rate of increase in total fecundity (based on ovarian egg counts) in O. leucostictus and O. niloticus was approximately proportional to the square of parent length. In S. galilaeus (Blay, 1981) and T. zillii (Dadzie and Wangila, 1980) fecundity increased by the power 2.36 of their

standard and total length, respectively. Recently, Payne and Collinson (1983) reported that total fecundity in O. niloticus and O. aureus increased at a rate proportional to $L^{2.25}$.

Further, in the present study total fecundity in O. niloticus increased with maternal weight (W) proportional to $W^{0.56}$ compared with $W^{0.40}$ for O. mossambicus (Table 3.6) suggesting that (a) total fecundity declines proportionally with weight, or (b) that small fish produce more eggs per unit body weight. This trend may also reflect on their adaptive reproductive strategy. Since tilapias generally respond to unstable environmental conditions by maturing at an earlier age (Noakes and Balon, 1982) when they will also be relatively more fecund, the biomass of newly colonised water bodies such as ponds, can therefore be increased rapidly.

In most fishes, the rate of increase in total fecundity has been reported to be proportional to body weight, that is to the cube of body length (Wootton, 1979), twice that found for both species in the present study. The lower values of 'b' for length and weight were probably due to fecundity estimates being based on actual number of eggs shed and the measurement of spent females rather than ovarian egg counts and total body weight including gonadal products. In other teleosts values for 'b' with respect to length range from 1 to 7, with a modal value of 3.25-3.75 (Wootton, 1979).

In the present study the rate of increase in egg production showed a similar trend to total fecundity. In O. niloticus the rate of increase in egg production was proportional to $L^{2.64}$ compared with

$L^{1.94}$ for O. mossambicus (Table 3.6) and was below the average value of $L^{2.69}$ reported by Wootton (1979) for Canadian fresh water species.

Interspecific differences in fecundity

Total fecundity, in relation to body weight, was higher for O. mossambicus than for O. niloticus. For example, the mean expected fecundity (derived from Table 3.6) for 100-200g O. mossambicus females ranged from 619-815 eggs compared with 527-780 for O. niloticus females of the same weight range. The higher fecundity of O. mossambicus, however, was at the expense of egg size. For the same weight range the expected mean egg weight was 1.92-2.35mg in O. mossambicus compared with 2.38-2.92mg in O. niloticus. Since the growth rate of O. niloticus is greater than O. mossambicus, however, O. niloticus would have a larger mean size at a given age and would therefore be able to produce more eggs.

The results of the present study suggest that earlier studies on tilapia fecundity may have overestimated reproductive potential since most of the data reported were based on ovarian egg counts of 'mature ovaries'. Lowe (McConnell) (1955) reported ovarian counts in O. niloticus to vary from 340 in 17cm (total length - TL) females to 1500 in 34cm TL females. Trewavas (1983) mentioned that a 7.5cm O. niloticus contained about 105 ovarian eggs. The number of ovarian eggs reported for O. mossambicus is also variable and ranges from 80-1754 for 8-25cm (TL) sized fish (see Trewavas, 1983). Estimates of total fecundity and relative fecundity based on spawned eggs

reported by Siraj et al. (1983) for O. niloticus were also higher than those obtained in the present study. Siraj et al., however, enumerated eggs by the water displacement method and due to the small volume changes involved (100 5mg fresh eggs displace only 0.5-0.6ml of water, personal observation), they may have overestimated the quantity of eggs. Their higher total fecundity estimates, especially for smaller fish, were also reflected in the egg to body weight ratio (EW:BW) calculated from their data. In the present trials the mean EW:BW for O. niloticus was 2.8% compared with the mean of 4% calculated from the data of Siraj et al. (1983). Further, the gonadosomatic index (GSI %) for many tilapia species rarely exceeds 5% (Fryer and Iles, 1972). In cases where direct egg counts were used, data were based on seine and gill netted specimens. Hence the reported numbers of orally-reared eggs and fry in O. mossambicus were low (Riedel, 1965). Bruton and Bolt (1975) noted 11-579 eggs in O. mossambicus females varying from 12.5-15.8cm (standard length).

A comparison of total fecundity between multiple spawners, such as the mouth-brooders, with annual spawners is inappropriate since fecundity is related to reproductive energy (Svardson, 1949). In the vast majority of teleosts, which are annual spawners (Scott, 1979), fecundity estimates represent the total potential annual egg production of individual fish, whereas in mouth-brooders, fecundity estimates of individual fish, based on either ovarian counts or direct egg counts, represent only a part of their annual potential egg production. By this comparison O. niloticus and O. mossambicus are regarded as less fecund than annual spawners (Macintosh, 1985). For a more accurate comparison of reproductive

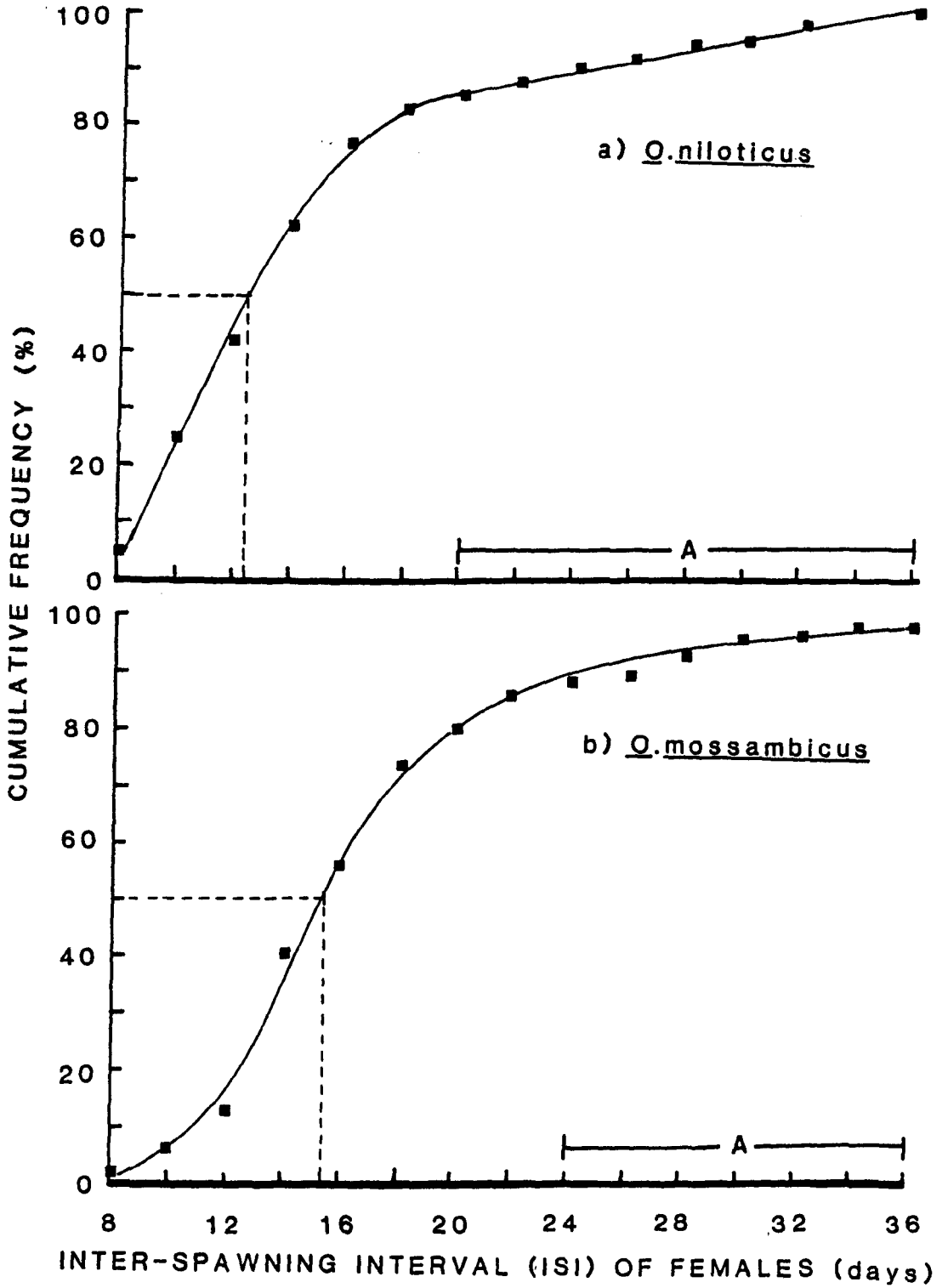
potential, however, spawning frequency should also be considered. In the present study the removal of eggs from individual females resulted in the majority of females spawning at 20 day intervals (Fig. 3.2). Assuming that a female can spawn 10 times annually and using the mean relative fecundity for O. niloticus and O. mossambicus obtained in the present trials (Table 3.1), approximately 52,000 and 76,650 eggs/Kg female can be expected annually from individual spawners. Assuming a survival rate of 85% (see Section 2.1) 44,200 and 65,000 swim-up fry/Kg female may be expected annually. In carp for example, the average fecundity of individual fish (based on ovarian counts) is approximately 100,000/Kg female (Bishai et al., 1974). Under hatchery conditions 50% survival to first-feeding is realistic (Macintosh, 1985). Therefore, 50,000 fry/Kg female may be expected annually from individual spawners. In natural environments survival of larvae and fry is even lower in other annual spawners, for example in jack mackerel, Trachurus symmetricus, which produce hundreds of thousands of eggs/Kg female the survival rate from fertilization to yolk-sac absorption was only 0.1%-0.5% (Hewitt et al., 1985). Therefore, based on an annual production and considering the survivorship of the progeny, fry production from individual O. niloticus and O. mossambicus females may approach species such as the carps. The differences in the number of eggs per spawn between these species may reflect their alternative reproductive strategies. In the tilapias their reproductive strategy would allow for the conservation of gonadal products during times of unfavourable spawning conditions thereby channelling their energy into growth. These larger fish may then subsequently produce more eggs of a larger size on the return of favourable spawning conditions.

FIGURE 3.2

Cumulative frequency distribution of inter-spawning intervals (ISI) for (a) O. niloticus, and (b) O. mossambicus females. The ISI are of females from which spawned eggs were removed for artificial rearing. A, refers to ISI of females bred in glass aquaria, the remainder were ISI of females spawned in 1m² and 2m diameter tanks.

(a) n = 108 from 46 females

(b) n = 113 from 26 females



Efficiency of egg production

The decrease in reproductive efficiency with growth in both species was indicated by a small but significant ($P < 0.01$) negative correlation between egg to body weight ratio (EW:BW) with age, weight and length (Table 3.6) but the differences in gonadal production between age-classes were not significantly ($P > 0.05$) different (Table 3.1). Therefore EW:BW allows a comparison of egg production between fish of different weights and species of the same age structure. Even though clutch weight in O. niloticus females was higher than in O. mossambicus females of a similar age, their EW:BW were similar (Table 3.1) and therefore not species specific.

In the present study gonadal production in O. niloticus and O. mossambicus females was found to average 2.8% and 3.1% of their body weight, respectively. These values are much lower than those reported for tilapia species based on GSI since EW:BW excludes the weight of spent gonads which can represent 1.7% of body weight (Peters, 1983). In O. mossambicus average GSI may occasionally be as high as 7% (Peters, 1983) and in T. variabilis it may be 5% (Fryer and Iles, 1972). On an annual basis, egg production in O. niloticus and O. mossambicus can potentially reach 28% and 31% of their body weight, respectively, under favourable spawning conditions. This compares favourably with the GSI of annual spawners of 20% for European perch, Perca fluviatilis, and brown trout, Salmo trutta (Bagenal, 1978), 20-35% for the cyprinid, Labeo spp. (Parameswaran, Selvaraj and Radhakrishnan, 1970) and 20-30% for the three-spined stickleback, Gasterosteus aculeatus (Wootton, 1974).

3.2 The Influence of Egg Size on Growth, Onset of Feeding, Point-of-No-Return and Survival of *O. niloticus* and *O. mossambicus* Fry Developing Solely on their Yolk Reserves

3.2.1 Introduction

The quality of eggs can be defined by their physical characteristics, i.e. size and weight, as well as their biochemical constitution. In the final analysis, however, it is the quality of the eggs in terms of both their hatchability and subsequent fry vigour and survival that is of crucial importance to hatchery management.

Even though eggs may originate from populations of mixed ages or from different geographical locations there is little variation in the biochemical composition of the major constituents in eggs between females of the same species (C. B. Cowey, pers. comm. ; J. C. A. Craik, pers. comm.). Whereas intraspecific variations in physical characteristics of eggs can be high (see review Bagenal, 1971). The well documented variability in egg size may therefore be more indicative of maternal differences.

In natural fish populations, interspecific differences in egg size, reflecting the adaptive radiation in life histories and reproductive strategies, may have evolved to ensure the survival of reproductively viable offspring (Wootton, 1984). One such strategy is the evolution of parental care, notably among the Cichlids, in which parental care is accompanied by a decrease in number of eggs and a corresponding increase in egg size.

The adaptive significance of intraspecific variation on egg size between individuals of the same weight and length and of the same strain is, however, less clear (Bagenal, 1978). One suggestion is that these differences may be due to the inherited genetic variation of parents (Kirpichnikov, 1981). In addition, intraspecific variation in egg size has also been attributed to the age and size structure of the breeding population (Blaxter and Hempel, 1963; Gall, 1974; Hulata et al., 1974), genetic stocks and strains (Hulata et al., 1974) and seasonality changes (Bagenal, 1971; Barton, 1981). Superimposed on these there may be compounding intraspecific relationships between egg size and fecundity (Bagenal, 1978; Wootton, 1979) and female nutritional status (Townshend and Wootton, 1984).

Despite these interacting factors, however, the observation that longer and heavier fry result from bigger eggs is well documented. Studies with brown trout, Salmo trutta (Dahl, 1918-1919; Bagenal, 1969b), rainbow trout, Salmo gairdneri (Pitman, 1979), Atlantic salmon, Salmo salar (Thorpe et al., 1984), chinook salmon, Oncorhynchus tshawytscha (Rombough, 1985), chum salmon, Oncorhynchus keta (Beacham et al., 1984), coho salmon, Oncorhynchus kisutch (Beacham et al., 1984), channel catfish, Ictalurus punctatus (Reagan and Conley, 1977), herring, Clupea harengus (Blaxter and Hempel, 1963) and jack mackerel, Trachurus symmetricus (Theilacker, 1981) have all shown that the growth of fry is linearly related to their yolk reserves and hence to egg size.

Further it has also been shown that fry from larger eggs show better survival (Blaxter and Hempel, 1963; Bagenal, 1969b; Gall, 1974;

Pitman, 1979), although this may be species dependent. For example, studies on Argentine anchovy, Engraulis anchoita (De Ciechowski, 1966), chinook salmon (Fowler, 1972), Japanese medaka, Oryzias latipes (Stanley, 1977), and rainbow trout (Springate and Bromage, 1985) have shown that fry survival is not affected by egg size.

More importantly, egg size may affect the time of hatching, the time at which maximum tissue weight of the fry is attained, and the time of transition to exogenous feeding which is regarded as a critical phase for the developing fry.

In view of the potential importance of egg size to subsequent fry performance, the objective of this trial was to determine if larger O. niloticus and O. mossambicus eggs yield larger fry and to examine whether egg size affects hatching time, feeding capabilities and susceptibility of the fry to starvation. To achieve this females from one genetic strain and of known age, spawning history and nutritional status were used.

3.2.2 Materials and Methods

Since egg size was found to be highly correlated with maternal age (Section 3.1), females from 0+, 1+ and 2+ age-classes were used as a source for different egg sizes. In addition, since the CV (%) of egg size within clutches was low in the majority of clutches (Section 3.1) the mean egg size of individual clutches was considered to be representative.

3.2.2.1 Egg supply and incubation

Individually tagged O. niloticus and O. mossambicus females from 0+, 1+ and 2+ age-classes were stocked in 1m² spawning tanks at a sex ratio of 3 females : 1 male and fed on a diet containing 40% crude protein (Pellet No. 4; Edward Baker Ltd., Bathgate, Scotland) at a rate equivalent to 2% of their body weight per day. To minimise paternal effects on the performance of developing fry, eggs of spawning females were fertilized by one conspecific male (age 14-15 months). The broodstock were allowed to spawn naturally, but where the size difference between the females and male was too large the females were stripped manually and fertilized with milt from the male as described previously (Section 2.1.2.2).

The eggs were removed from the buccal cavity of the female within 12 hours of her spawning and incubated in round-bottomed containers as described previously (Section 2.1) and the times to mass hatch noted. Individual egg clutches showing a high variation in egg size were first identified and the 'small' and 'large' eggs were removed and incubated separately as 'small' egg-size groups and 'large' egg-size groups. In addition, a random sample of 50 eggs was removed from each clutch, dried on absorbant paper, oven-dried at 50°C and the mean dry egg weight ($\pm 0.1\text{mg}$) determined.

Egg clutches were collected from at least five individual females from each age-class.

3.2.2.2 Fry maintenance

Three days after hatching, fry from individual egg clutches were transferred to 2 l glass containers held in a covered water bath maintained at 27°-28°C. An air stone connected to a compressed air supply provided aeration and water circulation in each rearing container. At least half the water in each container was replaced every two days with fresh water filtered through a 0.2µm pore filter (Microflow 650: Flow Laboratories, England). Water temperatures were recorded daily. The fry were not fed.

3.2.2.3 Growth and survival

To estimate fry growth 20 fry were sampled randomly at three day intervals from each clutch. The fry were killed in benzocaine (1:10,000 solution in water), rinsed in distilled water and their standard lengths ($\pm 0.1\text{mm}$) measured according to the method of May (1971). Due to the low numbers of eggs in the 'large' and 'small' egg-size groups only 10 fry were sacrificed at three day intervals.

The bodies of half of the fry from each sample were dissected from their yolk-sacs (if present) under a dissecting microscope (Olympus, CO11) and the bodies and fry (body + yolk, if present) were weighed as described previously (Section 2.2.2.2). Due to mortalities from starvation, fewer fry were available for sampling at 12, 15 and 18 days after hatching.

For survival studies 30 randomly sampled six day old fry were transferred from each maintenance vessel into 2 l glass containers holding

aerated, filtered water. Mortalities were monitored daily and the time to 50% survival (ST_{50}) determined.

3.2.2.4 Feeding capabilities of previously unfed fry

Feeding capability was defined as the percentage of fry in a sample that were able to ingest the presented diet. A random sample of 20 fry was transferred from each egg clutch into 500ml glass containers at three day intervals from hatching and fed in excess with finely ground ($< 300\mu\text{m}$) broodstock diet dyed with carmosine, E122. Two to three hours later the fry were killed in benzocaine (1:10,000 solution in water) and examined under the microscope for the presence of dyed food in the gastrointestinal tract. Onset of feeding was defined as the time when 50% of the fry had ingested food. The point-of-no-return was determined as the time when 50% of the fry were no longer able to ingest food.

3.2.2.5 Analysis of data

The degree of association and the regression analysis between the mean egg size of individual clutches and the subsequent fry growth and survival parameters were calculated using a computerised statistical package (Minitab, Pennsylvania State University).

3.2.3 Results

3.2.3.1 Egg size and time to hatching

Mass hatch of O. niloticus and O. mossambicus eggs incubated at 27°-28°C occurred within 96 hours of spawning and was independent of egg size.

3.2.3.2 Growth and survival of fry from within an egg clutch

The mean rearing temperatures for O. niloticus and O. mossambicus were 27.5°C and 29°C, respectively.

Only one O. niloticus and O. mossambicus egg clutch contained sufficient numbers of extreme-sized eggs to enable sampling for this trial. The mean dry egg weights and mean growth and survival traits of fry from these 'small' and 'large' egg-size groups of both species are presented in Tables 3.8 and 3.9.

Mean dry egg weights of the 'large' O. niloticus and O. mossambicus egg-size groups were 65% and 69% heavier, respectively, than eggs from the 'small' egg-size groups (Table 3.8). In both species fry from the 'small' egg-size group grew initially at a faster rate than fry from the large egg-size group (Table 3.9). By days 6 to 9 after hatching, however, their growth rate declined rapidly and was considerably lower than that of fry from the 'large' egg-size group. Consequently, at the stage of maximal body weight, O. niloticus and O. mossambicus fry from the 'large' egg-size groups

TABLE 3.8

Summary of mean growth characteristics¹ of unfed O. niloticus and O. mossambicus fry from 'small' and 'large' egg size-groups taken from the same egg clutch

	EGG-SIZE GROUP			
	<u>O. niloticus</u>		<u>O. mossambicus</u>	
	Small (2.35mg)	Large (3.88mg)	Small (1.37mg)	Large (2.32mg)
Size difference of eggs (% over 'small' eggs)	-	65	-	69
Maximal body growth				
Standard length (mm)	7.3	8.5	6.7	7.4
Dry body weight (mg)	1.4	2.5	1.0	1.6
Age at maximal growth (days)				
Standard length	9	9	9	12
Dry body weight	9	9	9	12
End of yolk-sac stage (days)	12	12	9	12
Survival time (ST ₅₀ - days)	13.5	16.5	14.5	18.5

¹ Mean rearing temperatures for O. niloticus and O. mossambicus were 27.5°C and 29.0°C, respectively.

TABLE 3.9

Mean specific growth rates of unfed O. niloticus and O. mossambicus fry from 'small' and 'large' egg size-groups taken from the same egg clutch

Species	Egg size-group (mean dry egg weight, mg)	Age of fry (days after hatching)			
		3 - 6	6 - 9	9 - 12	12 - 15
<u>O. niloticus</u>					
	Small (2.35)	38.9	1.9	-14.5	-1.5
	Large (3.88)	30.5	13.2	-6.4	-20.3
<u>O. mossambicus</u>					
	Small (1.37)	37.1	11.9	-9.6	-4.8
	Large (2.32)	32.7	22.1	1.1	-11.3

were 16% and 10% longer and 79% and 60% heavier, respectively, than fry from the 'small' egg-size groups (Table 3.8). In O. niloticus fry the maximum body weight was reached three days before their yolk reserves were fully utilized, whereas in O. mossambicus fry the time to maximum body weight coincided with yolk exhaustion (Table 3.8).

The survival times (ST_{50}) of O. niloticus fry from both egg-size groups were lower than those of O. mossambicus fry. Within species, however, O. niloticus and O. mossambicus fry from the 'large' egg-size group survived 3 and 4 days longer, respectively, compared with fry from the 'small' egg-size group (Table 3.8).

3.2.3.3 Growth of fry from females of different ages and hence from different egg sizes

Rearing temperatures for O. niloticus and O. mossambicus were $27.5^{\circ} \pm 0.5^{\circ}C$.

Fry length

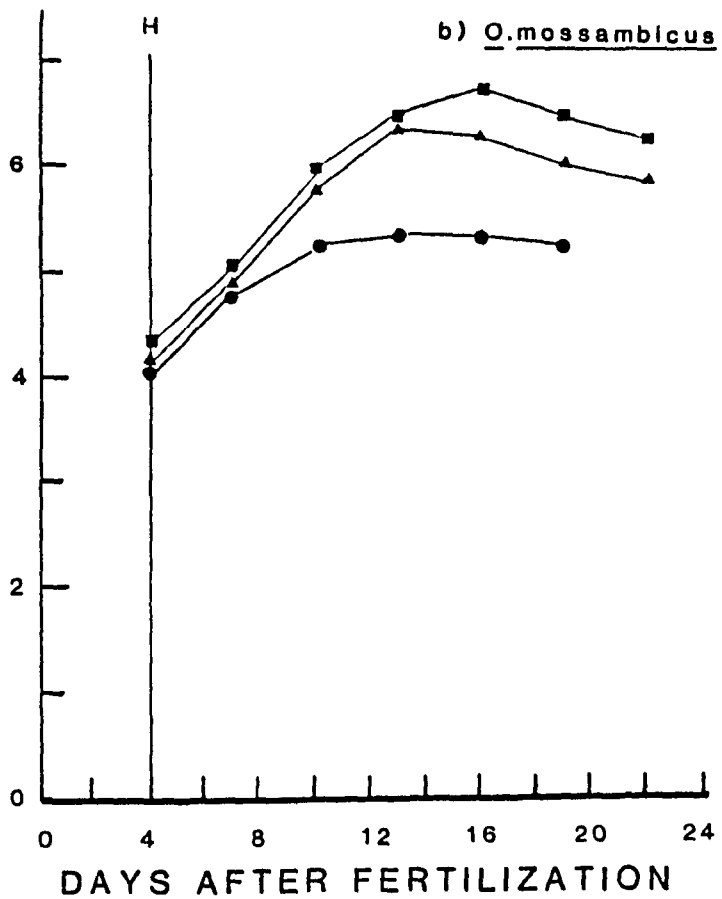
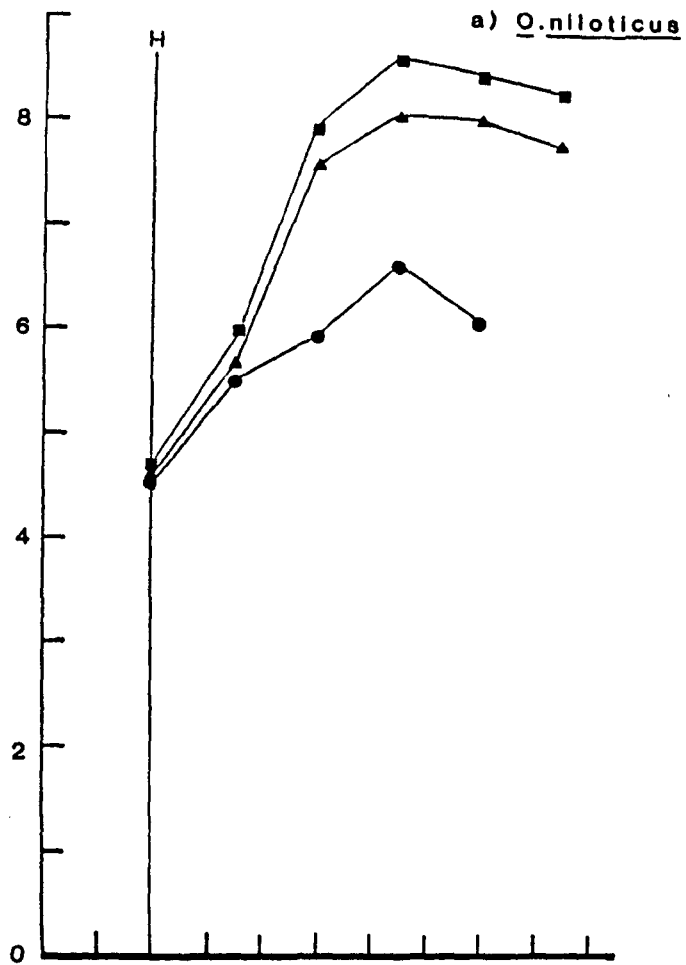
The temporal changes in standard length for O. niloticus and O. mossambicus fry from 0+, 1+ and 2+ females are shown in Fig. 3.3 and their mean growth traits are given in Table 3.10.

Initial increase in fry length was rapid in both species and was highest for fry from 0+ females (Table 3.10). Within 3 days of hatching O. niloticus and O. mossambicus fry attained 83% and 90% of their maximum body length, respectively. With the exception

FIGURE 3.3

Temporal changes in length of (a) O. niloticus, and (b) O. mossambicus fry developing solely on their yolk reserves. Curves relate to fry from: ● , 0+; ▲ , 1+; and ■ , 2+ broodfish. Mean dry egg weights of 0+ (7-9 months), 1+ (12-14 months) and 2+ (23-25 months) O. niloticus and 0+ (9-10 months), 1+ (13-14 months) and 2+ (24-25 months) O. mossambicus females were 1.70 mg, 2.91 mg and 3.74 mg, and 1.01 mg, 1.52 mg and 1.87 mg, respectively. H denotes hatching times of eggs.

MEAN STANDARD LENGTH OF FRY (mm)



of fry from 2+ O. mossambicus females, maximum body length was attained within 9 days of hatching. The mean maximum fry length and mean egg size of individual egg clutches were significantly related ($P < 0.001$). Mean maximum fry length accounted for 77% and 78% of the variation in egg size in O. niloticus and O. mossambicus, respectively (Fig. 3.4).

Body and yolk weights

Temporal changes in body and yolk dry weights of fry from 0+, 1+ and 2+ O. niloticus and O. mossambicus females are shown in Figs. 3.5 and 3.6 and their growth traits are given in Table 3.10.

On the third day after hatching O. niloticus fry from 0+, 1+ and 2+ females attained 46%, 24% and 26% of their mean maximum body weight of 1.0, 1.8 and 2.5mg, respectively. Similarly, over the same period, O. mossambicus fry attained 42%, 29% and 25% of their mean maximum body weight of 0.36, 0.58 and 0.78mg, respectively (Table 3.10).

The weight of fry originating from all three age-classes of O. niloticus and 0+ and 1+ O. mossambicus females increased to their maximum body weight within nine days of hatching, whereas fry from 2+ O. mossambicus females took a further three days to reach the same stage of development. By days 9, 12 and 12, however, O. niloticus fry from 0+, 1+ and 2+ fish, respectively, had utilized all their yolk reserves, while in O. mossambicus yolk exhaustion

TABLE 3.10

Mean growth characteristics of unfed O. niloticus and O. mossambicus fry developing solely on their yolk reserves: comparison of fry from 0+, 1+, and 2+ female broodfish

	Age-class of females (months)					
	<u>O. niloticus</u>			<u>O. mossambicus</u>		
	0+ (8-10)	1+ (12-14)	2+ (23-25)	0+ (9-10)	1+ (13-14)	2+ (24-25)
Mean dry weight of egg (mg) ¹	1.70 (0.14)	2.91 (0.09)	3.74 (0.09)	1.01 (0.04)	1.52 (0.05)	1.87 (0.02)
Fry growth at 3 days after hatching (% of maximum)						
Standard length (mm)	83	71	70	90	82	78
Dry body weight (mg)	46	24	26	42	29	25
Maximum fry growth ¹						
Standard length (mm)	6.6 (0.44)	7.9 (0.10)	8.5 (0.42)	5.3 (0.05)	6.0 (0.12)	6.7 (0.15)
Dry body weight (mg)	1.0 (0.06)	1.8 (0.09)	2.5 (0.04)	0.36 (0.03)	0.58 (0.02)	0.78 (0.05)
Nutrient reserves						
Dry weight difference of yolk at 3 days (% over 0+ eggs)	-	121	171	-	50	91
End of yolk-sac stage (days)	9	12	12	12	15	18
Age at maximal fry growth (days)						
Standard length	9	9	9	9	9	12
Dry body weight	9	9	9	9	9	12

¹ Means given with standard error of the mean

FIGURE 3.4

The relationship between mean dry egg weight of clutches and mean maximum fry length for (a) O. niloticus, and (b) O. mossambicus fry developing solely on their yolk reserves. Regression equations of the form $Y = a + bx$ fitted by the method of least squares were significant at $P < 0.001$. The coefficients in the regression equations and the 95% confidence limits were:

(a) $5.40 \pm (0.658)$ and $0.89 \pm (0.225)$ with $df = 20$

(b) $4.31 \pm (0.453)$ and $1.29 \pm (0.293)$ with $df = 22$

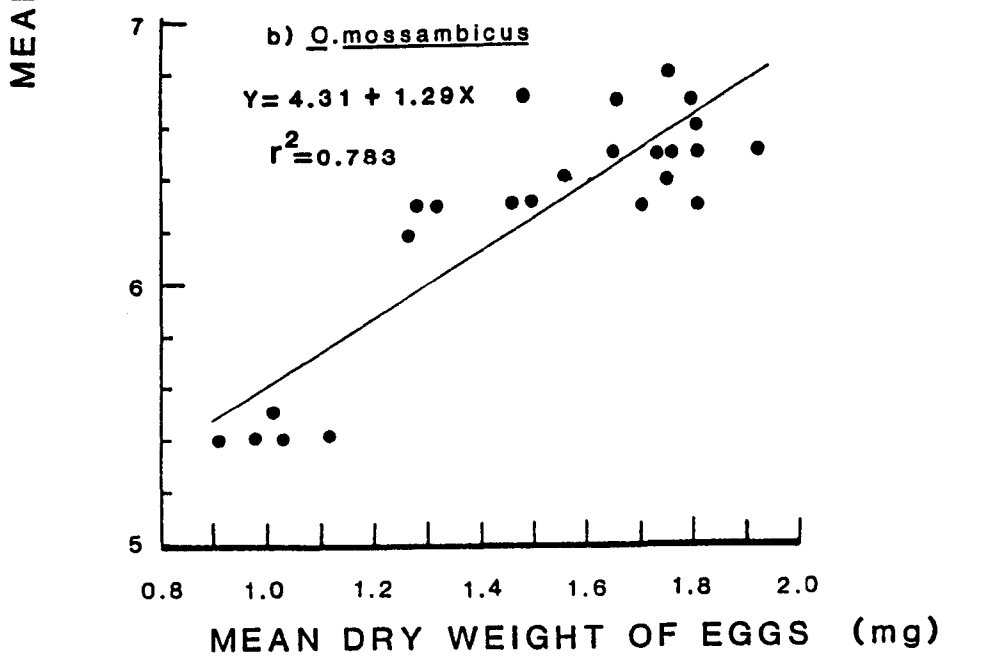
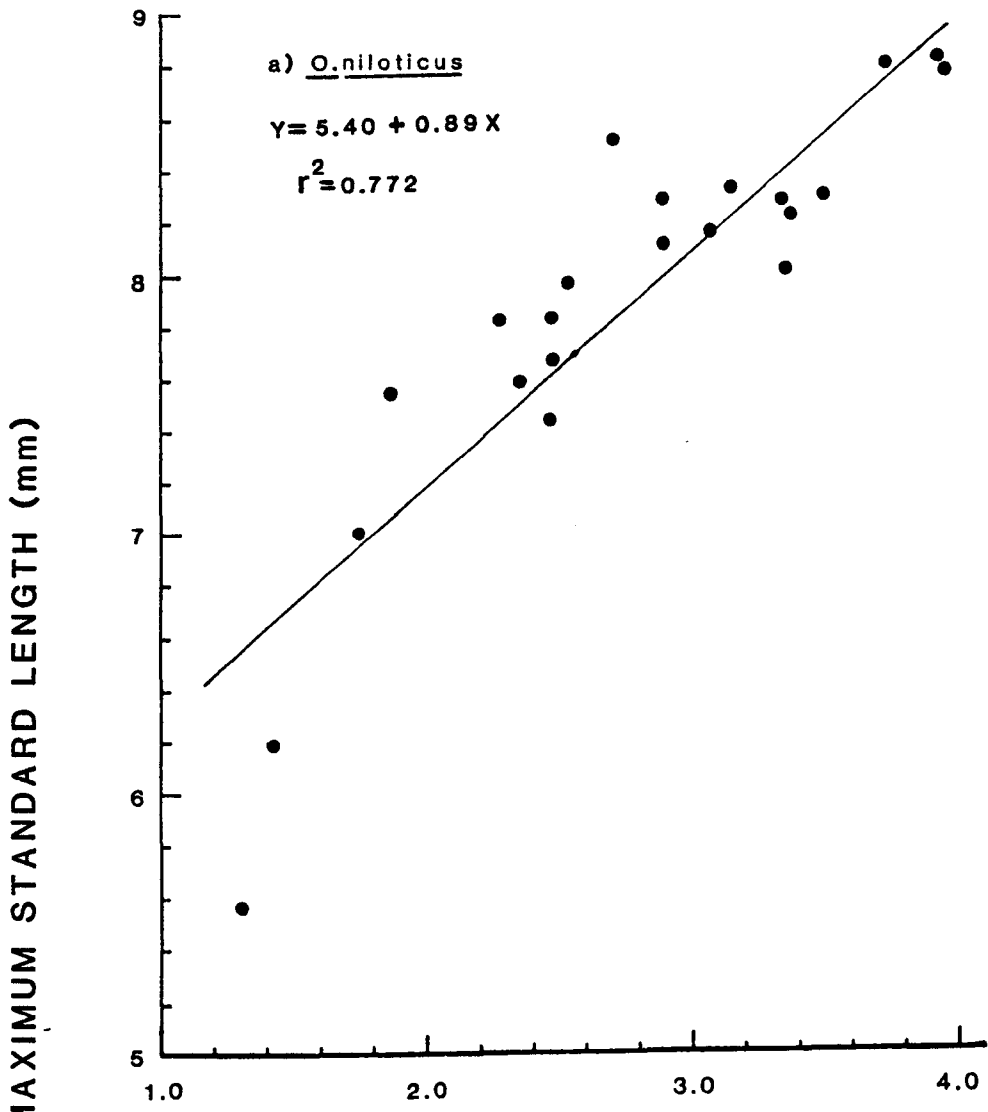


FIGURE 3.5

Temporal changes in body (fry less yolk) and yolk weights of O. niloticus fry developing solely on their yolk reserves. Fry derived from (a) 0+ (7-9 months); (b) 1+ (12-14 months); and (c) 2+ (23-25 months) females having mean dry egg weights of 1.70 mg, 2.91 mg and 3.74 mg, respectively. Upper curves relate to fry weights (body + yolk, if present). H denotes hatching time of eggs. Mean values based on clutches from five individual females.

O. niloticus

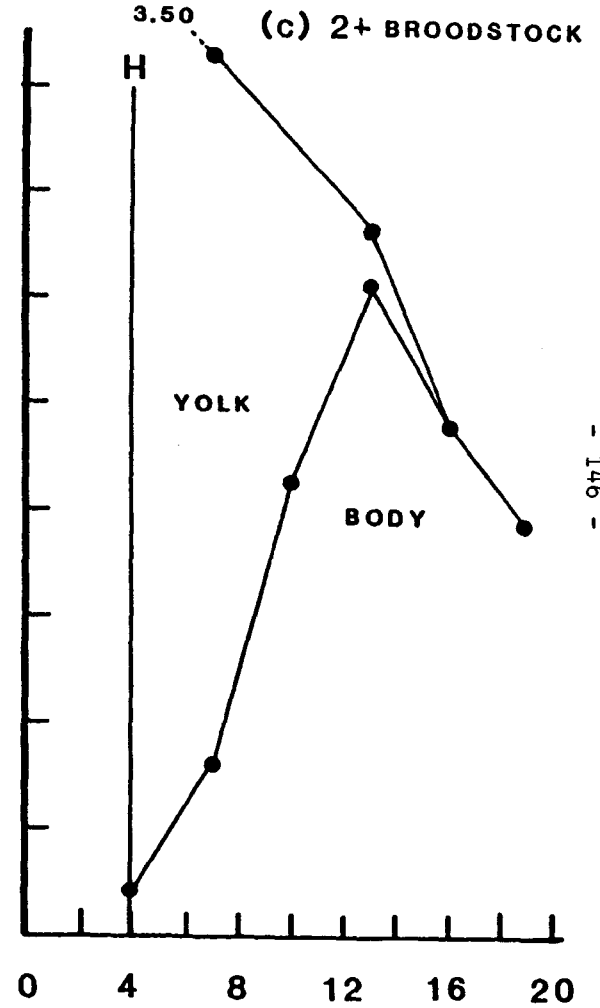
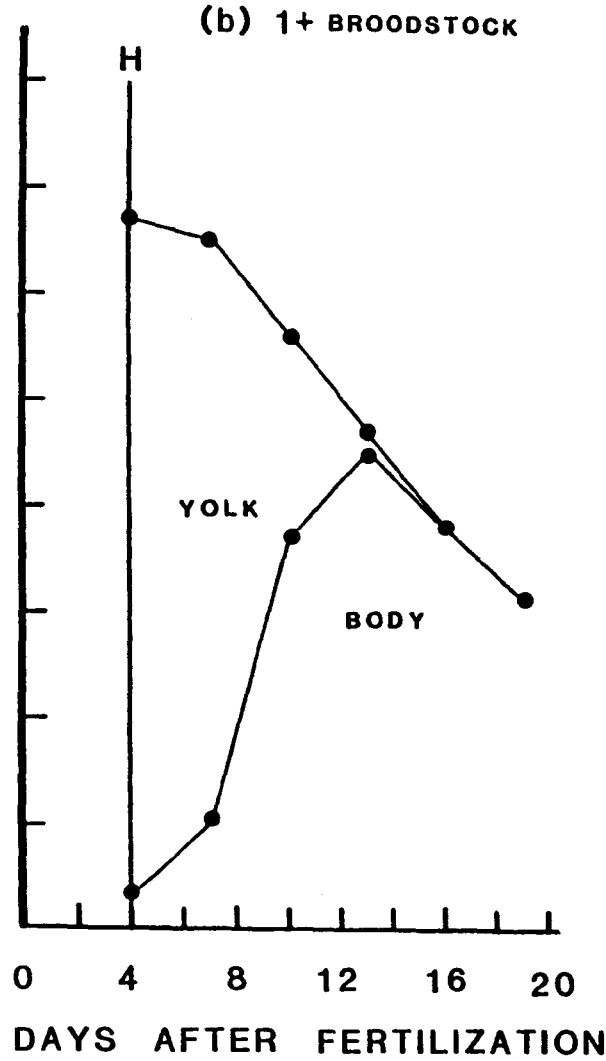
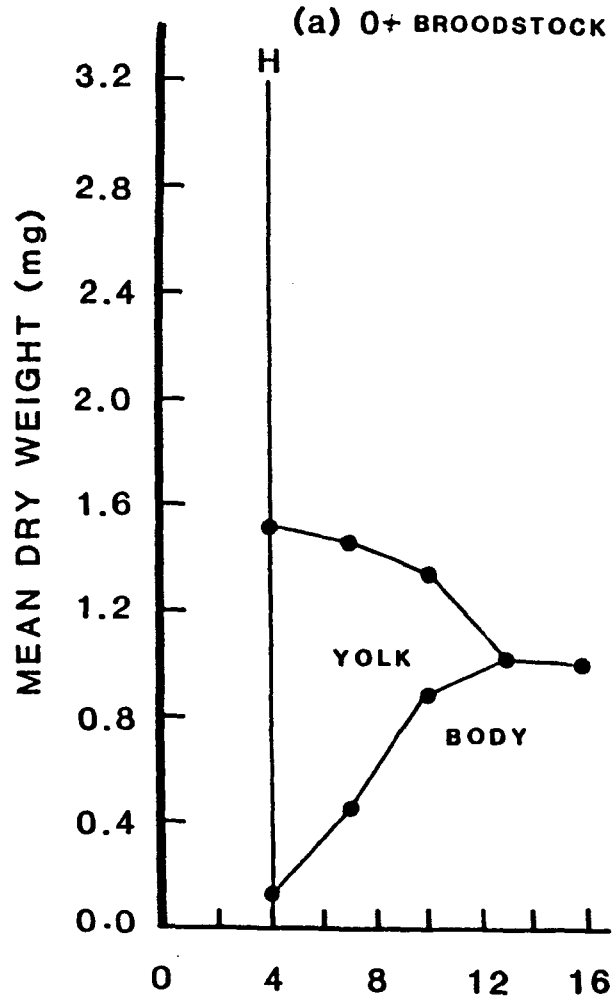
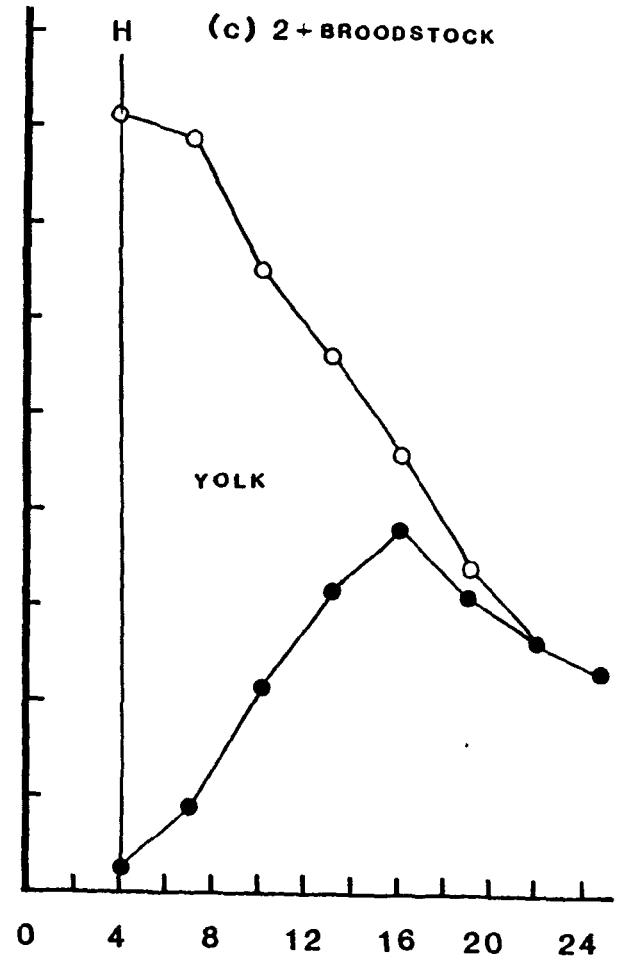
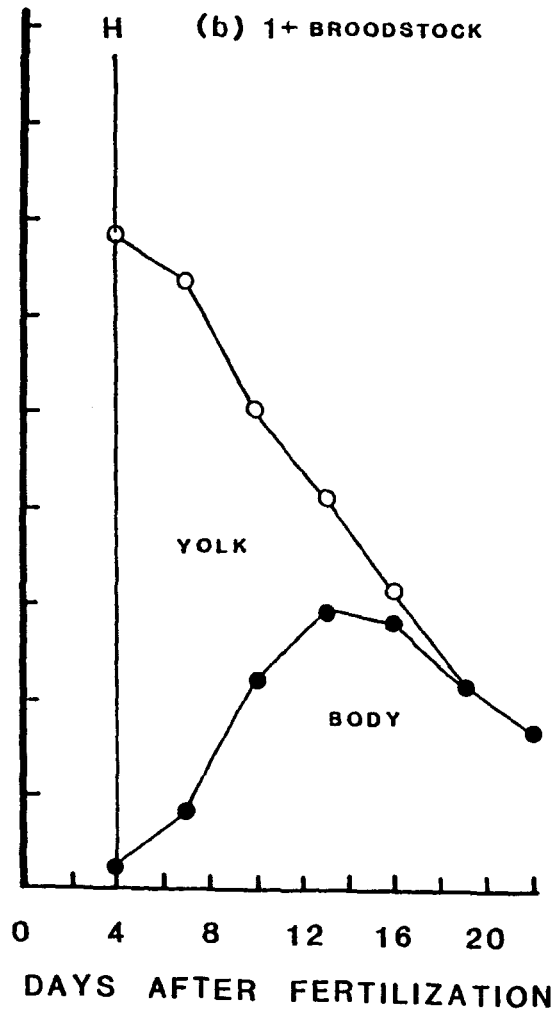
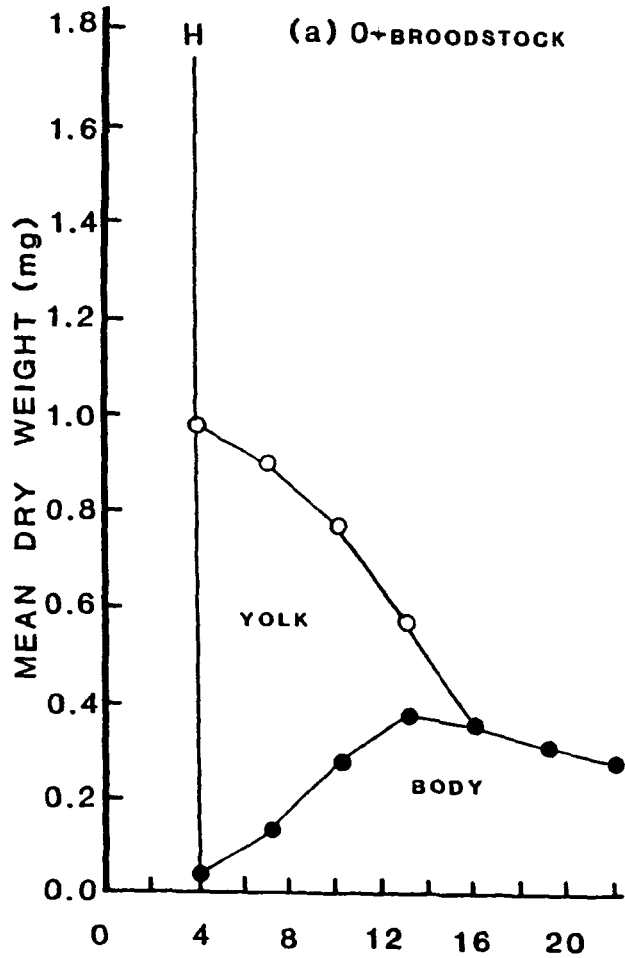


FIGURE 3.6

Temporal changes in body (fry less yolk) and yolk weights of O. mossambicus fry developing solely on their yolk reserves. Fry derived from (a) 0+ (9-10 months); (b) 1+ (13-14 months); and (c) 2+ (24-25 months) having mean dry egg weights of 1.01 mg, 1.52 mg and 1.87 mg, respectively. Upper curves relate to fry weights (body + yolk, if present). H denotes hatching time of eggs. Mean values based on clutches from five individual females.

O. mossambicus



occurred at days 12, 15 and 18 for fry from 0+, 1+ and 2+ females, respectively.

Overall, fry emerging from larger eggs grew to a significantly ($P < 0.001$) larger size (Fig. 3.7). In O. niloticus the average maximum dry body weight ranged from 0.89mg for fry from 1.30mg eggs (dry weight) to 2.55mg for fry from 3.46mg eggs, whereas in O. mossambicus the mean maximum dry body weight ranged from 0.33mg for fry from 0.91mg eggs to 0.90mg for fry from 1.80mg eggs (Fig. 3.7).

During fry development the average specific growth rate (calculated at three day intervals) declined with time in both species. This decrease, however, was greatest in fry from 0+ females (Table 3.11).

3.2.3.4 Feeding capabilities of previously unfed fry from females of different ages and hence from different egg sizes

Feeding capabilities (% of fry in sample capable of exogenous feeding) of O. niloticus and O. mossambicus fry are presented in Fig. 3.8. Onset of feeding commenced at five and six days after hatching in O. niloticus and O. mossambicus, respectively, and was independent of maternal age and hence egg size. The maximum feeding capabilities (maximum number in sample capable of ingesting food) of O. niloticus and O. mossambicus fry from 0+ females were, however, only 85% and 75%, respectively, compared with 96%-100% for fry from 1+ and 2+ females. The point-of-no-return of O. niloticus and O.

FIGURE 3.7

The relationship between mean egg sizes of clutches and mean maximum body (fry less yolk) weights of fry developing solely on their yolk reserves. (a) O. niloticus, and (b) O. mossambicus. Regression equations of the form $Y = a + bx$ fitted by the method of least squares were significant at $P < 0.001$. The coefficients of the regression equations and their 95% confidence limits were:

(a) $-0.06 \pm (0.217)$ and $0.66 \pm (0.073)$, with $df=21$

(b) $-0.123 \pm (0.115)$ and $0.51 \pm (0.075)$, with $df=23$

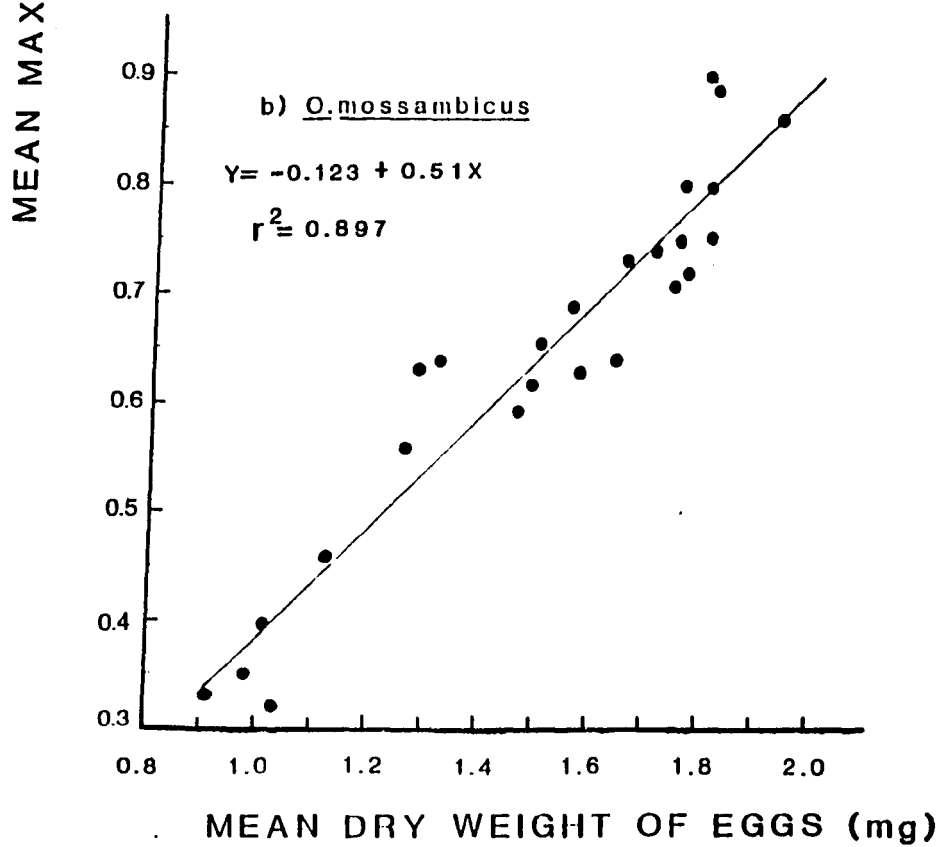
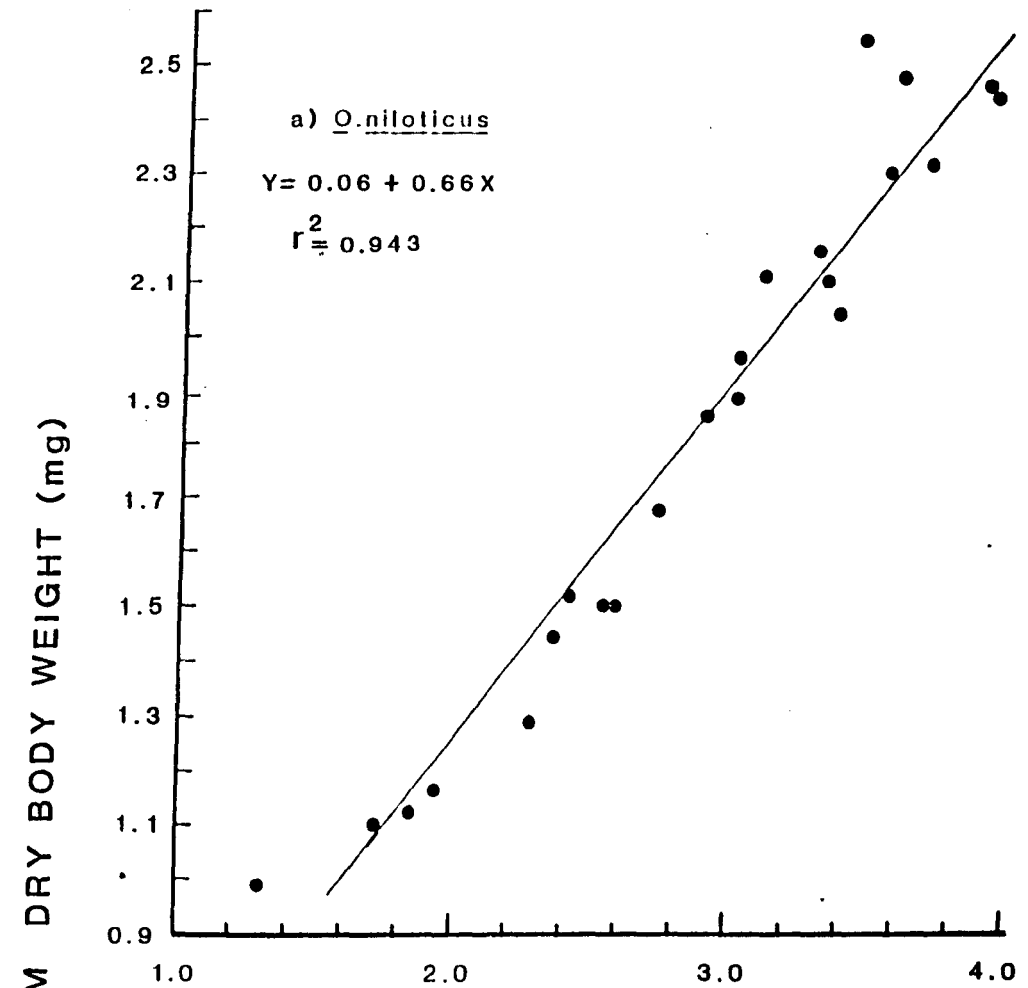


TABLE 3.11

Changes in the average specific growth rates¹ of unfed O. niloticus and O. mossambicus fry developing solely on their yolk reserves: comparison of fry from 0+, 1+ and 2+ female broodfish

Species	Broodstock age-class (months)	Mean dry egg weight (mg)	Age of fry (days after hatching)					
			0 - 3	3 - 6	6 - 9	9 - 12	12 - 15	15 - 18
<u>O. niloticus</u>								
	0+ (7-9)	1.70	52.3	22.4	3.5	-1.7	-	-
	1+ (12-14)	2.91	46.2	39.4	8.8	-5.9	-6.2	-
	2+ (23-25)	3.74	45.8	33.2	11.6	-8.3	-7.2	-
<u>O. mossambicus</u>								
	0+ (9-10)	1.01	58.6	18.3	10.9	-0.9	-0.3	-3.4
	1+ (13-14)	1.52	46.3	33.1	12.6	-1.9	-10.8	-5.4
	2+ (24-25)	1.87	47.1	32.1	15.1	4.6	-4.8	-6.1

$$^1 \text{ Specific growth rate (SGR-\%/day)} = \frac{\log_e W_{tx} - \log_e W_{to}}{tx - to}$$

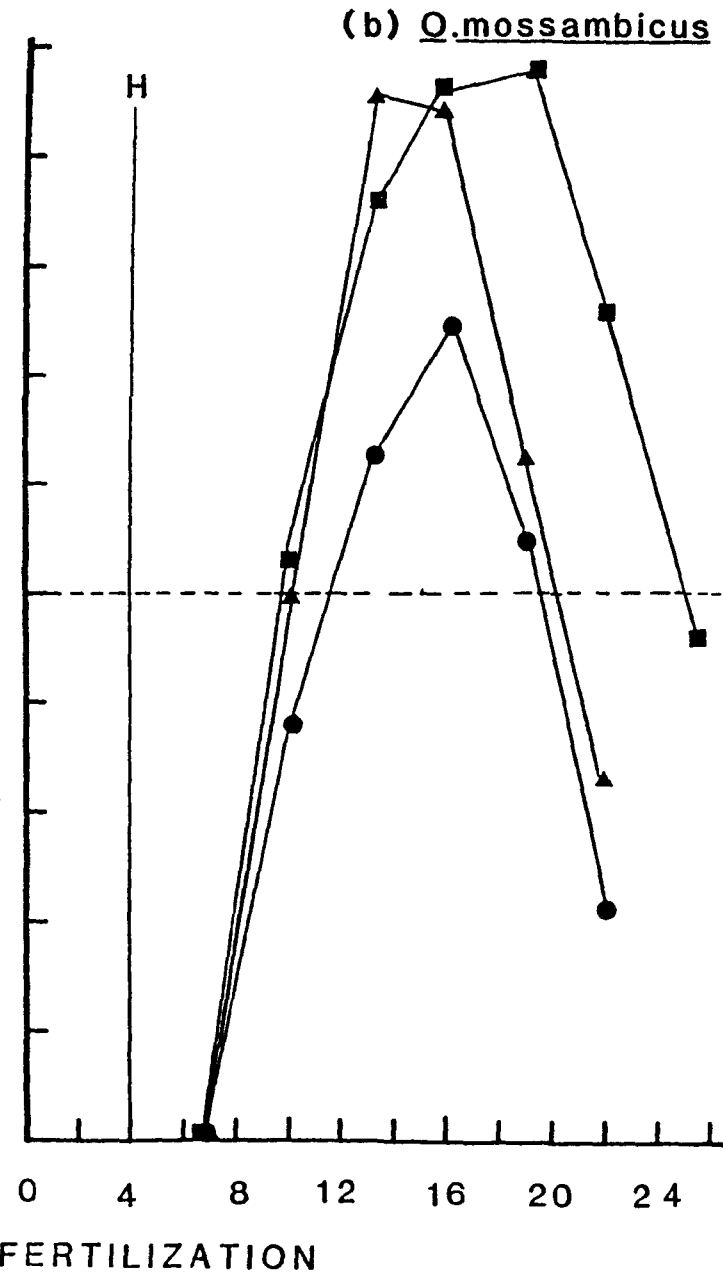
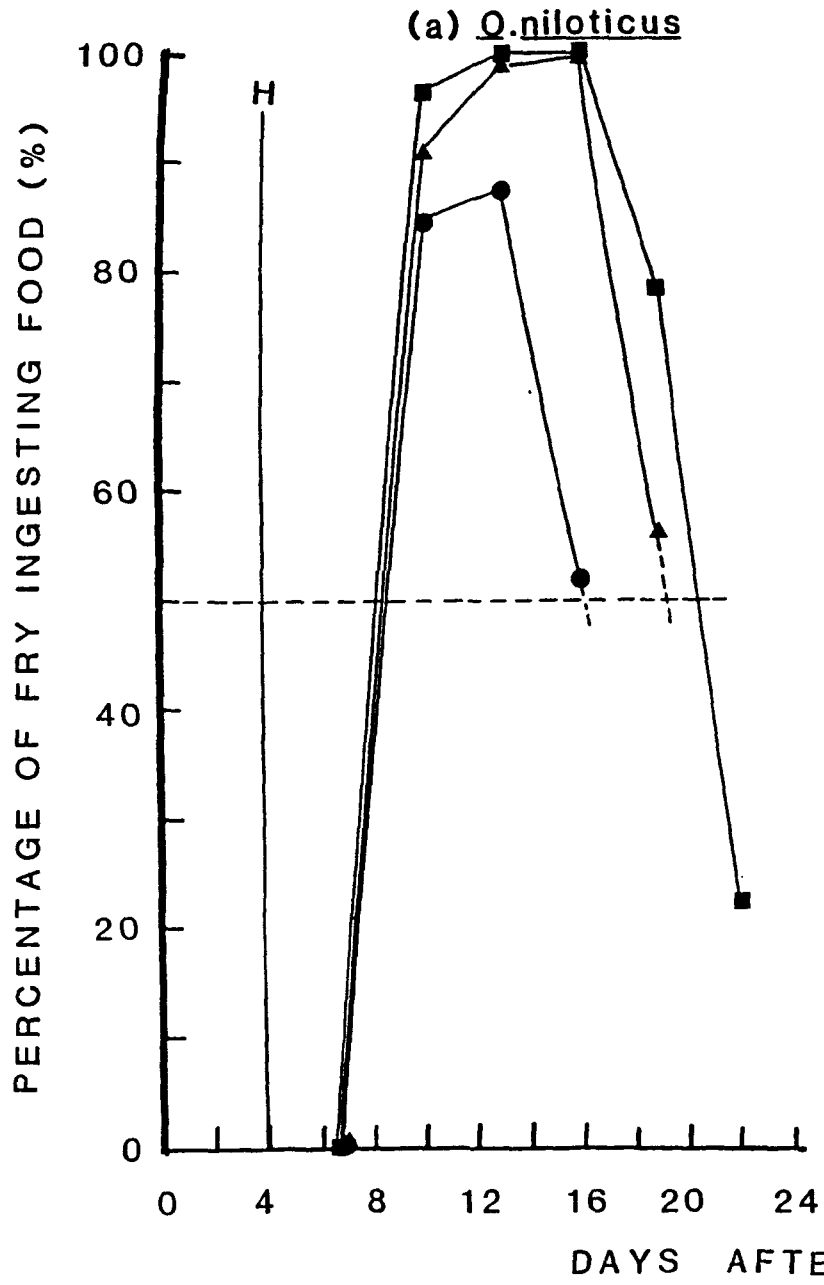
- where W_{tx} = final mean dry body weight at time tx
- W_{to} = initial mean dry body weight at time to
- tx = final time (days)
- to = initial time (days)

FIGURE 3.8

Temporal changes in the feeding capabilities of previously unfed fry developing solely on their yolk reserves.

(a) O. niloticus, and (b) O. mossambicus

Curves relate to fry from ● , 0+; ▲ , 1+; ■ , 2+; O. niloticus and O. mossambicus females having mean dry egg weights of 1.70 mg, 2.91 mg and 3.74 mg, and 1.01 mg, 1.52 mg and 1.87 mg, respectively. H denotes hatching time of eggs. Points of intersection of horizontal broken lines with curves marks onset of feeding and PNR. Mean values based on clutches from five individual females per age-class.



mossambicus fry from 0+, 1+ and 2+ females was reached within 12, 15 and 17 days and 15, 16 and 21 days after hatching, respectively (Fig. 3.8).

3.2.3.5 Survival of fry developing solely on their yolk reserves from females of different ages and hence from different egg sizes

The temporal changes in the survival of O. niloticus and O. mossambicus fry are shown in Fig. 3.9. Mortalities of fry from 0+, 1+ and 2+ females commenced at 11, 12 and 14 days after hatching in O. niloticus and 8, 14 and 16 days in O. mossambicus, respectively. Survival times (ST_{50}) of fry from 0+, 1+ and 2+ females were reached at 13.5, 16 and 17.5 days in O. niloticus and 15, 16 and 19.5 days in O. mossambicus, respectively.

The overall relationship between the ST_{50} times of O. niloticus and O. mossambicus fry from individual egg clutches with the mean dry egg weight of their clutch is shown in Fig. 3.10. In both species the survival times of fry were extended significantly ($P < 0.001$) by larger egg sizes (Fig. 3.10). The range in survival times was 12.5 days for O. niloticus fry from 1.65mg eggs (dry weight) to 18 days for fry from 3.98mg eggs, and 9.5 days for O. mossambicus fry from 0.91mg eggs to 21 days for fry from 1.93mg eggs (Fig. 3.10).

FIGURE 3.9

Survival of fry developing solely on their yolk reserves.

(a) O. niloticus, and (b) O. mossambicus

Curves relate to fry from ● , 0+; ▲ , 1+; ■ , 2+ females. Mean dry egg weights of 0+ (7-9 months), 1+ (12-14 months) and 2+ (23-25 months) O. niloticus, and 0+ (9-10 months), 1+ (13-14 months) and 2+ (24-25 months) O. mossambicus females were 1.70 mg, 2.91 mg and 3.74 mg, and 1.01 mg, 1.52 mg and 1.87 mg, respectively. Mean values based on clutches from five individual females per age-class.

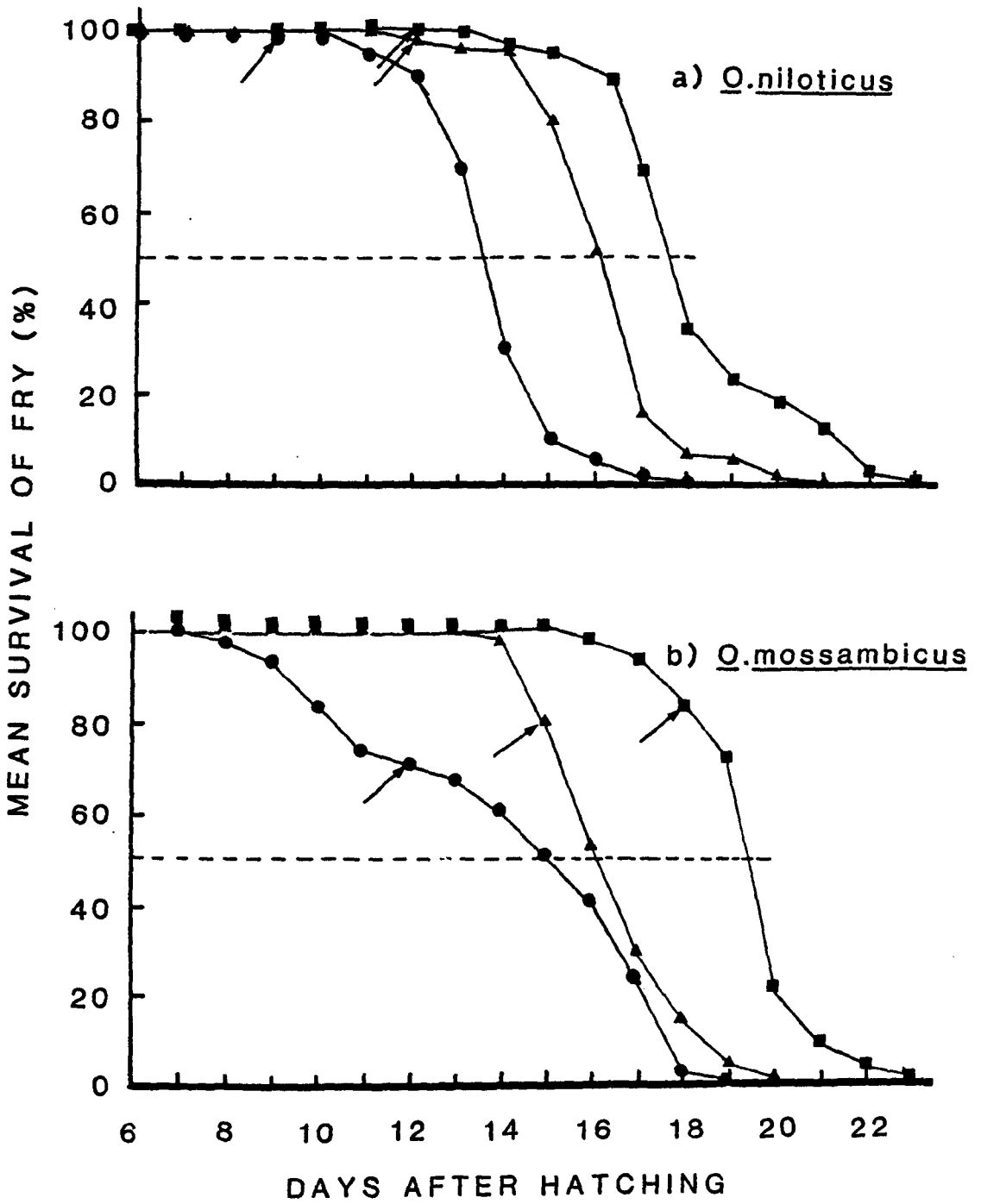
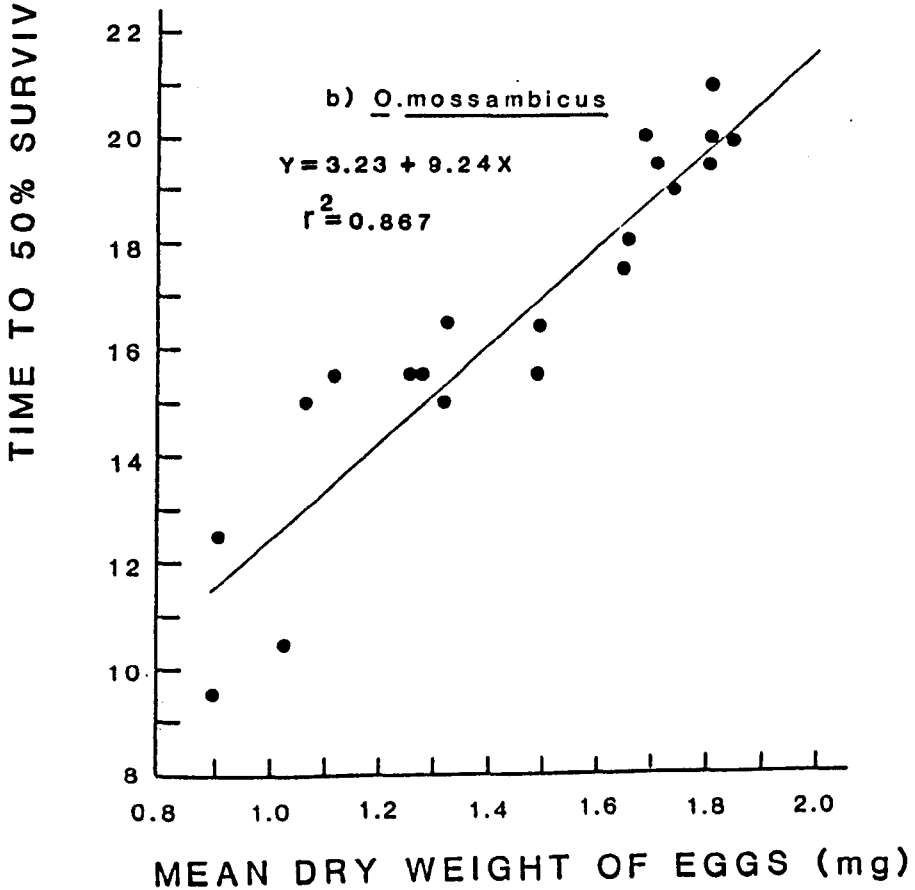
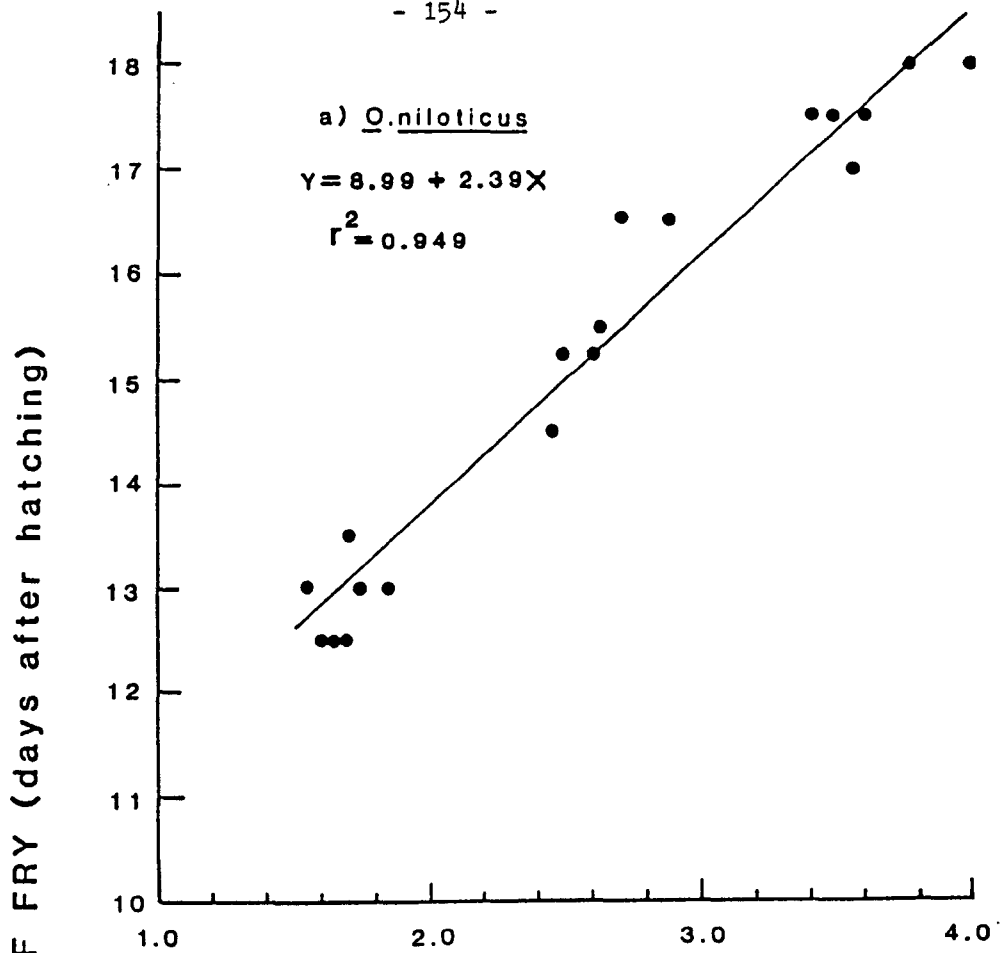


FIGURE 3.10

The relationship between the survival times (ST_{50}) of fry developing solely on their yolk reserves and their mean egg size. (a) O. niloticus, and (b) O. mossambicus. Regression equations of the form $Y = a + bx$, fitted by the method of least squares were significant at $P < 0.001$. The coefficients of the regression equations and their 95% confidence limits were

(a) $8.99 \pm (0.792)$ and $2.39 \pm (0.283)$ with $df = 17$

(b) $3.23 \pm (2.660)$ and $9.24 \pm (1.796)$ with $df = 18$



3.2.4 Discussion

3.2.4.1 The effects of broodstock age and hence egg size on the growth and survival of fry developing solely on their yolk reserves

In addition to maternal influences, the growth of fry relying upon their yolk reserves may be influenced by intraspecific paternal effects (Kirpichnikov, 1981), which was why in the present study the male genome was kept constant by the use of the same conspecific male. Further, differences in growth of developing fry may be due to variation in individual maternal genomes as well as in egg size (i.e. nutrient reserves). Previous studies did not investigate the rôle of these two influences (Fowler, 1972; Gall, 1974; Glebe, Appy and Saunders, 1979; Pitman, 1979; Thorpe et al., 1984; Springate and Bromage, 1985).

Therefore, in the present trial, egg clutches having sufficient numbers (for growth sampling) of 'large' and 'small' eggs were also used in assessing the effects of egg size on fry performance. In these egg-size groups the maternal genetic variability will be constant, therefore any growth differences of the subsequent fry from within clutches may be attributed to the quantity of yolk reserves. In the present study, where the mean egg weight of the 'large' egg-size group was 65% and 69% heavier than the 'small' egg-size group for O. niloticus and O. mossambicus, respectively, the growth rate as well as the maximum size of fry was dependent on the quantity of yolk reserves (Table 3.8 and 3.9). Further, the growth traits of fry from the 'small' and 'large' egg-size groups closely resembled

those of fry from other conspecific females with similar mean egg sizes (Tables 3.8 and 3.10).

The growth characteristics of unfed O. niloticus and O. mossambicus fry, from individual egg clutches were significantly ($P < 0.001$) influenced by mean egg weight of the clutch (Fig. 3.7). The yolk reserves of newly hatched fry from older females (producing bigger eggs) were considerably heavier than those of younger females (producing smaller eggs). For example, three day old fry from 2+ O. niloticus and O. mossambicus females possessed 171% and 91% more yolk, respectively, than fry from 0+ females (Table 3.10). Over the same period, fry from 0+ females of both species gained a higher proportion of their maximum body weight than fry from older females. Further, early growth was largely in length rather than weight (Table 3.10).

The time taken for fry to utilize all their yolk reserves differed between the two species and was dependent upon maternal age and hence egg size. In O. niloticus fry from 0+ females the yolk was consumed within 9 days of hatching and coincided with the time of maximum body growth, while fry from older females had sufficient yolk to last for a further three days past this time. In O. mossambicus, fry from 0+ females, however, the end of yolk-sac stage occurred on day 12; three days after maximum growth, compared to day 18; six days after maximum growth for fry from 2+ females (Figs. 3.5 and 3.6 and Table 3.10). The reasons for these longer yolk-sac periods in O. mossambicus fry are unclear. During the present study, however, it was observed that the swimming activities

of O. mossambicus fry were considerably less than O. niloticus fry as the yolk reserves approached exhaustion. This quiescence, resulting in a reduced metabolic demand, may in part account for the longer yolk-sac period in O. mossambicus compared with O. niloticus fry.

The mean weight and length of newly emergent fry of both species were not significantly ($P > 0.05$) influenced by egg size. In contrast, Barton (1981) reported a significant correlation between body length of newly emergent larvae and egg size in turbot, Scophthalmus maximus. In the present study, however, body length and weight increased significantly ($P < 0.01$) with egg size by six days after hatching in both species and at maximal body weight 94.3% and 89.7% of the variation in growth was attributable to egg size in O. niloticus and O. mossambicus, respectively (Fig. 3.7). At maximum growth the mean body weight of fry from 2+ O. niloticus and O. mossambicus females were 150% and 117% heavier, respectively, than that of fry from 0+ females. This positive correlation between fry size and egg size has also been reported for Atlantic salmon (Glebe et al., 1979; Thorpe et al., 1984), Arctic char, Salvelinus alpinus (Wallace and Aasjord, 1984), jack mackerel (Theilacker, 1981), herring (Blaxter and Hempel, 1963), and rainbow trout (Springate and Bromage, 1985).

If body weight alone is used as a measure of the effect of egg size on the growth performance of yolk-sac fry, changes in growth rate during rapid fry development may be omitted. For this reason SGR was also calculated (at three day intervals) to determine more clearly when the finite endogenous yolk reserves first become

limiting (Table 3.11). The SGR, which measured the rate of change in body weight, declined rapidly with time as the metabolic demands of the growing body on the finite yolk reserves increased. The growth rates of fry between days 6 to 12 are of interest. During the 6-9 day period, when the fry may still be reared in their parents' buccal chamber, the greater yolk reserves of O. niloticus and O. mossambicus fry from older broodfish will enable them to maintain a higher growth rate than that of fry from younger broodfish. When the fry ceased growing and began losing weight, (i.e. from day 9 onwards) the rate of body resorption during starvation was found to increase with egg size, which was probably due to their higher metabolic demand (Table 3.11).

With the exception of fry from 0+ O. mossambicus females, the rate of mortality following the end of the yolk-sac stage was rapid (Fig. 3.9). In O. mossambicus fry, the rapid rate of fry mortality coincided with yolk exhaustion, whereas in O. niloticus fry yolk was completely utilized 2-3 days prior to mass mortality. The delay in the onset of mortality to yolk exhaustion in O. niloticus may be due to the greater body reserves of fry which were larger than those of O. mossambicus fry.

In addition to the prolonged yolk-sac stage of larger fry, fry with heavier bodies derived from larger eggs were able to draw upon greater nutrient reserves and thereby significantly ($P < 0.001$) extend their survival times (Fig. 3.10). On a species basis, the lower survival times (ST_{50}) recorded for O. niloticus fry were probably due to the greater metabolic demand of their larger biomass and

the greater swimming activities observed during starvation compared with O. mossambicus. The trend of prolonged survival times of fry from larger eggs found here is in general agreement with the findings of earlier studies (Blaxter and Hempel, 1963; Bagenal, 1969b; Gall, 1974; Ware, 1975; Theilacker, 1981; Beacham et al., 1984). In contrast, no similar association between fry survival and egg size was observed for Argentine anchovy (De Ciechowski, 1966), Japanese medaka (Stanley, 1977) and chinook salmon (Fowler, 1972). The variability of the results of these authors may be due to differences between species or to the use of unfiltered water in which food particles may be present. Also, Springate, Bromage, Elliott and Hudson (1984) and Craik and Harvey (1984) suggest that in salmonids, the state of ripeness of stripped eggs may influence fry mortality.

The relevance of egg size to fry growth in O. niloticus and O. mossambicus should be evaluated together with the mouth-brooding strategy of these species. In the present study it was found that the time taken for O. niloticus and O. mossambicus females to first release their broods ranged from 12-18 and 12-16 days after spawning, respectively (see Chapter 4), which coincides with the most critical phase of fry growth (Fig. 3.10). During this buccal rearing period fry of both species and from all three broodfish age-classes may reach their maximum growth and may also begin to lose weight due to starvation (Table 3.11).

The practical management implications of delaying the time of irreversible release of fry on their growth and survival are considered in Section 3.4.

3.2.4.2 Influence of female age and hence egg size on the feeding capabilities of previously unfed fry

It has been suggested that the transition to exogenous feeding may be related to full swimbladder inflation (Doroshev et al., 1981). Doroshev et al., who reared O. mossambicus at 25°C, reported that the swimbladders of fry became fully inflated six days after hatching. In the present study buoyancy in both species was attained by days 5-6 at 27°-28°C and corresponded with the commencement of external feeding (Fig. 3.8). Commencement of exogenous feeding in fry was not affected by maternal age-class and hence egg size. The timing of this event demonstrates its significance to growth. The rapid decline in the growth rate of both species in the 6-9 day period (Table 3.11) suggests that most of the nutritional reserves are being used for maintenance rather than further growth. Therefore, if the commencement of feeding is delayed until after six days (i.e. 10 days after spawning at 27°-28°C) the fry may begin to starve. This may occur even when fry are still being reared naturally. Of interest is the maximum feeding capability of the fry (Fig. 3.8). The maximum number of fry capable of exogenous feeding was approximately 15% and 25% higher for fry from 1+ and 2+ females compared with fry from 0+ females in O. niloticus and O. mossambicus, respectively. In addition, the greater yolk reserves of fry from 2+ females delayed irreversible starvation (PNR) by four days compared with fry from 0+ females (Fig. 3.8), thereby affording fry from larger eggs a longer opportunity to locate food.

The ages of fry at swim-up, onset of feeding, maximum feeding and PNR however, occurred earlier at higher rearing temperatures (Section

2.2). Therefore under hatchery conditions artificially reared fry from small eggs may succumb to starvation stress even earlier at higher temperatures if not presented with food. Similarly, the likelihood of starvation may also be increased under natural rearing conditions if parents brooding fry from smaller eggs do not release their brood earlier for feeding.

3.3 The Influence of Egg Size on the Growth and Survival of O. niloticus and O. mossambicus Fry Fed on an Artificial Diet

3.3.1 Introduction

In the previous trial of the present study it was found that growth and survival of O. niloticus and O. mossambicus fry can be influenced significantly by egg size. It is not clear, however, whether egg size confers any continual or long-term growth or survival advantages to the fry. Some authors report that the initial growth advantages conferred on fry by larger yolk reserves are rapidly obscured during fry growth (Kincaid, 1972; Zonova, 1973; Reagan and Conley, 1977; Siraj et al., 1983; Thorpe et al., 1984; Springate and Bromage, 1985). In contrast, studies on rainbow trout by Millenbach (1950) and Pitman (1979) showed that initial advantage of egg size persisted through early life.

Therefore to complement the trials reported in Section 3.2 a study was conducted on the long term influence of egg size on the growth performance and survival of fry under carefully controlled hatchery conditions.

3.3.2 Materials and Methods

3.3.2.1 Supply of eggs

Oreochromis niloticus and O. mossambicus females from 0+, 1+ and 2+ age-classes were selected at random, tagged individually and then stocked in 2m diameter circular spawning tanks. To minimise

paternal effects on fry growth only one male was introduced into the spawning tank for each species. The fish were fed ad libitum and allowed to spawn naturally. Daily checks were made for brooding females. Eggs were collected within 12 hours of spawning from individual females and incubated in round-bottomed containers at $27.5 \pm 0.5^\circ\text{C}$ as described in Section 2.1. A random sample of 50 eggs was removed from each clutch for egg size determination as described in Section 3.2. Egg collection was terminated when spawnings from four different females of each age-class were obtained.

3.3.2.2 Experimental procedure

Yolk-sac fry were removed from the incubation containers prior to the swim-up stage and stocked in duplicate at 2/1 in 48-20 l plastic tanks connected to a recirculated water system. At least 20% of the water was replaced each week with clean, preheated water.

The fry were presented initially with powdered ($<500\mu\text{m}$) diet (Pellet No.3: Edward Baker, Bathgate, Scotland) in excess six times a day. After two weeks the particle size of the diet was increased to $500-1000\mu\text{m}$ and the feeding frequency was decreased to four times per day. Fry were weighed at 20 day intervals from hatching on a digital top pan balance (Mettler PC4400) to an accuracy of 0.01g. Prior to weighing the fish were held in a net suspended in the rearing tanks and starved for 12 hours to allow the contents of their guts to evacuate.

The survival of fry in each group was monitored daily.

3.3.2.3 Biometrics

The mean specific growth rate (SCR - % weight change/day) of 20, 40 and 60 day old fry from all three age-classes was compared statistically using a computerised one-way analysis of variance method (Minitab, Pennsylvania State University). In addition, the data for mean fry weights at 20, 40 and 60 days were pooled from all age-classes and analysed against their mean dry egg weight using a computerised least-squares regression method (Minitab, Pennsylvania State University) for each species.

3.3.3 Results

Each batch of fry was reared for only 60 days due to the early maturity of these species which occurred at an age of 50 - 60 days.

Calculation of correlation coefficients revealed no significant association between mean fry survival and mean egg size in O. niloticus and O. mossambicus ($r = 0.132$ and 0.169 , respectively, with $df = 10$; $P > 0.05$).

For both species the weight of 20, 40 and 60 day old fry was strongly associated with egg size (Table 3.12). After the first 20 days, however, the degree of association, although remaining significant,

TABLE 3.12

Degree of association (r^2) between egg size and fry weight, showing the prolonged effect of egg size on early growth in O. niloticus and O. mossambicus fry

	n	Age after hatching (days) ¹		
		20	40	60
<u>O. niloticus</u>	12	0.856***	0.623*	0.586*
<u>O. mossambicus</u>	12	0.894***	0.635*	0.570*

¹ Significance levels of r^2 (coefficient of determination)

- * P < 0.05
- ** P < 0.01
- *** P < 0.001

TABLE 3.13

Mean specific growth rates¹ of O. niloticus and O. mossambicus fry from 0+, 1+ and 2+ female broodfish

Species	Age-class (months)	Mean dry egg weight (mg)	Growth period (days after hatching) ^{2,3}		
			20	40	60
<u>O. niloticus</u>					
	0+ (5-7)	1.93	16.9 (1.0) ^a	11.0 (0.3) ^a	9.5 (0.5) ^a
	1+ (11-13)	2.79	18.1 (0.4) ^b	11.9 (0.7) ^{a,b}	10.1 (0.4) ^a
	2+ (24-26)	3.68	20.9 (0.9) ^c	12.3 (0.4) ^b	10.0 (0.5) ^a
<u>O. mossambicus</u>					
	0+ (7-10)	1.30	15.6 (0.8) ^a	11.0 (0.4) ^a	8.7 (0.3) ^a
	1+ (15-16)	1.70	18.6 (0.3) ^b	11.6 (0.3) ^a	8.8 (0.6) ^a
	2+ (22-24)	2.43	19.9 (0.5) ^c	11.5 (0.4) ^a	9.4 (0.5) ^a

¹ Specific growth rate (SGR, %/day) see footnote Table 3.11

² Standard errors of the means are given in parenthesis

³ Figures with the same superscript are insignificantly different (P > 0.05)

decreased with time. This decrease in the proportion of variation in growth explained by egg size was also reflected in the SGR (Table 3.13). At 20 days the SGR of fry between all three age-classes were significantly different ($P < 0.05$), being highest for fry from 2+ females (Table 3.13). By 60 days even though the growth rate of fry showed an increasing trend with female age the differences were not significant ($P > 0.05$).

3.3.4 Discussion

The results of this trial suggest that the initial advantages in growth due to egg size extend into post yolk-sac fry. The growth rate and the absolute size of 20 day old fry were significantly higher for fry derived from larger eggs (Tables 3.12 and 3.13). There was, however, a reduction of the initial advantages in growth rate later in development. In both species, the SGR of 60 day old fry from all three age-classes of females was similar ($P > 0.05$). The earlier advantages in higher SGR on absolute fish size, however, enabled the association between egg size and weight of 40 and 60 day old fry to remain significant, although at a lower level of probability (Table 3.12).

In contrast Siraj et al. (1983) reported that the length of 20 and 40 day old O. niloticus fry from three age-classes of females were not significantly different, a result probably reflecting on the thrice daily ad libitum feeding regime and a stocking density of 7/1. In the present study, fry were stocked at a density of 2/1 and fed in excess six times daily from the age when they were first

capable of ingesting food to ensure that food was not limiting (see Sections 2.2 and 3.2).

Early maturity in hatchery reared O. niloticus and O. mossambicus fry may also obscure the effect of egg size on fry growth. In the present trial there was evidence of sexual maturity by 40 days after hatching and the strong territorial behaviour of males in tanks resulted in an increase in fish-size variation. In addition, the variability in sex ratios between tanks and spawns may have also affected mean fry size. This may explain the declining degree of association observed between egg size and fry growth during the trial (Table 3.12).

The decline of early advantages in fry growth resulting from egg size has been reported for other fish species. Under similar rearing conditions, Hayes and Armstrong (1942) noted that size differences in Atlantic salmon fry resulting from egg size were obscured after 35 days, while Thorpe et al. (1984) reported that the size advantage at first feeding was lost by the end of the first growing season. In channel catfish such advantages were lost after 30 days (Reagan and Conley, 1977).

Of particular interest to hatchery producers may be in the finding that fry survival was not affected by egg size. This is in agreement with studies on Atlantic salmon (Glebe et al., 1979; Thorpe et al., 1984) and carp (Zonova, 1973; Tomita, Iwahashi and Suzuki, 1980). Fowler (1972), on the other hand, reported reductions in the survival of chinook salmon fry derived from larger eggs.

3.4 The Influence of Delayed Initial Feeding on Growth and Survival of *O. niloticus* and *O. mossambicus* Fry from Different Maternal Age-Classes

3.4.1 Introduction

In *O. niloticus* and *O. mossambicus* fry the maximum body weight and the period of maximum exogenous feeding occur in advance of complete yolk exhaustion (Section 3.2). During this critical ontogenic period associated with the transition from yolk nutrition to exogenous feeding, the growth and survival of tilapia fry, like other fish larvae, may closely follow the quantity and quality of available food (Gamble, MacLachlan and Seaton, 1985; Hewitt et al., 1985). This together with the mouth-brooding mode of parental care of species within the genus *Oreochromis* has profound implications for hatchery production of high quality tilapia fry.

Information regarding the vulnerability of fish larvae to periods of total or partial food deprivation is well documented for species of commercial importance (Blaxter, 1963; Hempel and Blaxter, 1963; O'Connell and Raymond, 1970; Wyatt, 1972; Bilton and Robins, 1973; Gamble et al., 1985; Hewitt et al., 1985). It has been shown that the growth potential of fry subjected to delayed initial feeding may be seriously impaired. For example, Bilton and Robins (1973) reported that sockeye salmon fry fed only for the last eight weeks out of their first 12 weeks had significantly lower weights and lengths than fry fed throughout the 12 weeks. Further, May (1971) reported that in the grunion, *Leuresthes tenuis*, the growth rate of larvae decreased with delay of initial feeding. In addition,

the tolerance of larvae or fry to food deprivation may be related to rearing temperature (Blaxter and Hempel, 1963) and may also be related to the quantity of yolk reserves in eggs (Blaxter and Hempel, 1963; Hempel and Blaxter, 1963).

Outwith the salmonids, information on the effects of delayed initial feeding on fry growth and survival is lacking for freshwater species and especially for the Oreochromis species. Many authors have observed that the period of oral incubation of O. niloticus and O. mossambicus females can vary between individuals and between successive broods and a range within 10-21 days is common (Baerends and Baerends-Van Roon, 1950; Hofstede and Botke, 1950; Panikkar and Tampi, 1954; Russock and Schein, 1977). Consequently, during the final phase of maternal rearing feeding opportunities of orally reared fry may be reduced. Thus the growth potential of these fry may not be fully realised, particularly if they are fry from small eggs. For example, in the present study it was found that the SGR of fry from small eggs (i.e. from 0+ females) decreased faster during days 6-9 compared with fry from large eggs (i.e. from 2+ females) (Section 3.2).

In view of the implications of oral incubation in the context of hatchery practices, the effects of delaying initial feeding on the growth and survival of O. niloticus and O. mossambicus fry from eggs of three age-classes of females were evaluated.

3.4.2 Materials and Methods

3.4.2.1 Design and maintenance of the fry rearing system

Four independent rearing units, each consisting of two rows of six 2 l water-tight compartments were set up as shown in Fig. 3.11. In order to reduce disturbance of the fry the rearing units were surrounded with black polythene sheeting.

To minimise extraneous food material entering the rearing units from the biofilters or header tank, a filter trap and a secondary header tank were incorporated into the design. These were covered with black polythene sheeting to exclude algal growth. The filter trap was cleaned and the filter medium (polymer filter wool; Armitage Bros., Nottingham, England) changed weekly.

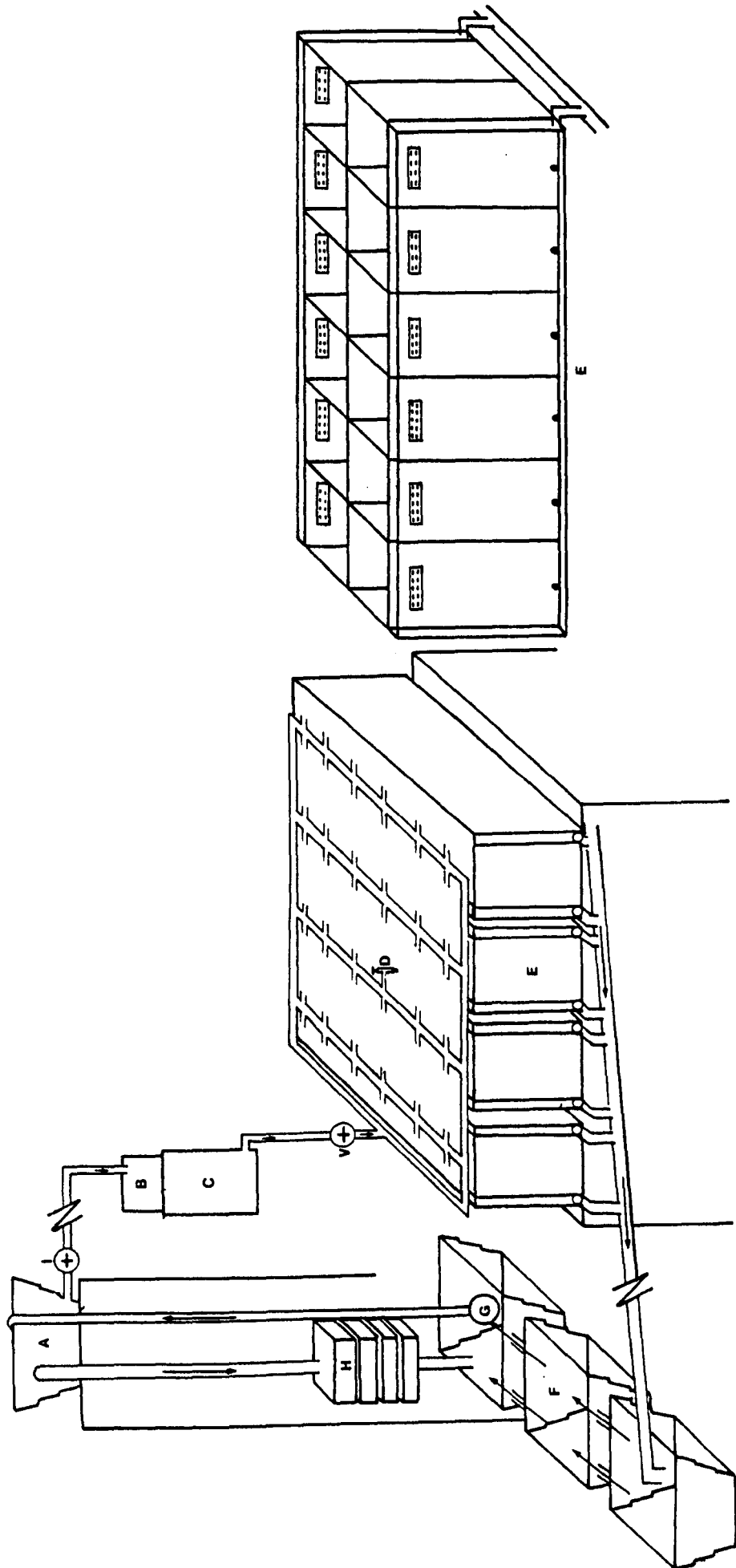
A common water ring main leading from the secondary header tank provided water at uniform pressure to all compartments. The flow rate to each compartment was controlled with a modified two-way aquarium air valve. A preliminary trial was conducted to determine the optimum stocking density and flow rate for the system for maximum fry growth and survival. These were 10 fish/l and 100ml/min and were maintained during the experiment.

The water temperature was maintained at $27.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ with five 200 watt thermostatically controlled heaters submerged in the header tank and by heating the fry room with a 2kw thermostatically controlled fan heater. Aeration was achieved by placing two 15cm air

FIGURE 3.11

Layout of the recirculatory system and rearing units used in the delayed initial feeding trials. Drawing not to scale. Arrows indicate direction of water flow.

- A - 125 l primary header tank
- B - 5 l filter trap
- C - 20 l secondary header tank
- D - Valves to individual 2 l compartments
- E - Rearing units
- F - 225 l sedimentation tanks
- G - Pump
- H - Percolating filter trays
- I - Control valves



stones connected to a compressed air supply in the secondary header tank. The hatchery was illuminated by six 60 watt fluorescent lamps controlled to provide a 12 hour day : 12 hour night photo-regime.

3.4.2.2 Source of eggs and fry

To obtain eggs of various sizes naturally fertilized egg clutches were collected from individual 0+, 1+ and 2+ O. niloticus and O. mossambicus females which were stocked at a ratio of 3 females : 1 male in 2m diameter spawning tanks. The eggs were removed gently from the buccal cavity of brooding females and reared artificially in round-bottomed containers for five days as described previously (Section 2.1). A random sample of 50 eggs was removed from each clutch, oven dried and weighed for egg size determination as described previously (Section 3.1.2.3).

3.4.2.3 Experimental design

To determine the effect of delayed initial feeding on the growth and survival of fry, five day old fry reared from each egg clutch of four individual 0+, 1+ and 2+ females were allocated to separate rows within the rearing units. Twenty five day old fry were transferred into each of the first five compartments of the row and any surplus into compartment six, which served as a 'supply'.

Any dead fry were replaced from the 'supply' compartment of the row prior to commencement of the trials which began the next day. The age at which fry in the compartments of each row were fed was

delayed progressively. Fry were initially fed at six days after hatching (the time when they were first capable of ingesting food, at 28°C) and 9, 12, 15 and 18 days after hatching.

They were fed in excess four times a day with a ground and sieved (<500µm) commercial diet (Pellet No. 3; Edward Baker Ltd., Bathgate, Scotland); uneaten food and faeces were siphoned out daily. The feeding trials were terminated when the fry were 20 days old.

Prior to each feed each container was inspected for mortalities; dead fry were removed with a pipette and recorded.

3.4.2.4 Sampling procedure and biometrics

To obtain initial weights for the fish, 20 six day old fry were removed from the 'supply' compartment, killed in benzocaine (1:10,000 solution in water) and rinsed in distilled water. The yolk-sacs (if present) of half the sample were removed and the body and fry (body + yolk, if present) weighed to an accuracy of 0.1mg on a Mettler balance (H80) as described previously (Section 2.2.2.2).

At the end of the trial, the surviving fry from each compartment were transferred into clean water and allowed four hours to evacuate the contents of their gastrointestinal tract before they were killed in benzocaine. Their standard lengths and weights were measured to an accuracy of 0.1mm and 0.1mg, respectively, with a calibrated dissecting microscope and a pan balance (Mettler H80).

In order to evaluate the effects of delaying initial feeding on the growth potential of fry originating from the three age-classes, the specific growth rate was calculated between six and 20 days after hatching, irrespective of the period of feeding, using the formula:-

$$\text{SGR (\%/day)} = \frac{\log_e W_{20} - \log_e W_6}{\Delta T}$$

where W_6 = mean wet weight (mg) of six day old fry
 W_{20} = mean wet weight (mg) of 20 day old fry
 ΔT = 14 days (the trial period)

The rate of change in specific growth rate between fry from three maternal age-classes was compared according to the co-variance method described by Sokal and Rohlf (1969).

In addition, to compare the condition of the fry from the various treatments Fulton's condition factor (K) (Bagenal and Tesch, 1978) was determined according to the following:-

$$K = \frac{W100}{L^3}$$

where W = mean wet weight (mg) of 20 day old fry
 L = mean standard length (mm) of 20 day old fry

Probit analysis, following the reasoning of Sprague (1969), was applied to determine the impact of delaying initial feeding on final mean fry survival.

3.4.3 Results

3.4.3.1 Growth of fry

The mean wet weights and mean standard lengths of 20 day old O. niloticus and O. mossambicus fry from the delayed initial feeding trials are given in Table 3.14, and presented graphically in Fig. 3.12. The mean SGR (%/day) of 20 day old fry are shown in Table 3.15 and Fig. 3.13.

Oreochromis niloticus fry from 0+, 1+ and 2+ females initially fed six days after hatching attained mean weights of 79.3mg, 126.8mg and 191.8mg and mean lengths of 13.6mm, 15.4mm and 17.6mm, respectively, on day 20. As initial feeding was delayed for up to 12 days after hatching, mean final weights and lengths decreased linearly to 11.9mg, 31.6mg and 68.7mg and 8.5mm, 10.9mm and 13.3mm, respectively (Table 3.14; Fig. 3.12(a)).

Similar trends were observed for O. mossambicus fry from the three maternal age-classes, but their mean weight was lower than equivalent O. niloticus fry. Fry from 0+, 1+ and 2+ O. mossambicus females initially fed six days after hatching attained mean weights of only 18.1mg, 41.4mg and 48.1mg and mean lengths of 9.9mm, 11.3mm and 11.9mm, respectively, which declined linearly to 6.9mg, 27.8mg and 27.4mg and 7.6mm, 10.2mm and 10.1mm as initial feeding was delayed progressively to 12 days after hatching (Table 3.14; Fig. 3.12(b)).

In addition, incremental biomass change of fry decreased linearly with delay in the time of initial feeding as indicated by the

TABLE 3.14

The influence of delayed initial feeding on the growth¹ of *O. niloticus* and *O. mossambicus* fry produced from different egg sizes derived from 0+, 1+, and 2+ female broodfish

Species	Broodstock age-class (months)	Mean dry egg weight (mg) \bar{x} (SEM)	Age of fry at initial feeding (days after hatching) ²									
			6		9		12		15		18	
			Length \bar{x} (SEM)	Weight \bar{x} (SEM)	Length \bar{x} (SEM)	Weight \bar{x} (SEM)	Length \bar{x} (SEM)	Weight \bar{x} (SEM)	Length \bar{x} (SEM)	Weight \bar{x} (SEM)	Length \bar{x} (SEM)	Weight \bar{x} (SEM)
<i>O. niloticus</i>	0+ (6-8)	1.73(0.04)	13.6(0.4)	79.3(6.5)	11.3(0.2)	38.4(2.3)	8.5(0.2)	11.9(1.4)	8.1(-)	8.4(-)	-	-
	1+ (12-14)	2.62(0.08)	15.4(0.3)	126.8(5.4)	13.4(0.3)	82.4(5.4)	10.9(0.1)	31.6(2.1)	9.2(0.3)	16.8(2.2)	-	-
	2+ (23-27)	3.53(0.06)	17.6(0.2)	191.8(5.2)	15.9(0.4)	139.7(10.4)	13.3(0.4)	68.7(9.1)	10.8(0.4)	33.1(3.4)	9.5(0.1)	18.9(1.4)
<i>O. mossambicus</i>	0+ (9-10)	1.10(0.08)	9.9(0.1)	18.1(0.4)	9.7(0.3)	14.2(0.8)	7.6(0.1)	6.9(0.4)	-	-	-	-
	1+ (16-17)	1.48(0.08)	11.3(0.3)	41.4(4.1)	10.7(0.2)	34.1(2.3)	10.2(0.2)	27.8(1.3)	8.9(0.5)	18.1(3.0)	-	-
	2+ (22-24)	1.93(0.07)	11.9(0.3)	48.1(4.5)	11.6(0.6)	44.0(5.8)	10.1(0.3)	27.4(3.1)	9.0(0.4)	20.4(3.2)	8.5(0.2)	14.1(0.4)

¹ Length and weight measured as standard length (mm) and wet weight (mg)

² Standard error of the mean (SEM) = $\frac{sd}{\sqrt{n}}$ where sd = standard deviation and n = no. of trials (4)

TABLE 3.15

The effect of delayed initial feeding on the mean specific growth rates (%/day)¹ of 20 day old O. niloticus and O. mossambicus fry from 0+, 1+ and 2+ females having different mean egg sizes

Broodstock age-class (months)	Species	Mean dry egg weight (mg) \bar{x} (SEM)	Age of fry at initial feeding (days after hatching) ²				
			6 \bar{x} (SEM)	9 \bar{x} (SEM)	12 \bar{x} (SEM)	15 \bar{x} (SEM)	18 \bar{x} (SEM)
0+							
(6-8)	<u>O. niloticus</u>	1.73 (0.04)	17.8 (1.5)	12.6 (1.4)	4.0 (1.2)	1.8 (-)	-
(9-10)	<u>O. mossambicus</u>	1.10 (0.08)	16.2 (0.5)	12.5 (0.9)	7.3 (0.8)	-	-
1+							
(12-14)	<u>O. niloticus</u>	2.62 (0.08)	18.8 (0.8)	15.8 (1.3)	8.9 (1.2)	4.2 (2.3)	-
(16-17)	<u>O. mossambicus</u>	1.48 (0.08)	19.2 (0.6)	17.4 (0.7)	16.0 (0.9)	12.8 (2.4)	-
2+							
(23-27)	<u>O. niloticus</u>	3.55 (0.06)	20.3 (0.6)	17.9 (1.0)	12.8 (2.2)	7.6 (2.1)	3.6 (1.1)
(22-24)	<u>O. mossambicus</u>	1.93 (0.07)	19.4 (0.9)	17.1 (0.8)	14.6 (1.5)	11.8 (2.6)	9.9 (1.0)

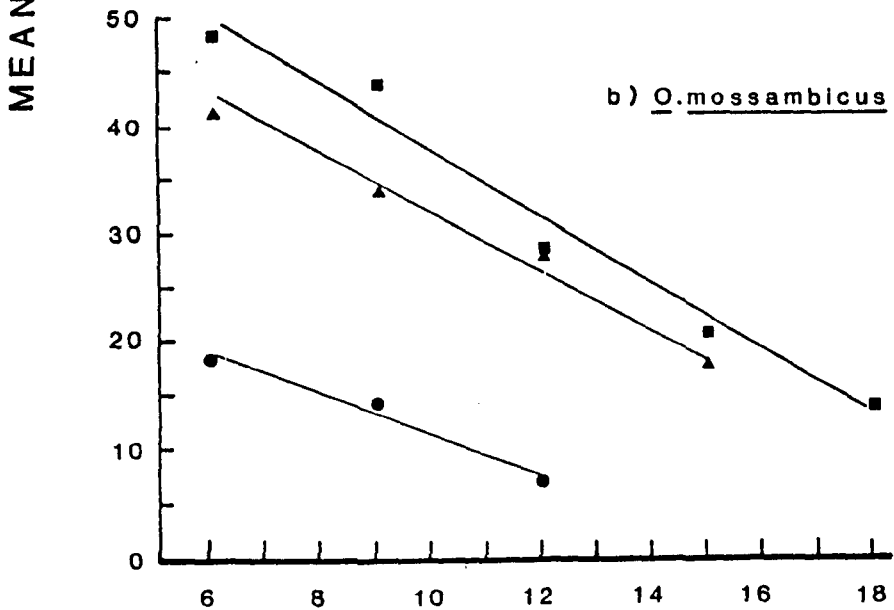
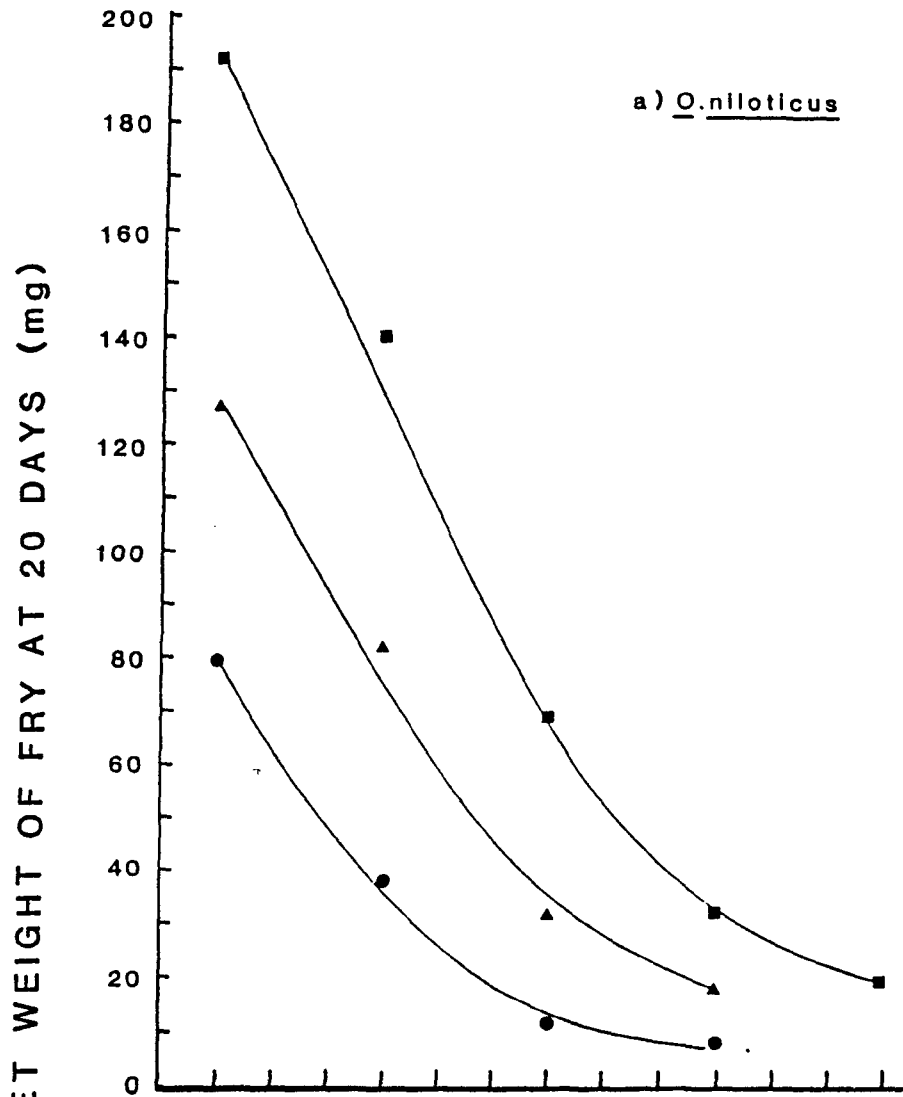
$$^1 \text{SGR (\%/day)} = \frac{\log_e W_{20} - \log_e W_6}{\Delta T}$$

where W_{20} = mean wet weight (mg) of 20 day old fry
 W_6 = mean wet weight (mg) of 6 day old fry
 ΔT = 14 days (the trial period)

$$^2 \text{Standard error of the mean (SEM)} = \frac{sd}{\sqrt{n}} \quad \text{where } sd = \text{standard deviation and } n = \text{no. of trials}$$

FIGURE 3.12

Effect on growth between 6 and 20 days post-hatching of delaying the time of initial feeding of previously unfed fry. (a) O. niloticus, and (b) O. mossambicus. Curves, fitted by eye, relate to fry from ●, 0+; ▲, 1+; and ■, 2+ females. Mean dry weights of eggs from 0+ (6-8 months), 1+ (12-14 months) and 2+ (23-27 months) O. niloticus, and 0+ (9-10 months), 1+ (16-17 months) and 2+ (22-24 months) O. mossambicus females were 1.73 mg, 2.62 mg and 3.55 mg, and 1.10 mg, 1.48 mg and 1.93 mg, respectively. Mean values based on clutches from four individual females per age-class.



AGE AT INITIAL FEEDING (days after hatching)

FIGURE 3.13

Influence of delaying the time of initial feeding on the mean specific growth rate between 6 and 20 days post-hatching of (a) O. niloticus, and (b) O. mossambicus fry. Lines, fitted by the method of least squares, relate to fry from ●, 0+; ▲, 1+ and ■, 2+ females. Mean dry weight of eggs from 0+ (6-8 months), 1+ (12-14 months) and 2+ (23-27 months) O. niloticus, and 0+ (9-10 months), 1+ (16-17 months) and 2+ (22-24 months) O. mossambicus females were 1.73 mg, 2.62 mg and 3.55 mg, and 1.10 mg, 1.48 mg and 1.93 mg, respectively. All relationships significant at $P < 0.05$. Mean values based on clutches from four individual females per age-class.

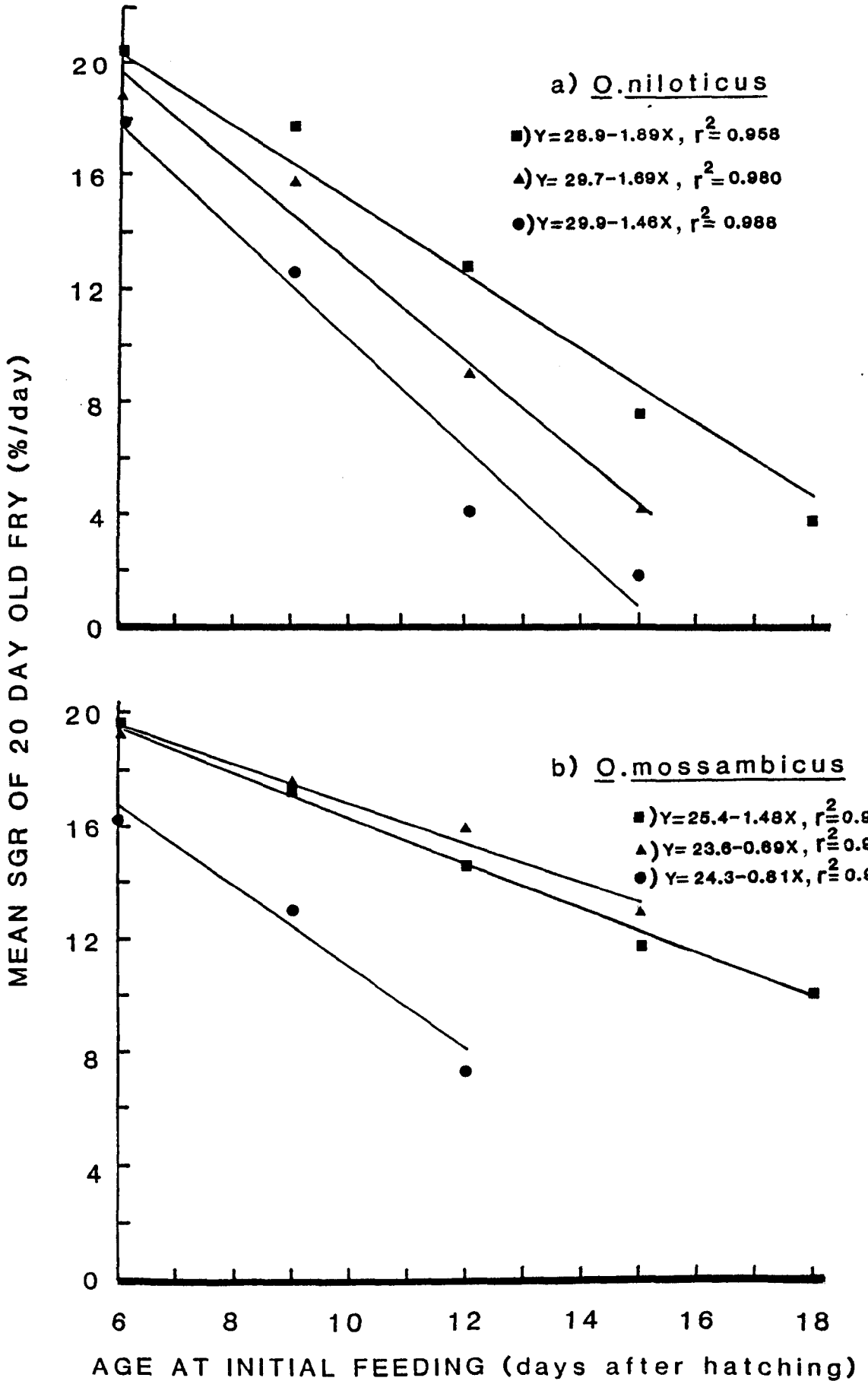


TABLE 3.16

Influence of delayed initial feeding on the mean condition (K values)¹ of O. niloticus and O. mossambicus fry from 0+, 1+ and 2+ female broodfish at 20 days post-hatching

Species	Broodstock age-class (months)	Mean dry egg weight (mg) \bar{x} (SEM)	Age of fry at initial feeding (days after hatching)				
			6	9	12	15	18
<u>O. niloticus</u>							
	0+ (6-8)	1.73 (0.04)	3.15	2.66	1.94	1.37	-
	1+ (12-14)	2.62 (0.08)	3.47	3.42	2.44	2.16	-
	2+ (23-27)	3.55 (0.06)	3.52	3.48	2.92	2.63	2.20
<u>O. mossambicus</u>							
	0+ (9-10)	1.10 (0.08)	1.87	1.52	1.57	-	-
	1+ (16-17)	1.48 (0.08)	2.87	2.78	2.62	2.55	-
	2+ (22-24)	1.93 (0.07)	2.85	2.82	2.66	2.54	2.30

¹ Condition factor (K) based on Fultons ratio:

$$K = \frac{100W}{L^3}$$

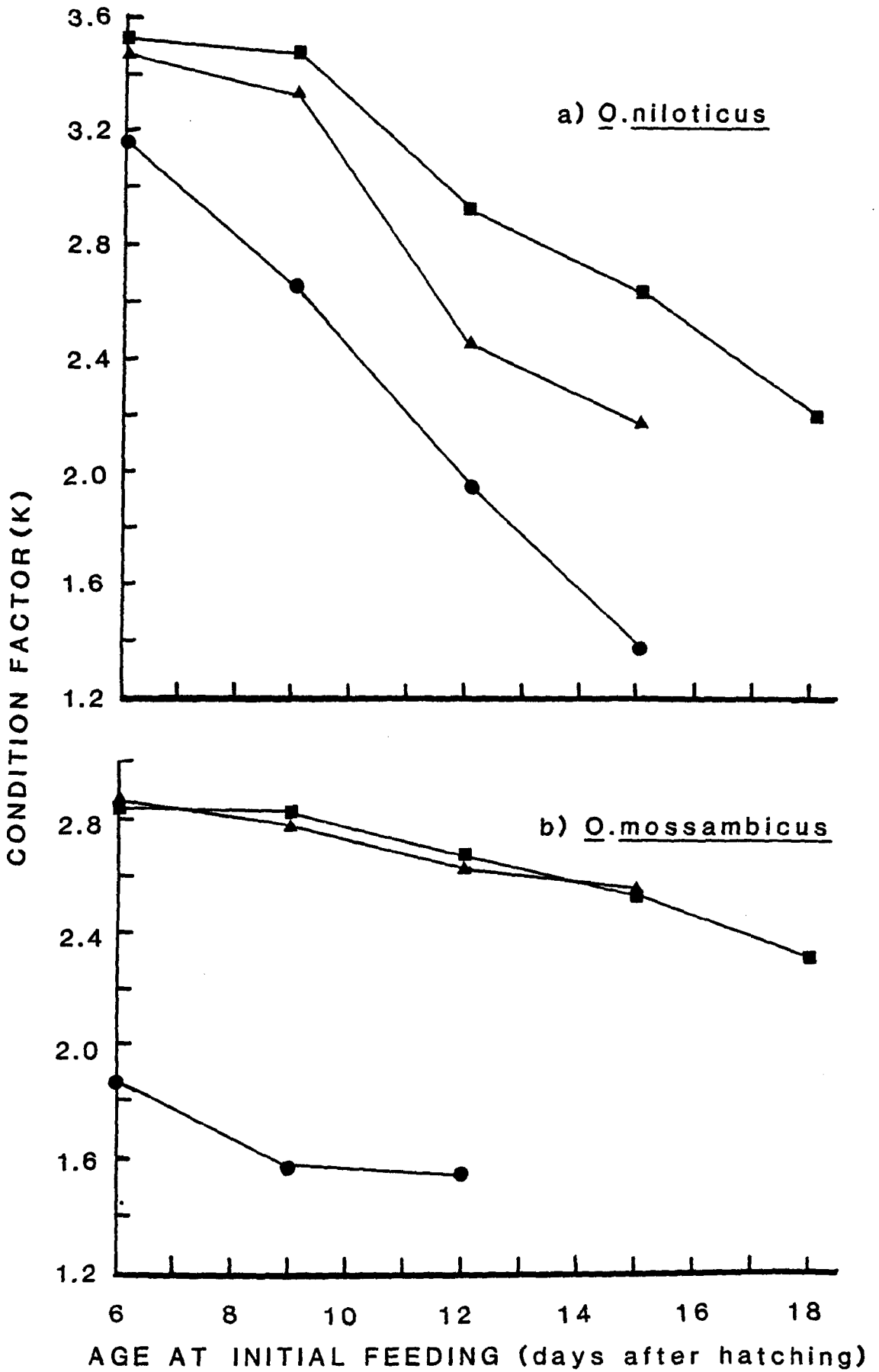
where W and L are wet weight (mg) and standard length (mm) respectively

FIGURE 3.14

The condition at 20 days post-hatching of fry which were initially fed at various ages. (a) O. niloticus, and (b) O. mossambicus. Curves, fitted by eye, relate to ● , 0+; ▲ , 1+; and ■ , 2+ females. Mean dry weight of eggs from 0+ (6-8 months), 1+ (12-14 months) and 2+ (23-27 months) O. niloticus, and 0+ (9-10 months), 1+ (16-17 months) and 2+ (22-24 months) O. mossambicus females were 1.73 mg, 2.62 mg and 3.55 mg, and 1.10 mg, 1.48 mg and 1.93 mg, respectively.

$$K = \frac{100W}{L^3}$$

where W = mean wet weight of fry at 20 days (mg),
L = standard length (mm)



negative slopes of the regression lines in Fig. 3.13. In O. niloticus the rate of decrease in the mean SGR's of 20 day old fry, resulting from delayed initial feeding, was significantly different ($P < 0.05$) between the three maternal age-classes, being highest for fry from 0+ females, producing the smallest eggs. Similarly, the rate of decrease in mean SGR's of 20 day old O. mossambicus fry from 0+ broodfish were significantly higher ($P < 0.05$) than fry from 1+ and 2+ females. No significant differences ($P > 0.05$), however, were found between fry from 1+ and 2+ O. mossambicus females (Fig. 3.13).

Similarly, the condition of fry from the three maternal age-classes also decreased as initial feeding was delayed beyond six days after hatching (Table 3.16 and Fig. 3.14). In both species, however, the rate of decline in fry condition, as indicated by the negative slopes of the lines relating weight and length to delayed initial feeding times, was higher for offspring from younger broodfish (i.e. small eggs). Further, under the various delayed initial feeding treatments fry from older females (i.e. larger eggs) were able to maintain a higher condition than fry from younger females (i.e. smaller eggs). These differences were clearer in O. niloticus than in O. mossambicus (Fig. 3.14).

Oreochromis niloticus fry from all three maternal age-classes had higher condition factors than O. mossambicus fry (Fig. 3.14).

3.4.3.2 Survival of fry

The temporal patterns of survival for fry from 0+, 1+ and 2+ O. niloticus and O. mossambicus females fed initially at 6, 9, 12, 15 and 18 days after hatching are shown in Figs. 3.15 and 3.16, respectively, and survival times (ST_{50}), estimated from the survival curves, are presented in Table 3.17.

The survival trends of O. niloticus and O. mossambicus fry from the same maternal age-classes were similar (Figs. 3.15 and 3.16). Throughout the trial period, over 80% of O. niloticus and O. mossambicus fry from all maternal age-classes survived a delay in initial feeding of six and nine days. Delaying the initial feeding for a further three days was critical for fry from 0+ O. niloticus and 0+ and 1+ O. mossambicus females and survival times (ST_{50}) were 14.5, 13.5 and 14.5 days, respectively (Table 3.17). For fry from 1+ O. niloticus females, however, a delay in the initial feeding of 15 days was critical. Over 50% of the fry from 2+ O. niloticus and O. mossambicus females survived. A delay in initial feeding of 18 days, however, was critical for fry from 2+ females of both species.

The final percentages of 20 day old fry from 0+, 1+ and 2+ O. niloticus and O. mossambicus females surviving the various periods of delay before initial feeding are presented as probit plots in Fig. 3.17. In both species, fry from the older females and hence larger eggs showed enhanced survival. Initial feeding at six days in O. niloticus fry from all three maternal age-classes and at 7.5,

FIGURE 3.15

Influence of delaying the time of initial feeding on the pattern of survival of O. niloticus fry between 6 and 20 days post-hatching. Fry from: (a) 0+ (1.78 mg eggs), (b) 1+ (2.62 mg eggs) and (c) 2+ (3.55 mg eggs) females. Age of fry at initial feeding: ○ , 6; □ , 9;

● , 12; ▲ , 15; and ■ , 18 days after hatching.

Mean values based on clutches from four individual females per age-class.

O. niloticus
(a)

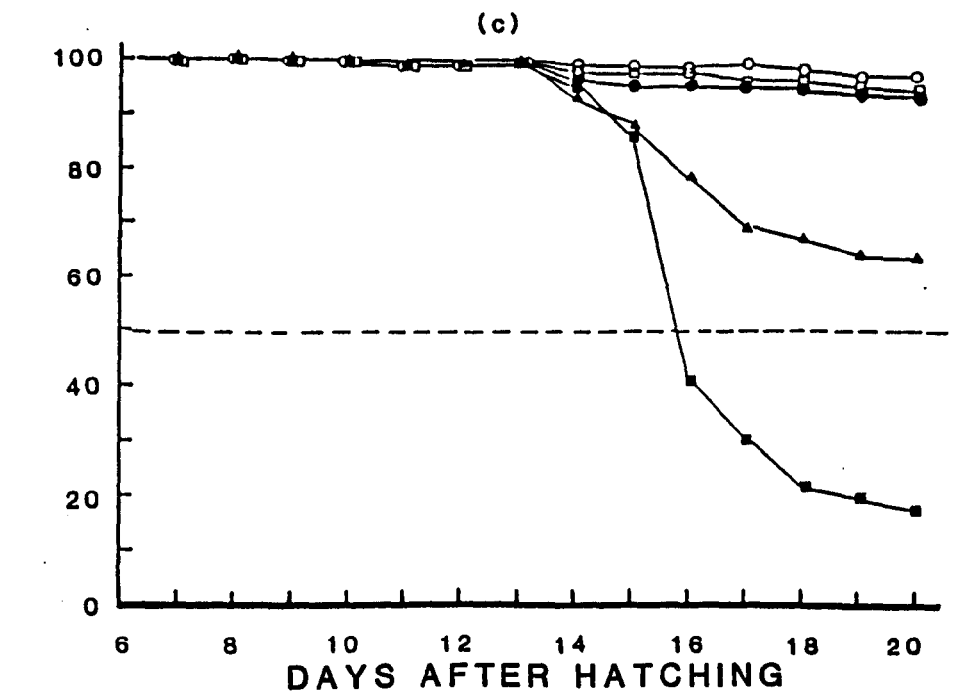
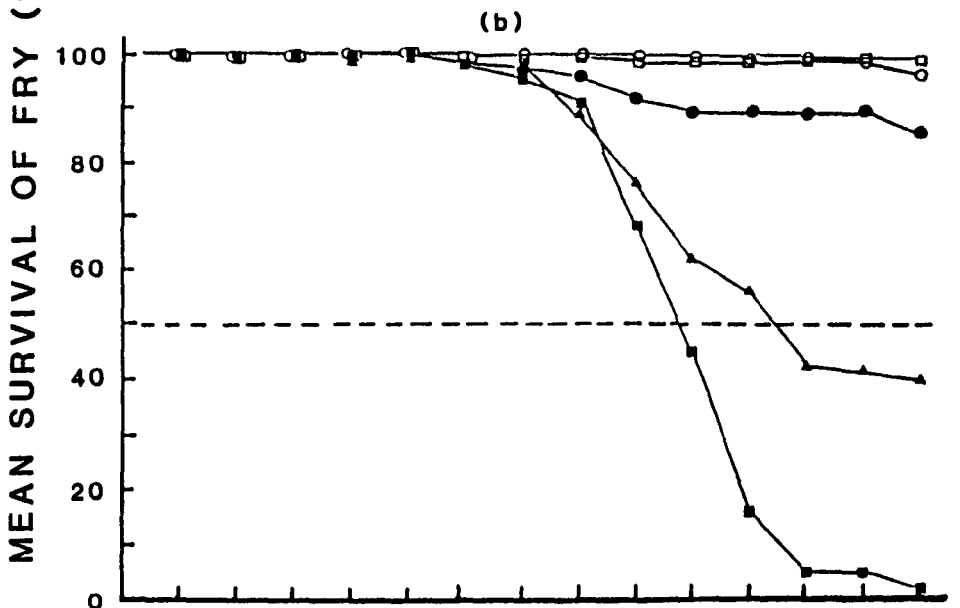
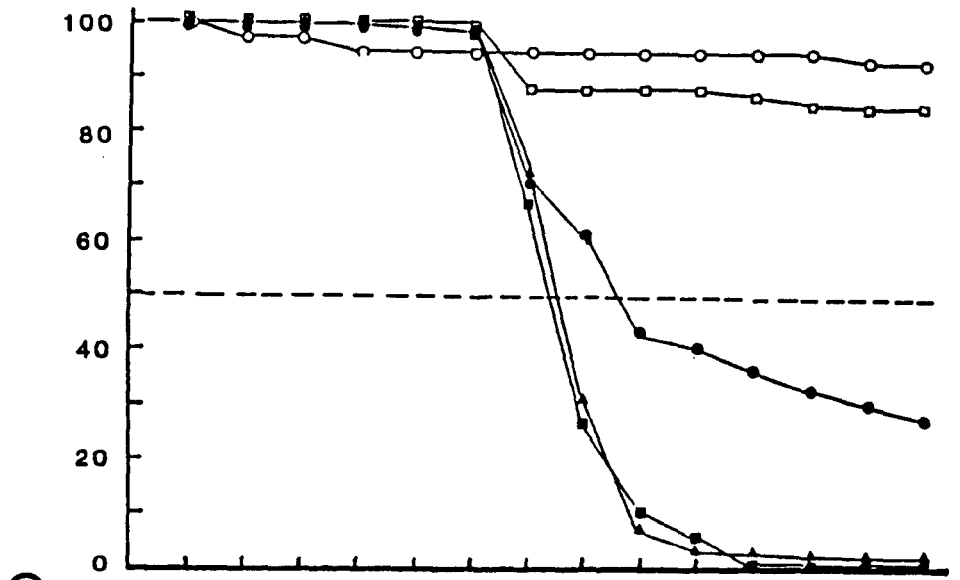


FIGURE 3.16

Influence of delaying the time of initial feeding on the pattern of survival of O. mossambicus fry between 6 and 20 days post-hatching. Fry from: (a) 0+ (1.10 mg eggs), (b) 1+ (1.48 mg eggs) and (c) 2+ (1.93 mg eggs) females. Age of fry at initial feeding: ○ , 6; □ , 9; ● , 12; ▲ , 15; and ■ , 18 days after hatching. Mean values based on clutches from four individual females per age-class.

O. mossambicus

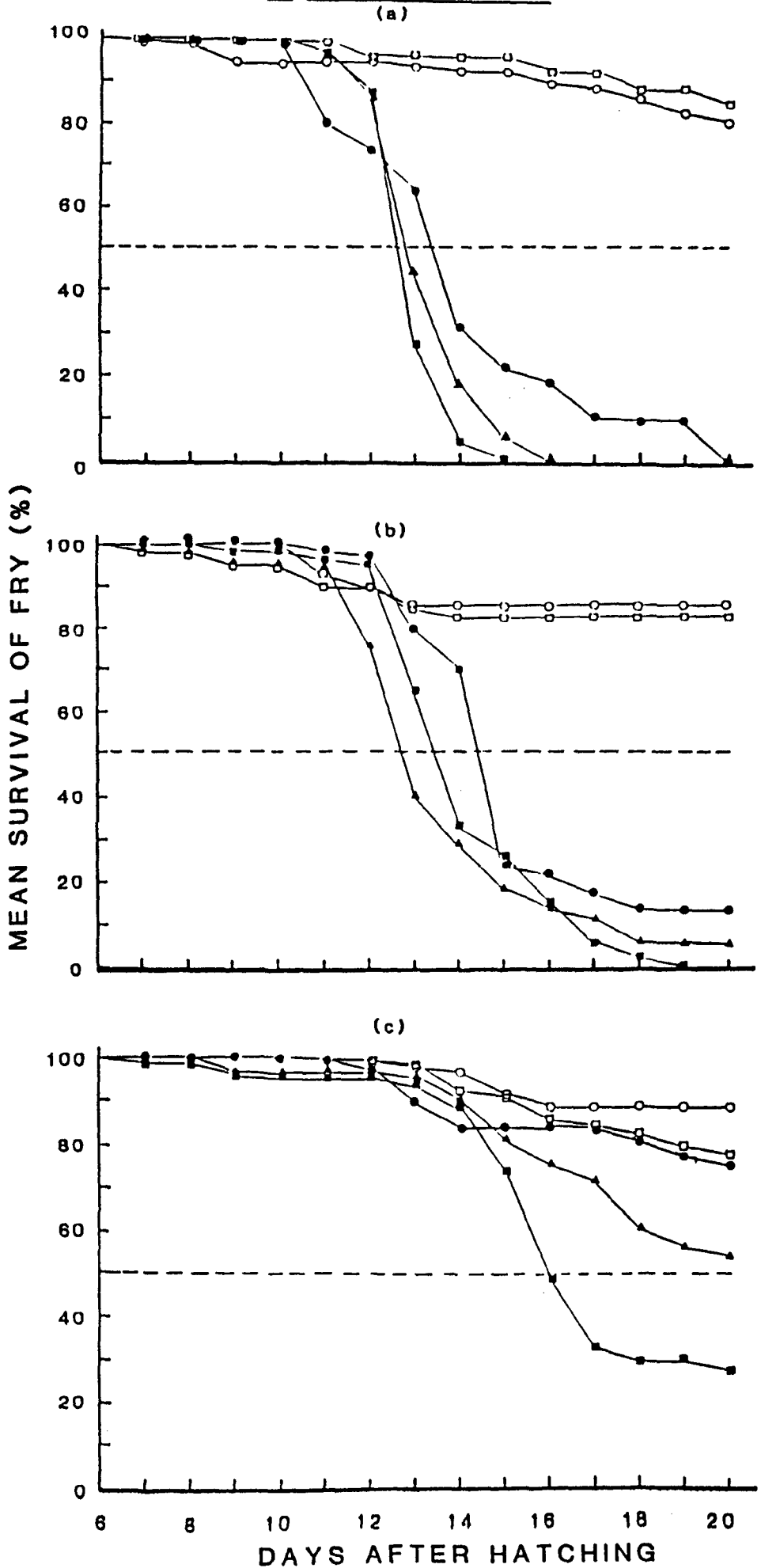


FIGURE 3.17

Effect of delaying the time of initial feeding on the overall mean survival of fry between 6 and 20 days post-hatching. (a) O. niloticus, and (b) O. mossambicus.

Curves, given as probit plots, relate to fry from ● , 0+; ▲ , 1+ and ■ , 2+ females. Mean dry weight of eggs from 0+ (6-8 months), 1+ (12-14 months) and 2+ (23-27 months) O. niloticus, and 0+ (9-10 months), 1+ (16-17 months) and 2+ (22-24 months) O. mossambicus females were 1.73 mg, 2.62 mg and 3.55 mg, and 1.10 mg, 1.48 mg and 1.93 mg, respectively. The intersection of the upper and lower broken lines indicate time of initial feeding of fry giving 90% and 50% survival, respectively. Mean values based on clutches from four individual females per age-class.

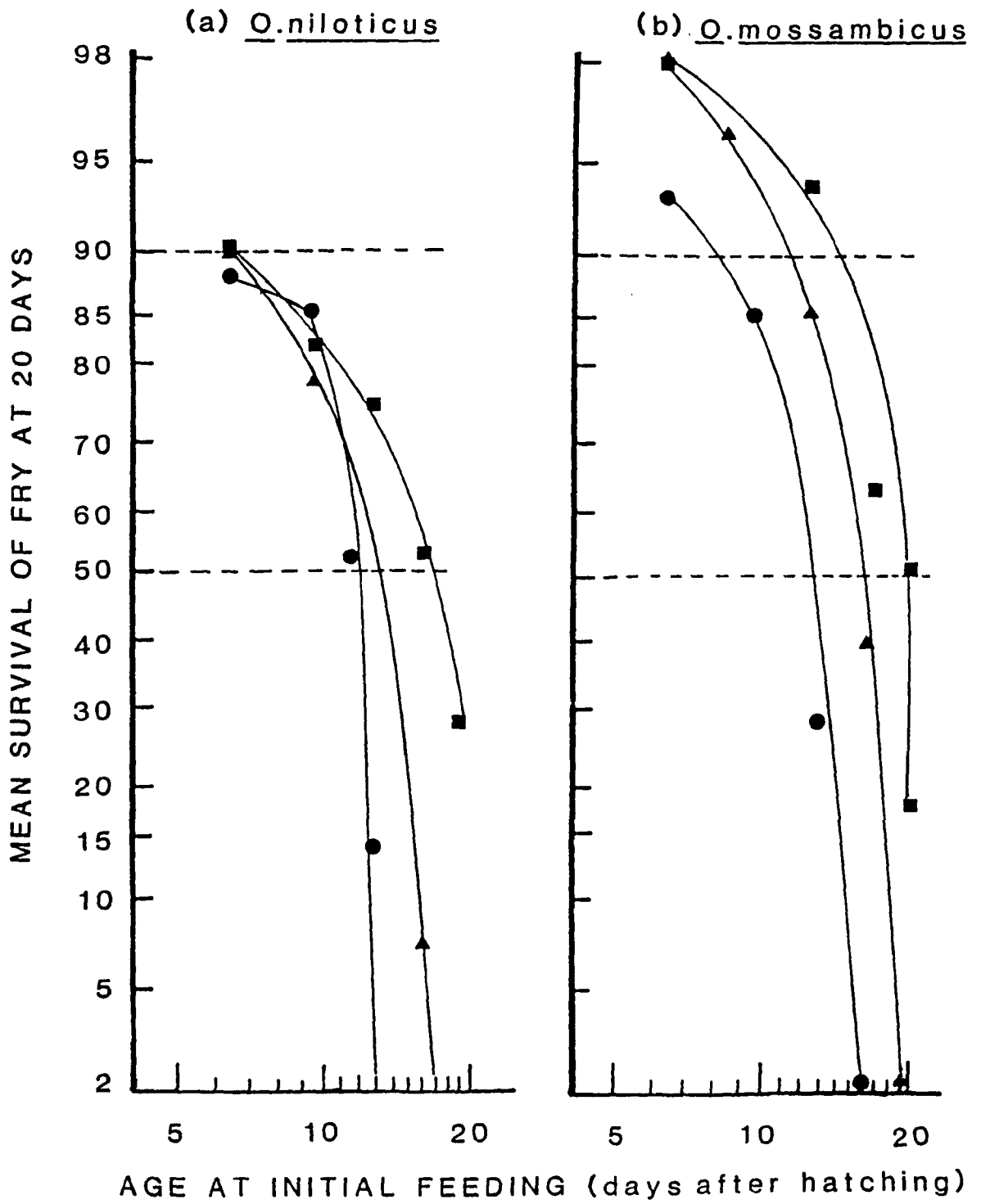


TABLE 3.17

The result of delayed initial feeding on the mean time to 50% survival (ST_{50})¹ of O. niloticus and O. mossambicus fry produced from 0+, 1+ and 2+ female broodfish²

Species	Broodstock age-class (months)	Mean dry egg weight (mg)	Age at first feeding (days after hatching)				
			6	9	12	15	18
<u>O. niloticus</u>							
	0+ (6-8)	1.73 (0.04)	-	-	14.5	13.5	13.5
	1+ (12-14)	2.62 (0.08)	-	-	-	17.5	15.5-16.0
	2+ (23-27)	3.55 (0.06)	-	-	-	-	15.5-16.0
<u>O. mossambicus</u>							
	0+ (9-10)	1.10 (0.08)	-	-	13.5	12.5-13.0	12.5-13.0
	1+ (16-17)	1.48 (0.08)	-	-	14.5	12.5-13.0	13.5
	2+ (22-24)	1.93 (0.07)	-	-	-	-	16.0

¹ ST_{50} values extrapolated from Fig. 3.15 and Fig. 3.16

² Trials were conducted for 20 days from hatching

11 and 13.5 days in O. mossambicus fry from 0+, 1+ and 2+ females, respectively, resulted in 88%-90% survival at the end of the trial period. Delaying initial feeding by 11, 12 and 16, and 12, 15 and 18 days in O. niloticus and O. mossambicus fry from 0+, 1+ and 2+ females, respectively, resulted in only 50% survival at the end of the trial (Fig. 3.17).

3.4.4 Discussion

3.4.4.1 The effects of delayed initial feeding on fry survival

In the teleost species in which parental investment in offspring is limited to production and shedding of gametes, it has been shown that larval survival is controlled by predation and starvation and during periods of food shortages by the interaction of these factors (O'Connell and Raymond, 1970; Shepherd and Cushing, 1980; Hunter, 1984; Hewitt et al., 1985). Recently, Hewitt et al. (1985) demonstrated that at sea the predation on pre-feeding larvae of jack mackerel accounted for daily mortalities of between 50%-80%, while starvation during exogenous feeding resulted in losses of about 45% per day. In contrast, the mouth-brooding tilapia species have evolved a precocial life style (Noakes and Balon, 1982) possibly a mechanism to reduce predation mortality on yolk-sac fry. Consequently, mortalities associated with starvation may be of greater importance in these species. If during natural rearing, however, the first release of fry by brooders is delayed, even by a few days, and if food is not immediately available or provided, the newly

released fry may then become weak and be highly susceptible to predation and cannibalism.

In the present study it was found that the survival of hatchery reared fry of both species over the first 20 days was dependent on their age at initial feeding as well as on maternal age and hence egg size (Figs. 3.15 and 3.16). In both species initial feeding of fry from 0+ females after nine days post-hatching did not halt mortalities. This suggests that even though these fry may be capable of ingesting food (Fig. 3.8) they may nevertheless die because of irreversible physiological damage to organs such as the liver and pancreas (Stroband and Dabrowski, 1979). In fry from 2+ females of both species this occurred with a delay in initial feeding of over 15 days from hatching.

The influence of delayed initial feeding on fry survival can be seen more clearly in Fig. 3.17. A delay in the initial feeding of O. niloticus and O. mossambicus fry from 0+, 1+ and 2+ females of 11, 12 and 16 days, and 12, 15 and 18 days, respectively, resulted in only 50% survival at the end of the trial. For commercial hatchery practice, however, a 90% survival rate of O. niloticus and O. mossambicus fry during their early development would be practically attainable if feeding of fry commences no later than five to six days from hatching, that is 9-10 days from spawning at 28°C. In addition, the overall survival may also be improved by the use of older broodstock which produce larger eggs (Fig. 3.17).

3.4.4.2 The effects of delayed initial feeding on fry growth

In teleost species where parental investment in reproduction is limited to the production and shedding of gametes, the growth of fry during and after the transition to exogenous feeding is determined largely by the abundance and quality of available food (Houde and Schekter, 1980; Hunter, 1981; Gamble et al., 1985). In mouth-brooding fish such as those within the genus Oreochromis, however, early fry growth may also be influenced by the length of the maternal oral rearing period.

Under hatchery conditions the growth performance of O. niloticus and O. mossambicus fry reared from the egg stage was found to be dependent on the period of delay to initial feeding as well as maternal age and hence the egg size. In both species the maximum growth rate of fry over the trial period was attained when fry were initially fed immediately after they were first capable of exogenous feeding (i.e. 5-6 days post-hatch, at 28°C). If initial feeding was delayed even by a few days fry growth declined (Fig. 3.12). Such trends were also found by May (1971) for grunion larvae and by Bilton and Robins (1973) for sockeye salmon. In addition, it was found in the present study that the rate of decrease in SGR of fry when subjected to various periods of delayed initial feeding was greater in fry from younger females (which produce small eggs) although this decrease varied between species (Table 3.15 and Fig. 3.13). For example, when O. niloticus and O. mossambicus fry from 0+ broodfish were initially fed at nine days post-hatching the mean weights of 20 day old fry decreased by 52% and 22%,

respectively, compared with only 27% and 9% for fry from 2+ broodfish (Table 3.14). Further, when initial feeding was delayed for 12 days from hatching, 20 day old fry from 0+, 1+ and 2+ females realised only 15%, 25% and 36% of their maximum growth potential, respectively, in O. niloticus and 38%, 67% and 56% in O. mossambicus.

In addition, a delay in the initial feeding of fry in both species of six to 18 days post-hatching also decreased their condition (i.e. weight to length relationship) (Fig. 3.14). The rate of decline in condition, however, was higher for fry from younger broodfish. In both species, the larger yolk reserves of fry from the larger eggs of older females helped to reduce starvation stress. Therefore, for each period of delayed initial feeding these fry were in a better condition at the end of the trial. The lower rate of decrease in condition of O. mossambicus fry compared with O. niloticus may be due to the lower swimming activity and hence metabolic demand of O. mossambicus fry during the starvation period. It should be noted that condition factors were based on Fulton's ratio ($K = W/L^b$) which assumes that the growth of fry in all treatments was isometric (i.e. $b = 3$) and may therefore be less accurate than if 'b' values were derived from fry weight/length relationships. This could not be determined because fry from each treatment were bulk weighed at the end of the trial.

The loss in fry growth potential and condition, especially during the first 12 days after hatching (i.e. 16 days after spawning, at 28°C), may also be found in naturally reared fry. During this period fry which are capable of exogenous feeding may still be held in

the buccal cavity of the brooder. Baerends and Baerends-Van Roon (1950) reported that O. mossambicus fry were first released 10-12 days after spawning while Panikkar and Tampi (1954) and Russock and Schein (1977) reported 10-14 days. During the present study, however, the time from spawning to first observed release varied from 11 to 18 days in females spawned in 1m² and 2m diameter spawning tanks (Table 4.3). In addition, it was observed that the total buccal rearing period varied between females and between successive broods of the same individual. The results of this trial suggest that if the final release of fry by brooders is delayed beyond 10 days post-spawning (at 28°C) the growth of fry as well as their condition is reduced.

CHAPTER 4

CONSEQUENCE OF REPRODUCTIVE BEHAVIOUR ON THE
VIABILITY OF OREOCHROMIS EGG CLUTCHES AND
THE QUALITY OF FRY

4.1 Introduction

There is now general agreement that the mouth-brooding specialisation of parental care among tilapias of the genus Oreochromis has evolved from substrate spawning (Fishelson, 1966; Fryer and Iles, 1972; Trewavas, 1973; Barlow, 1974; Balon, 1984). The evolutionary pathways from monogamy and biparental care through to polygyny (male spawning with many females) and polyandry (female spawning with many males) and maternal brooding are still much debated (Noakes and Balon, 1982).

In this section the implications of the sexual behaviour of O. niloticus and O. mossambicus broodstock under cultured conditions on the viability of the egg clutch, early fry development and the quality of the fry in terms of growth and survival are considered.

To ensure paternity of offspring, Oreochromis males, unlike substrate spawners, establish and vigorously defend nest sites on common breeding grounds in which sexual display, courtship and eventually shedding and fertilization of gametes occur. The females, on the other hand, visit the nest sites only during spawning and select one or a few males to fertilize her spawn. Consequently, infighting among males to monopolize females may result in a few dominant males being highly successful in spawning with females.

When courtship between a male and female is successful the female remains in the male's nest and deposits her spawn in several batches (up to 20), which are immediately fertilized by the male, over a

period of 45 minutes to two hours (Trewavas, 1983). To sire as many offspring as possible one would expect the male to maximise his reproductive success by shedding sufficient numbers of sperms to fertilize each batch of eggs and to ensure that all eggs of the spawning female are shed in his nest.

The first experiment reported here was conducted to test under hatchery conditions the hypothesis that since males fertilize several batches of eggs in succession during a spawn, the numbers of viable sperms may decline and result in a decrease of the fertility rates of subsequent egg clutches.

The females, on the other hand, may mate with more than one male (polyandry) (Trewavas, 1983). She collects the eggs into her buccal cavity and leaves the nest site to rear her clutch. During the rearing period fertile eggs hatch in the buccal cavity and the fry tissue volume increases progressively resulting in a rise in total clutch volume. Since the bodies of newly hatched sac-fry are fragile they may be easily damaged in the buccal cavity. Further, the extent of damage may also be associated with the number of viable eggs and hence clutch volume in relation to the buccal volume. Indeed, Aronson (1949) and Baerends and Baerends-Van Roon (1950) suggested that the discrepancy in numbers between egg fecundity and fry produced may be explained by a limiting buccal cavity volume.

A second investigation was to determine whether yolk-sac fry are damaged during oral rearing and if so, to assess the extent and nature of fry damage and to establish whether maternal buccal volume is limiting to the number of eggs and fry reared.

During the final phase of natural rearing the surviving fry, which are held at high densities in the buccal cavity, may not be released even though they may be free-swimming and capable of exogenous feeding (Table 3.8). Initially, the fry are released occasionally by the mother for feeding and should danger threaten they retreat into the safety of the buccal cavity. The rearing period up to first release of fry by brooders is nevertheless variable and can range from 10-21 days after spawning (Hofstede and Botke, 1950; Panikkar and Tampi, 1954; Russock and Schein, 1977). The interacting effects of fry density in the buccal cavity, varying periods of time to first release and reduced feeding opportunity during subsequent brooding may reduce the growth potential of fry when yolk reserves are limiting. The final experiment reported here was therefore conducted to evaluate the consequence of oral rearing on the quality of fry by comparing the growth (weight) of naturally and artificially reared fry from the same clutch ('siblings'), at the time of first observed release from maternal care.

4.2 Materials and Methods

4.2.1 Effects of male spawning frequency on fertility rates of naturally spawned egg clutches

Experimental procedure

To obtain fish in reproductive condition, twenty O. niloticus and O. mossambicus females and ten conspecific males were held separately in four 1m² tanks. They were fed three times daily at a rate equivalent to 2% of their body weight per day for two weeks on a commercial diet (Pellet No. 4; Edward Baker, Bathgate, Scotland).

The females were starved for 24 hours prior to the commencement of the trials. These fish were then examined for swollen abdomens and enlarged genitals which were indicative of advanced ovarian development. Eight such females were first tagged with floy tags and colour coded with subcutaneous injections of alcian-blue dye before they and a dominant conspecific male from the holding tank were transferred into a 2m diameter circular spawning tank. The broodstock in the spawning tanks were fed ad libitum three times daily.

Observations were made daily for spawning activities and the order of individual spawning females in any one day recorded. Twelve hours after the last spawning of the day the brooders were netted and the egg clutches removed to a clean container. The tag number of each brooder was recorded and the females were returned to the spawning tank.

A random sample of 100 eggs from each clutch was preserved in Bouin's fluid for 30 minutes; the numbers of developing and unfertilized eggs were then counted (for definitions of these stages see Section 2.2.3.1).

4.2.2 Evaluation of fry losses associated with oral rearing

Experimental procedure

Oreochromis niloticus and O. mossambicus broodstock (12-18 months) were stocked in 1m² spawning tanks and 100 l glass spawning aquaria at a ratio of 3 females : 1 male. Females were tagged and colour coded as described previously (Section 4.2.1). The water temperature of the spawning tanks and aquaria was maintained at 27.5° ± 0.5°C and broodstock were fed three times daily on a commercial diet (Pellet No. 4; Edward Baker, Bathgate, Scotland) at a rate equivalent to 1%-2% body weight/day. The tanks were observed several times daily for breeding activity and the spawning dates of individual females recorded. Brooding females were allowed to rear their eggs for five to 12 days. Clutches were then removed carefully with a double netted net (a larger meshed inner and smaller meshed outer net) and transferred into large petri dishes (15cm diameter) containing clean warm water.

Each clutch was then carefully but quickly (within five minutes) sorted to separate damaged and undamaged fry. The damaged fry were checked under a binocular microscope for the presence of a heart beat. Damaged fry with a beating heart were assumed to be very recently damaged as a result of

handling; these fry were therefore added to the undamaged fry total. The numbers of damaged fry were recorded and the types of damage noted. Photographs of the various types of damage were taken using a Pentax camera mounted on a binocular microscope.

In addition, to investigate whether the pharyngeal teeth pads are exposed in the buccal cavity during oral rearing, photographs were taken of the pharyngeal region of the buccal cavity of brooding females and mature non-brooding females using a rigid endoscope connected to a camera (Storz Equipment, Rimmer Brothers Ltd., London, England) with a cold xenon light source through a flexible glass fibre cable. The females were anaesthetized in benzocaine (1:5,000 solution in water) and the mouth rinsed with a detergent solution to remove mucus. The endoscope was inserted carefully into the mouth and the mouth closed around it. Photographs were taken of the pharyngeal region with the buccal floor in the raised position and also in the lowered position by depressing the hydroid arch.

4.2.3 Estimation of female buccal cavity volume and egg and fry clutch volumes

4.2.3.1 Technique used to estimate buccal cavity volumes

Buccal cavity volumes were determined indirectly by water displacement of buccal casts made from expandable foam ('Handy Foam Plus' - FEB (Great Britain) Ltd., Manchester, England). When this liquid foam comes into contact with air a reaction with atmospheric water vapour causes it to expand. Upon full expansion it sets and cures

permanently, the complete process taking approximately 45-60 minutes. The casts produced are waterproof and do not shrink (FEB (Great Britain) Ltd., personal communication). Preliminary trials were conducted to assess the quantities of foam required to make a buccal cast with minimal expansion of foam beyond the normal shape of the buccal cavity during oral rearing.

Various sizes of O. niloticus and O. mossambicus females were killed in benzocaine (1:4,000-5,000 solution in water). The fish were then tagged with numbered floy tags and their weights and standard lengths recorded. The mouth of the fish was then opened and the buccal cavity injected with foam. Holding the opercula shut and raising the floor of the buccal cavity ensured that excess foam was extruded through the mouth and that foam was introduced to all areas of the oral cavity. The mouth of the fish was then held shut by a pin inserted through the upper and lower jaws to allow the cast to mould into the normal shape of the mouth. To prevent abnormal lateral displacement of the opercular bones the opercula were held between a wedge. During the setting stage (20-25 minutes) a syringe needle with the tip bent to form an 'L' shape, was inserted gently into the body of the cast to facilitate later handling. The tag number of the fish was recorded on the syringe needle for later identification. Casts were allowed to expand, set and cure for a total of 90 minutes. Buccal casts were then removed by cutting along the floor of the oral cavity and lower jaw, washed in running water and dried at room temperature.

4.2.3.2 Measurement of buccal cast volumes

The apparatus developed for the measurement of cast volume by water displacement is shown in Fig. 4.1. To facilitate the displacement of water, the surface tension of water was reduced by adding a few drops of liquid detergent. This water was reused for each buccal volume measurement.

Water was added to the beaker until it began to spill through the long side arm directly into a container on a digital top pan balance (Mettler PC400); approximately 10 minutes were allowed for complete drainage. The balance was then tared and a cast was lowered gently into the beaker and held with a clamp. The displaced water, representing the volume of the cast, was allowed to drain completely and the weight of water was recorded (Fig. 4.1). This was repeated three times for each cast and the average taken.

4.2.3.3 Egg and fry clutch volumes

Eggs from the various sizes of O. niloticus and O. mossambicus broodfish were stripped manually into petri dishes and fertilized with milt from conspecific males as described previously (Section 2.1). The fertilized eggs were then washed with clean warm water and allowed to harden for one hour before being drained on absorbant paper. The volumes of egg clutches were determined by the displacement method using a 5ml graduated measuring cylinder to an accuracy of 0.1ml. The eggs were then counted and incubated in round-bottomed containers as described in Section 2.1.

FIGURE 4.1

Assembled apparatus used to estimate the volume of buccal cavities. On lowering the cast (C), water equivalent to the cast volume is displaced into a collecting beaker (B) (previously tared on the balance) and weighed. Weights converted to volumes using $1g = 1ml$.



To determine the volume of a clutch of swim-up fry they were killed in benzocaine (1:10,000 solution in water) and dried lightly on absorbant paper to remove surface water. One hundred fry were counted and their volume measured by displacement in a 5ml graduated measuring cylinder to an accuracy of 0.1ml. Maximum fry volume was calculated assuming that all eggs in a clutch developed into swim-up fry.

4.2.3.4 Analysis of data

The weights of water displaced by buccal casts were converted to volumes (i.e. 1g = 1ml) and the association between mean buccal cavity, egg clutch and maximum fry clutch volumes and female length was determined by regression analysis using a computerised statistical package (Minitab, Pennsylvania State University).

4.2.4 Growth of artificially and naturally reared fry at the time of first release from maternal care

4.2.4.1 Experimental procedure

Twelve to eighteen-month old O. niloticus and O. mossambicus females were tagged individually with floy tags and stocked in 1m² spawning tanks at a ratio of 3 females : 1 male. To facilitate easy identification of individual fish, females were injected subcutaneously with alcian-blue dye on the dorsal surface.

The broodstock were fed on a commercial diet (Pellet No. 5; Edward Baker, Bathgate, Scotland) at a rate equivalent to 1%-2% body

weight/day, three times daily. In addition, when the broodstock were fed, ground and sieved (< 500µm) broodstock diet was added in excess as a source of food for any fry released by females.

The tanks were observed daily for spawnings. Brooding females were encouraged gently into a deep net and their mouth quickly held shut to prevent any release of eggs. The mouth was then partially opened with thumb and forefinger to collect some eggs (approximately 100) before returning her to naturally rear the remainder of the clutch. The date of the spawning and her tag identification were noted. To minimise the mixing of fry from different females, the eggs of brooding fish that spawned within two days of the last accepted spawning were removed and discarded. The clutches from seven individual O. niloticus and eight O. mossambicus females were used.

4.2.4.2 Artificial rearing of eggs and fry

The eggs that were removed from the female were reared artificially in round-bottomed containers as described in Section 2.1. Hatching of artificially incubated eggs occurred within four days of spawning; the fry were held in the incubators for five days. The fry were then stocked at 10/1 in the rearing units described in Section 3.4 (Fig. 3.11) and fed in excess four times daily on ground and sieved (< 500µm) broodstock diet. Feeding was terminated when the naturally reared fry from the corresponding female were first released; this date was recorded.

4.2.4.3 Growth of fry

The naturally reared fry and their artificially reared 'siblings' were removed with a net from the spawning tanks and rearing units, respectively, and transferred into clean water for four to five hours to allow for evacuation of the gut contents. They were then killed in benzocaine (1:10,000 solution in water), rinsed in distilled water and the standard lengths, mean moisture levels and mean dry weights of 20 randomly sampled fry determined in duplicate as described in Section 2.2.2.2. The mean fry weights of artificially and naturally reared 'siblings' were compared statistically using Student's 't' test.

4.2.4.4 Water quality

Temperature and pH in the spawning tanks, incubation and rearing systems were maintained at 27.5 ($\pm 0.5^{\circ}\text{C}$) and 6.00-6.50, respectively. Analysis of water from the above systems was performed at fortnightly intervals; ammonia, nitrite and nitrate levels were similar in all three systems.

4.3 Results

4.3.1 Effects of male spawning frequency on fertility rates of naturally spawned egg clutches

The effects of male spawning frequency on the numbers of unfertilized and developing O. niloticus and O. mossambicus eggs are given in Tables 4.1 and 4.2. In each species a maximum of four spawnings per day was obtained. The effect of male mating frequency on the fertility rates of egg clutches is illustrated in Fig. 4.2.

In both species, the numbers of developing eggs in a clutch declined rapidly with an increase in male spawning frequency in any one day irrespective of male age (Tables 4.1 and 4.2). The numbers of developing eggs in a clutch were dependent on the spawning frequency of males and the relative numerical position of the spawning female in that day. For example, when O. niloticus females number 4377 and 4384 spawned second and fourth on 24/2/84 at least 80% and 22% of their eggs were fertilized, respectively. On 8/3/84, however, the same females spawned first and second and the numbers of developing eggs in their clutches were 96% and 72%, respectively (Table 4.1). A similar trend was observed for O. mossambicus female number 4889 which spawned twice during the trial (Table 4.2).

4.3.2 Fry losses associated with oral rearing

The mean cumulative percentages of damaged O. niloticus and O. mossambicus fry, associated with the various periods of oral rearing are shown in Fig. 4.3.

TABLE 4.1

The consequence of repeated matings by O. niloticus males on the viability of naturally spawned egg clutches

Male No. (age)	Date of spawnings	Number of matings/day	Spawning order of females ¹	Egg viability (% of clutch)		
				Non-viable ² eggs (%)	Unfertilized ³ eggs (%)	Developing ⁴ eggs (%)
1 (26 months)	24/2/84	4	4383	3	1	96
			4377	4	16	80
			4383	5	26	69
			4384	8	70	22
	4/3/84	2	4375	0	0	100
			4381	3	27	70
	8/3/84	2	4377	4	-	96
			4384	7	21	72
2 (8 months)	6/7/84	3	4894	5	11	84
			4895	4	33	63
			4891	3	90	7

¹ Numbers refer to tag identification

² Defined as those eggs in which germinal disc was not visible

³ Defined as eggs remaining in the germinal disc stage

⁴ Eggs in blastula - embryonic shield stage

TABLE 4.2

The consequence of repeated matings by *O. mossambicus* males on the viability of naturally spawned egg clutches

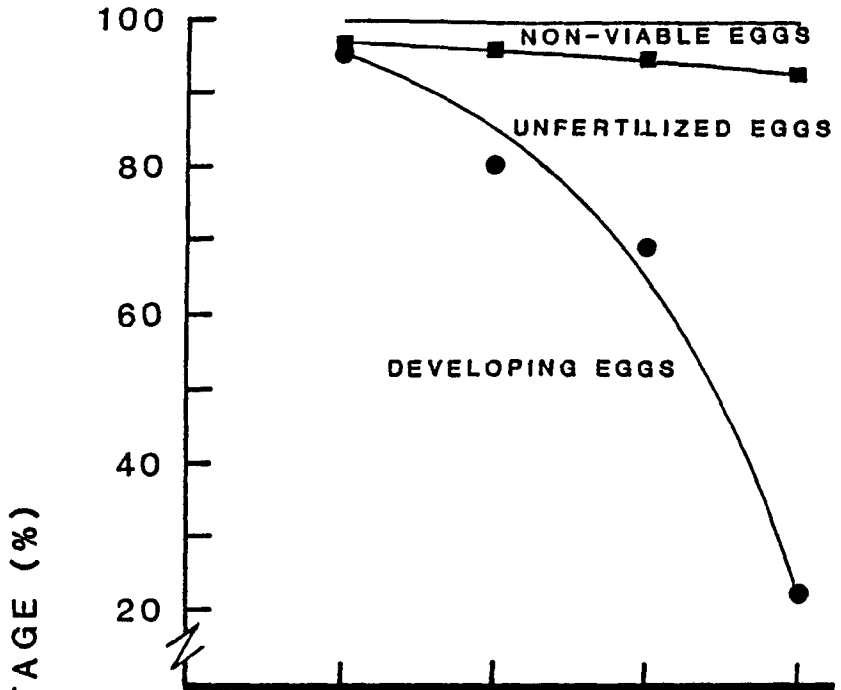
Male no. (age)	Date of spawnings	Number of matings/day	order of females ¹	Egg viability (% of clutch)		
				Non-viable ² eggs (%)	Unfertilized ³ clutch (%)	Developing ⁴ eggs (%)
1 (16 months)	27/4/84	4	4820	2	6	92
			4890	5	15	80
			4441	3	41	56
			4277	2	70	28
	29/4/84	2	4272	6	14	80
			4068	2	30	68
2 (9 months)	2/6/84	2	4882	2	19	89
			4889	4	33	63
	10/7/84	2	4889	1	14	85
			4881	2	27	71

1-4 Footnotes as in Table 4.1

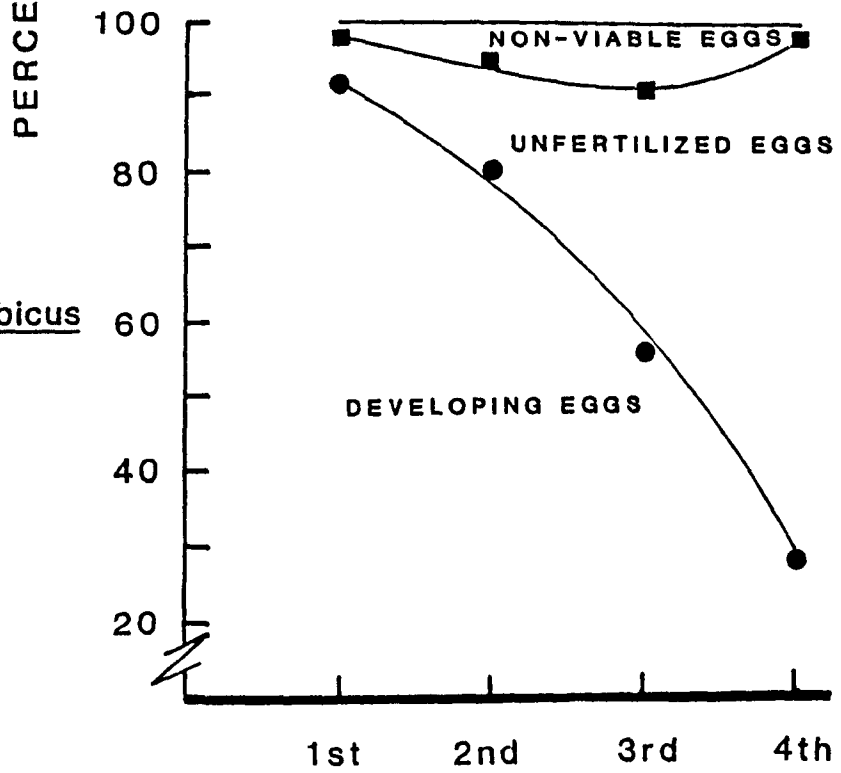
FIGURE 4.2

Effect of repeated matings during a single day by an individual male on the percentage of developing (●) and unfertilized (■) eggs in the resultant clutches. Curves fitted by eye

(a) O. niloticus



(b) O. mossambicus



ORDER OF CLUTCH FROM REPEATED MATINGS
OF A SINGLE MALE IN A DAY

In both species the loss of newly hatched fry increased linearly between five to eight days post-spawning. Thereafter the rate of fry loss decreased and by day 10 was nearly zero (Fig. 4.3). In O. niloticus as well as O. mossambicus the numbers of damaged fry in individual clutches were not significantly ($r^2 = 0.042$ and 0.167 with $df = 15$ and 18 , respectively, $P > 0.05$) correlated to the total number of fry in the clutch.

Examples of types of fry damage are shown in Fig. 4.4. In both species the dislodgement of yolk from the yolk-sac epithelium (shown in Figs. 4.4b and c) was the most common type of damage and accounted for 81%-87% of the total number of damaged fry. In addition, damage to the eyes occurring either together with the loss of yolk (Fig. 4.4c), or on its own, accounted for between 11%-16%. A smaller proportion of fry showed evidence of physical crushing (Fig. 4.4d). In these specimens, damage to the head region was often accompanied by haemorrhages on the body and around the yolk-sac blood sinuses (see Fig. 4.4d).

Photographs of the pharyngeal region of brooding and non-brooding O. niloticus females are shown in Fig. 4.5. In both species exposure of the pharyngeal teeth pads was greater with the buccal floor in the lowered position. The degree of exposure, however, was greater in mature non-brooding females than in brooding females.

FIGURE 4.3

Physical damage to fry within the buccal cavity associated with natural rearing under hatchery conditions.

(a) O. niloticus, and (b) O. mossambicus. ● , denotes average and vertical bars show the range of fry damage during each brooding period. Note large range for O. niloticus at 6 days was due to the inclusion of two parents kept in 100 l aquaria in which high numbers of fry were damaged, the remaining broodfish were kept in 1m² and 2m diameter tanks

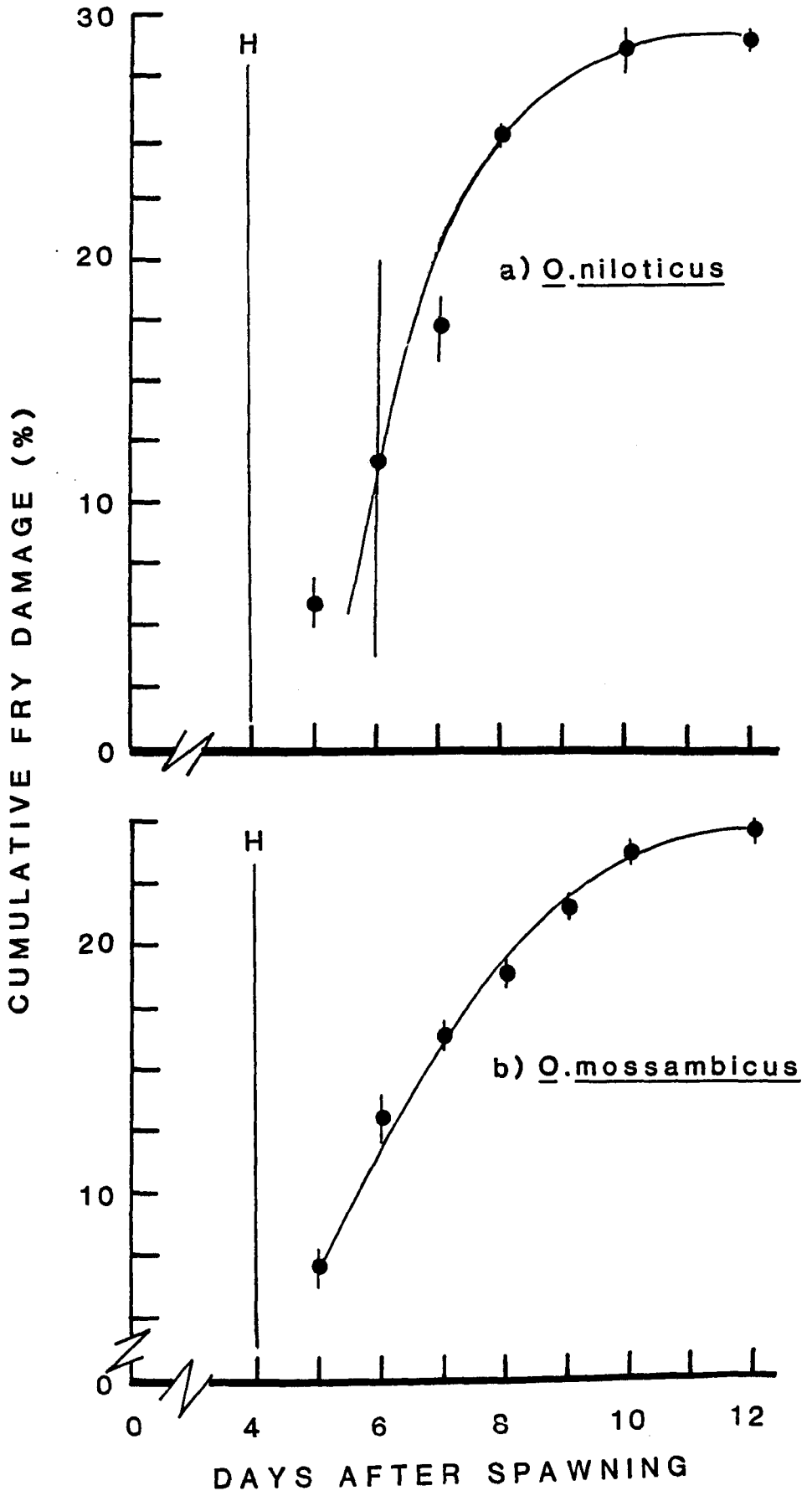


FIGURE 4.4

Examples of physical damage identified in naturally reared O. niloticus clutches.

- (a) Normal (undamaged) fry
- (b) & (c) Fry with dislodged yolk-sac and eye damage
- (d) Fry with damage to head

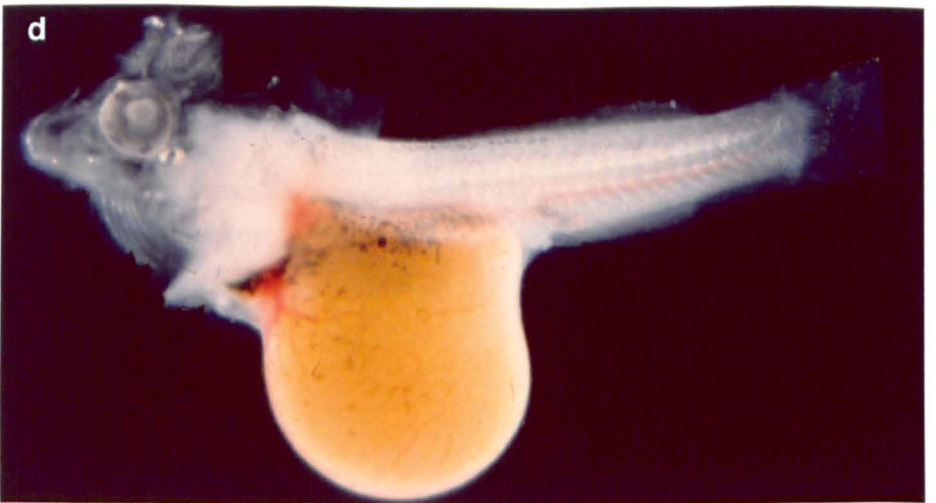
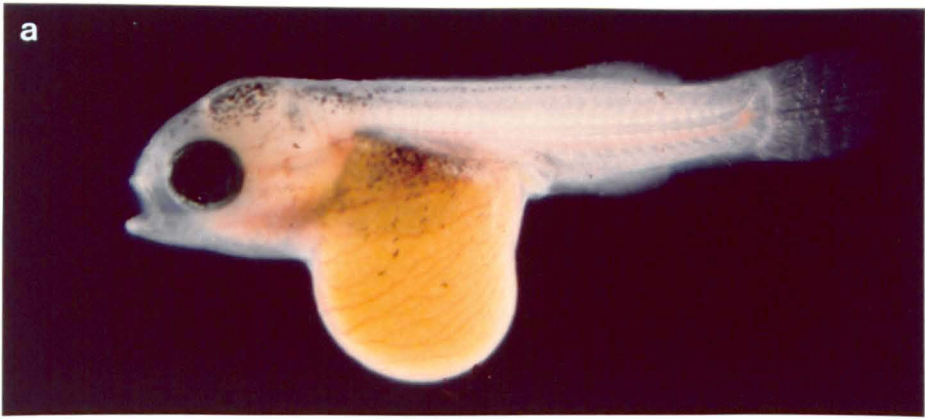
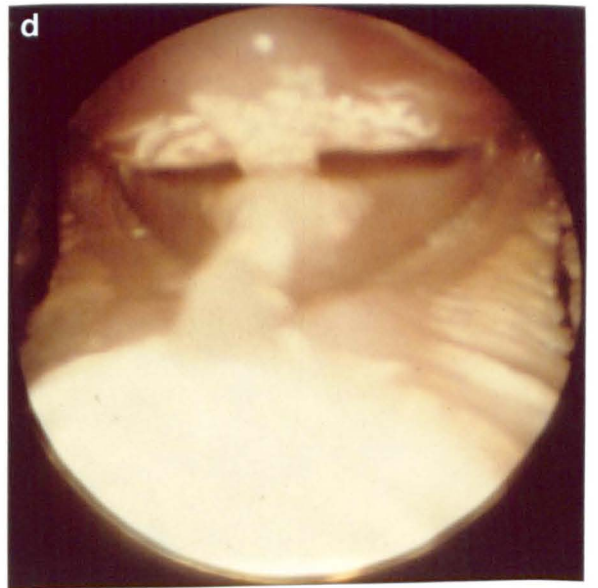
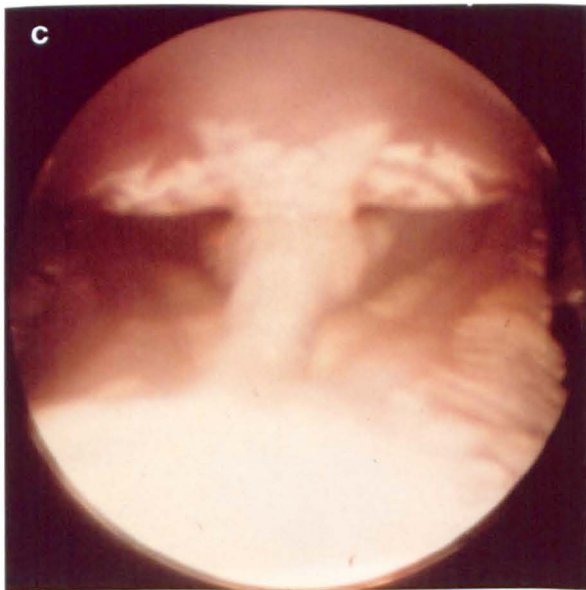
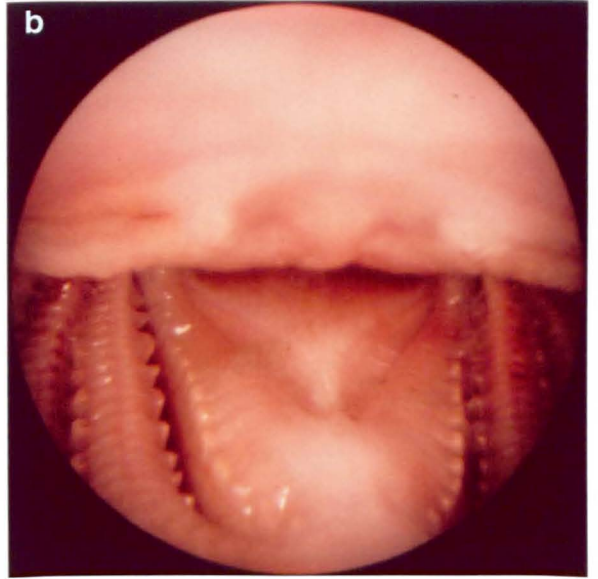
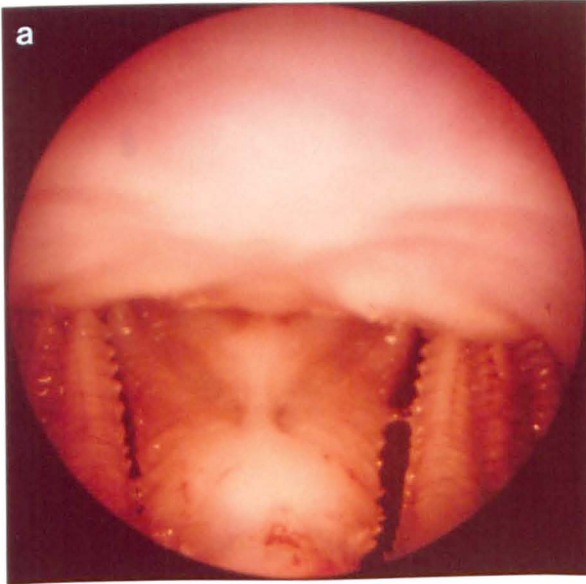


FIGURE 4.5

Endoscopic view of the pharyngeal region of mature O. niloticus females.

TOP: Mature non-spawning female (weight 387g) with (a) the floor of the buccal cavity in normal raised position, and (b) with the floor of the buccal cavity and hydroid bone in the lowered position.

BOTTOM: A brooding female (440g) with (c) the buccal floor in the raised position, and (d) with the buccal floor and hydroid bone in the lowered position. Brooding female had been rearing her clutch for five days



4.3.3 Buccal cavity and egg and fry clutch volumes

Examples of buccal casts from females of various sizes are shown in Fig. 4.6. The relationship between buccal cavity volume, total egg clutch volume and total fry clutch volume with female length are given in Fig. 4.7.

In O. niloticus and O. mossambicus brooders oral cavity volumes increased significantly ($P < 0.001$) with maternal length (Fig. 4.7). For 11-12cm O. niloticus females egg clutch volumes were only 10%-25% of buccal cavity volumes in comparison to 15%-40% for O. mossambicus egg clutch volumes. The differences between egg clutch and buccal volumes, however, increased with maternal length in both species (Fig. 4.7). During fry development, however, the total clutch volume increased and by the swim-up stage maximum fry clutch volume (assuming all eggs develop into fry) increased by 90%-100%. The total fry clutch volumes were nevertheless considerably lower than the buccal cavity volumes (Fig. 4.7).

4.3.4 Comparison between the growth of artificially and naturally reared 'siblings' at the time of first release from maternal care

The growth traits of artificially and naturally reared 'siblings' are shown in Table 4.3. The time to first observed release of fry from the buccal cavity varied between females and ranged from 11 to 18 days post-spawning for O. niloticus and 11 to 16 days for O. mossambicus.

FIGURE 4.6

Examples of foam casts of the buccal cavity of Oreochromis females of various sizes.

(a) O. niloticus, and (b) O. mossambicus

x0.7

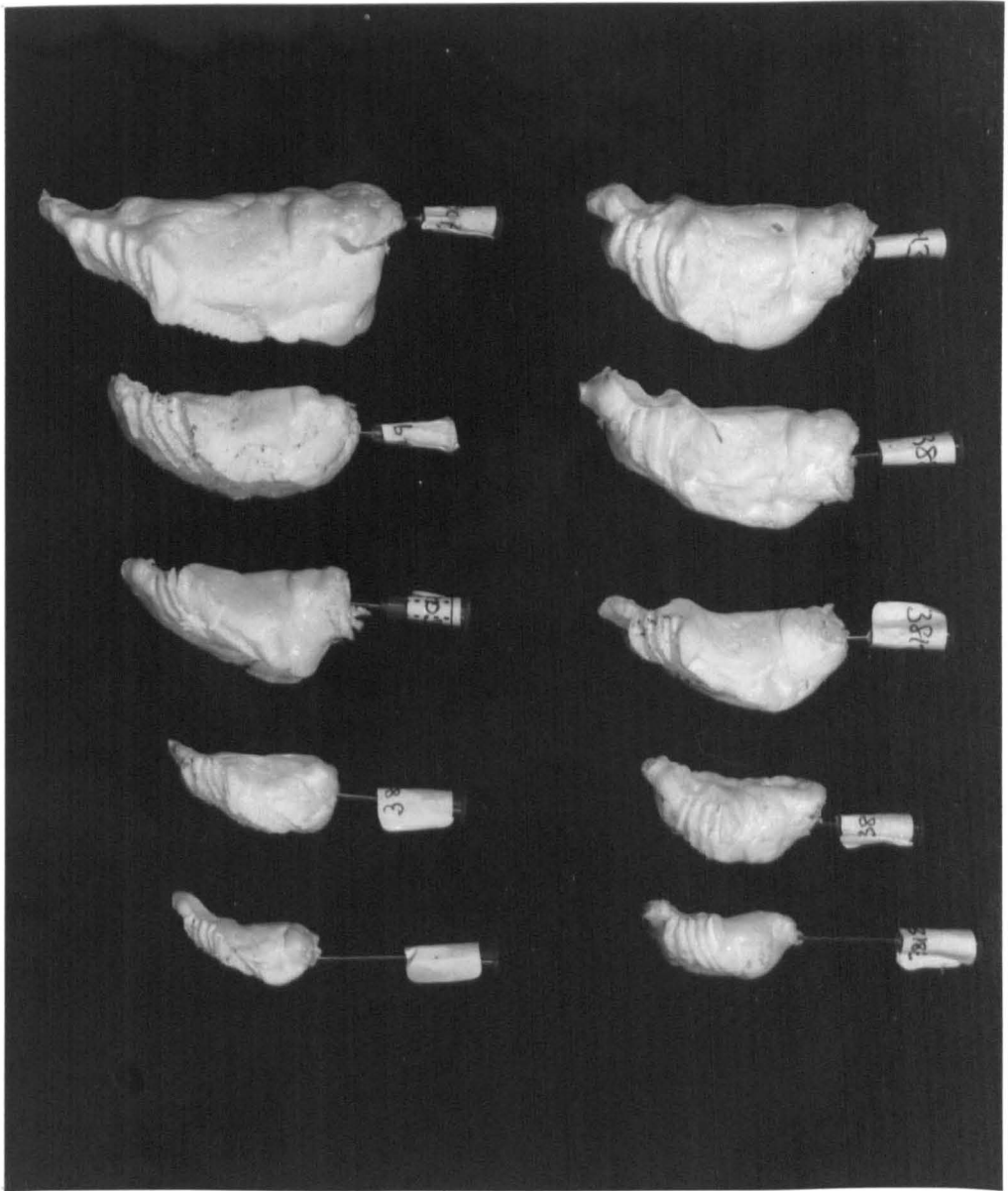
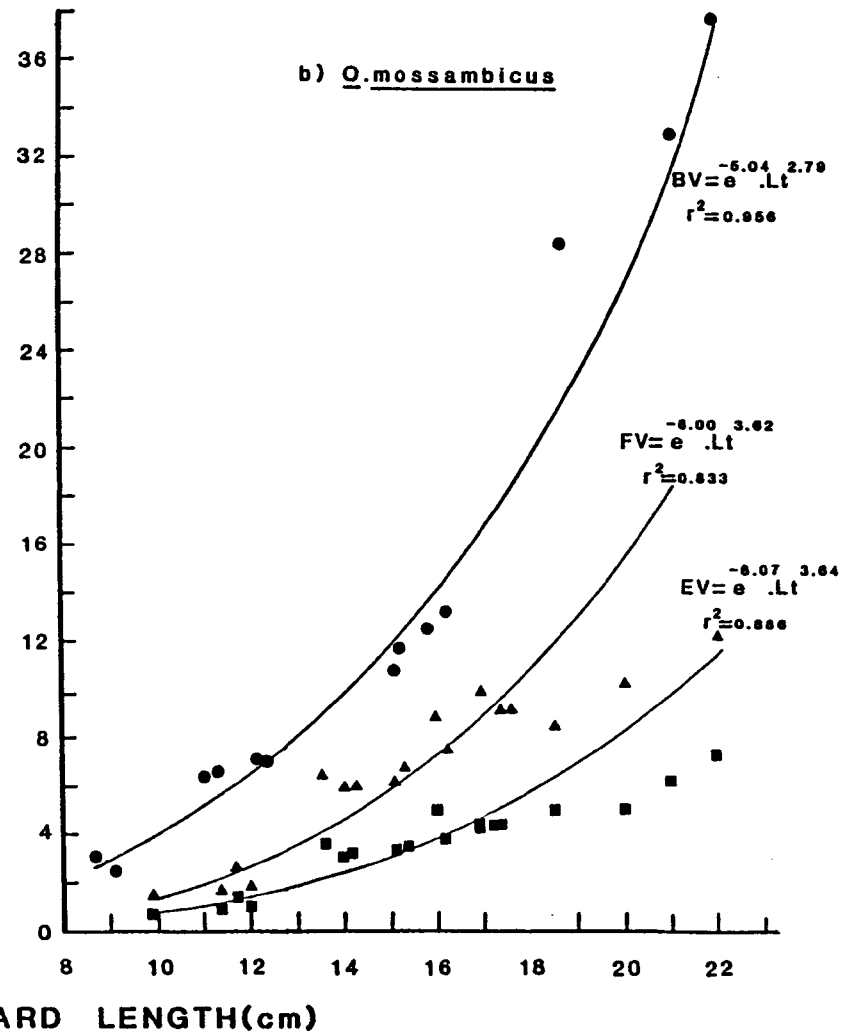
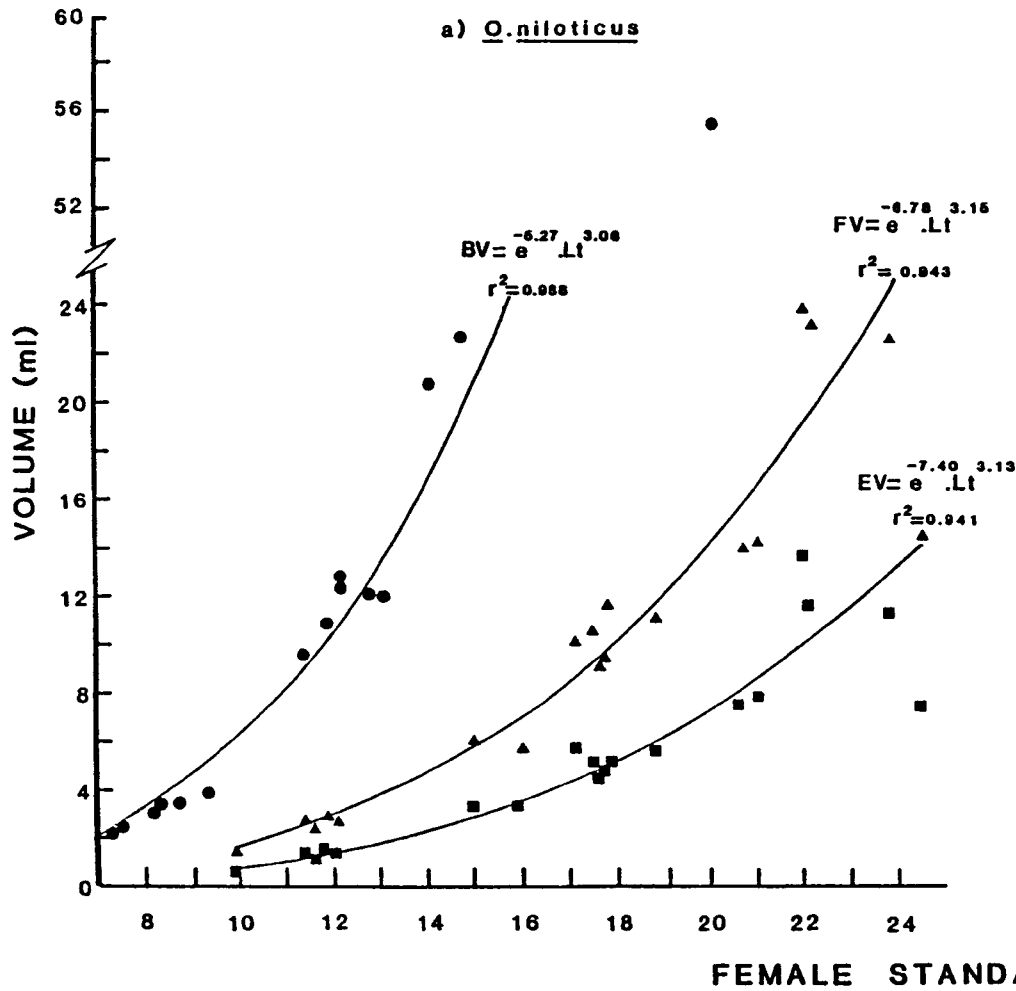


FIGURE 4.7

Comparison between the buccal volume (BV), total egg volume (EV) and total fry volume (FV) of females of various sizes. (a) O. niloticus, and (b) O. mossambicus. Curves relate to: ● , buccal volume; ■ , egg volume; ▲ , fry volume. Equations given are based on the natural logarithmic transformation of data



In all clutches, artificially reared O. niloticus and O. mossambicus fry were significantly ($P < 0.05$) longer and heavier than their naturally reared 'siblings' (Table 4.3 and Fig. 4.8). The mean weights of artificially and naturally reared 'siblings' from clutches first released at different times after spawning are shown in Fig. 4.8. Longer periods of oral rearing to first release in O. niloticus brooders resulted in greater differences in mean fry growth between artificially and naturally reared 'siblings' than in O. mossambicus 'siblings'. The differences between artificially and naturally reared 'siblings' in mean length and mean weight ranged from 12.5% to 36.4% and 13.8% to 211.0%, respectively, for O. niloticus fry, and 4.9% to 23.0% and 11.7% to 119.0% for O. mossambicus fry.

The mean moisture levels of naturally reared fry were higher than those of their artificially reared 'siblings' in most (6 out of 7) O. niloticus clutches and in some (3 out of 8) O. mossambicus clutches.

TABLE 4.3

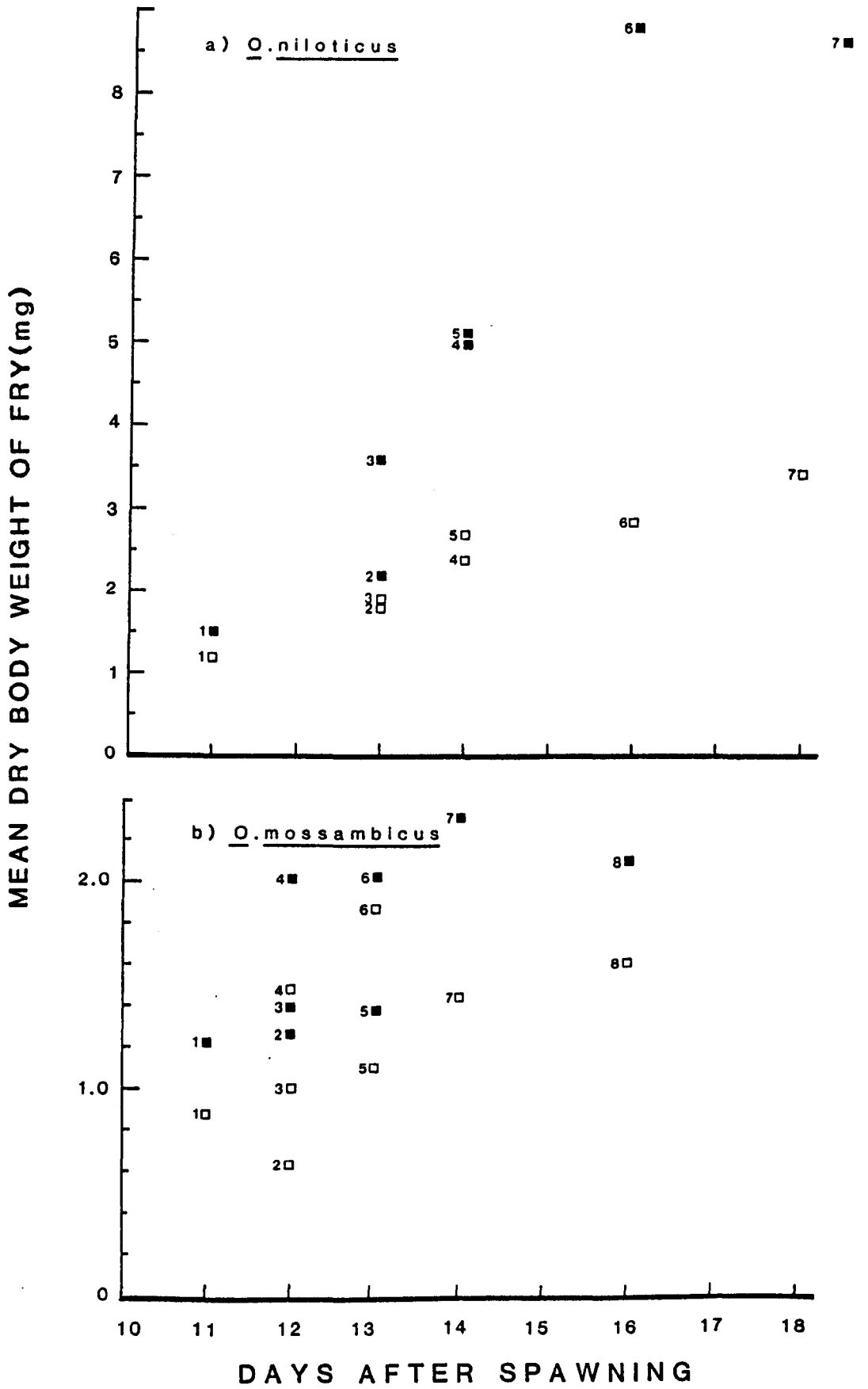
Comparison between the size of artificially and naturally reared *O. niloticus* and *O. mossambicus* 'siblings' from the same clutch at the time of first observed release by brooding females

Species	Female no.	Time of first observed release (days) after spawning)	Method of fry rearing						Difference in fry size (% over naturally reared fry)	
			Artificial			Natural				
			Mean standard length (mm)	Mean dry ¹ body weight (mg)	Moisture (%)	Mean standard length (mm)	Mean dry ¹ body weight (mg)	Moisture (%)	Length	Weight
<i>O. niloticus</i>	1	11	6.9	1.52	83.5	6.1	1.22	84.4	13.1	24.6
	2	13	7.2	2.15	80.3	6.4	1.89	85.0	12.5	13.8
	3	13	7.9	3.56	82.2	6.8	1.81	83.7	16.2	96.7
	4	14	8.9	5.01	83.3	7.2	2.45	82.0	23.6	104.5
	5	14	9.0	5.12	83.1	7.5	2.52	83.9	20.0	103.2
	6	16	10.5	8.80	82.7	7.7	2.83	83.4	36.4	211.0
	7	18	9.9	8.57	83.1	7.9	3.43	84.3	25.3	149.9
<i>O. mossambicus</i>	1	11	7.2	1.22	83.8	6.7	0.89	83.2	7.5	37.1
	2	12	8.2	2.11	83.2	7.5	1.49	84.6	9.3	41.6
	3	12	7.5	1.38	83.8	6.1	0.63	86.1	23.0	119.0
	4	12	7.2	1.25	84.4	6.6	1.00	83.3	9.1	25.0
	5	13	7.4	1.39	81.9	6.8	1.10	82.5	8.8	26.4
	6	13	8.5	2.10	84.2	8.1	1.88	84.1	4.9	11.7
	7	14	8.6	2.33	84.4	7.4	1.45	85.5	16.2	60.7
	8	16	8.1	2.10	82.9	7.0	1.60	80.0	15.7	31.3

¹ Comparisons of mean weights using Students 't' test showed significant differences ($P < 0.05$) between artificially and naturally reared fry from each clutch

FIGURE 4.8

Comparison between the mean body (fry less yolk) weights of artificially and naturally reared 'siblings' from the same clutch. (a) O. niloticus, and (b) O. mossambicus. Open and closed squares refer to naturally and artificially reared 'siblings', respectively



4.4 Discussion

In Oreochromis species the arena or lek type mating system, in which spatially structured aggregations of sexually active males are visited by ripe females, is a feature of their breeding behaviour. In such breeding systems where intra-sexual competition for mates leads to social hierarchy, the inter-male variance, as in higher vertebrates, is extreme (Van Rhijn, 1973; Rippen and Boag, 1974; Robel and Ballard, 1974). One would expect dominant males to increase their number of offspring sired through multiple matings. It is generally assumed, however, that since the male increases his reproductive success by multiple spawning, he is also capable of repeatedly fertilizing eggs (Lowe (McConnell), 1955, 1959; Fryer and Iles, 1972; Perrone and Zaret, 1979; Baylis, 1981).

The results obtained in the present study for O. niloticus and O. mossambicus do not support this assumption. Here, under controlled hatchery conditions it was shown that although a male may readily court and successfully mate with several females in a day, his ability to increase the total number of fry sired decreases. The fertilizing capability of males is inversely related to the number of spawnings in a day (Tables 4.1 and 4.2). For example, when O. niloticus and O. mossambicus males spawned four times in a day, the numbers of developing eggs (= fertile eggs) in the last clutch decreased by 64% and 74%, respectively, when compared with the first spawning.

Since the majority of non-developing eggs were characteristic of the mature unfertilized eggs described in Section 2.2.3.1, the

observed reductions in numbers of developing eggs with increasing male spawning frequency were probably due to depletion of viable sperms in the lobules of the testes, rather than differences in egg quality between females. After an elaborate courtship, a spawn may be released in up to 20 batches (Trewavas, 1983). To ensure his reproductive success, one would expect the male to maximise his effort of shedding sperms to fertilize as many eggs as possible in each batch. Consequently, it is conceivable that even in dominant males the testes would become spent rapidly on repeated matings within a day, especially as the male gonadosomatic index is only 4%-6% in the tilapias (Fryer and Iles, 1972; Siddiqui, 1977; Dadzie and Wangila, 1980; Blay, 1981). Moreover, the effect on clutch fertility of a rapid decline in the concentration of sperms may be exacerbated by the short life of sperms in fresh water of about one minute (Stoss and Donaldson, 1983).

A key factor relating clutch fertility to male spawning frequency is the rate of replacement production of sperms by the testes. In general spermatogenesis requires less time than oogenesis (Baylis, 1981). When a male has discharged most of his milt, the testes immediately undergo active spermatogenesis (Dadzie, 1969). There is, however, a period before which the male is capable of again achieving maximum fertilization. In the cichlid, Aequidens portalegrensis, this period is reported to be four days (Polder, 1971). It can be seen from the present trials that the short inter-spawning interval of males between multiple spawnings in a single day was insufficient to replace the discharged sperms. Within two days, however, the fertilizing capability of an O. mossambicus male that

had spawned four times in one day increased from 28% to 80% (Table 4.2). In O. niloticus a 'rest' period of seven days raised the fertilizing capability of a male that also had spawned four times in one day from 22% to 100% (Table 4.1).

Although observations relate to only four males, it was found that the fertility rate of clutches declined with multiple male spawnings in both species and in males of different ages. This indicates that under hatchery conditions a variation in the fertility rates of egg clutches and therefore in the levels of fry production from females may be affected by male spawning frequency. Thus variation in fertility rates may account for some of the reported differences between fecundity (based on ovarian counts) and number of fry brooded (Lowe (McConnell), 1955, 1959; Riedel, 1965; Welcomme, 1967; Marshall, 1979). The decline in fertility rates of eggs with an increase in male spawning frequency has also been reported in the lemon tetra, Hyphessobrycon pulchripinnis (Nakatsuru and Kramer, 1982). In this species, where the female spawns on average 23 times a day every four days, it was found that as the total daily spawning acts of a male increased from five to 45 the median percentage of developing eggs decreased from over 80% to 0%.

In contrast to the present findings, however, Peters (1971) reported that short intervals between matings by tilapia males can occur without apparently reducing their fertilizing capabilities. He reported that a single O. mossambicus male defended a spawning site for 11 months with no apparent decline in fertility in spite of frequent spawning. Similarly, Polder (1971) found that male

Aequidens portalegrensis could spawn again within the same day without loss in fertilizing capability. In both of these studies, however, the fertility rate of egg clutches and the order of spawning females were not given.

In view of the possible reduction in male reproductive success in Oreochromis during successive matings, the spawning of females with more than one male (polyandry) may have evolved as a mechanism to maximise female reproductive success. Electrophoretic evidence for multi-paternal fry from the same clutch has been reported for O. niloticus (Hulata, Rothbard and Avtalion, 1981). In addition, it may be possible for a female to detect and discriminate against a recently spawned male. There is evidence for this in the lemon tetra. In this species it was found that females show a preference for males that have not recently spawned (Nakatsuru and Kramer, 1982). Female reproductive success and hence fry production may also be increased by the close contact between sexes during spawning. Oreochromis females may snap up sperms from the genital papilla of the male thus increasing the probability of fertilization (Bohrer, 1953; personal observation). This behaviour is particularly well developed within the Oreochromis subgenus Nyasalapia, e.g. O. macrochir, the males of which have well developed genital tassels to attract females to snap up sperms (Fryer and Iles, 1972).

Another factor that may affect fry production during natural rearing is damage to fry within the buccal cavity. In the present trial the number of hatchlings damaged in the oral cavity was found to increase linearly for the first eight days after spawning (Fig.

4.3). By the twelfth day a total of 29% and 25% of fry in O. niloticus and O. mossambicus broods, respectively, were damaged. The fry damage was probably due to physical injury sustained during the churning of the clutch by the female.

It was suggested in Section 2.1.4.1 that the pharyngeal 'lobes', situated adjacent to the pharyngeal teeth pads, and the proliferation of the tissue between the 'lobes' may preclude the pharyngeal teeth pads from the buccal cavity thus minimising mechanical damage from the teeth to eggs and fry. Photographic evidence from the oral cavity shows that the degree of exposure of the pharyngeal teeth in the buccal cavity of brooding females is less than in mature non-brooding females, both when the floor of the buccal cavity is in the raised position (Figs. 4.5a and c) and when the buccal floor and hydroid bones are lowered (Figs. 4.5b and d). In the brooding female when the buccal floor is raised, i.e. during opercular flushes, the pharyngeal tissues obscure the teeth minimising damage in the clutch. However, tissue proliferation does not completely obscure the teeth when the buccal floor is in the lowered position. Therefore, it is conceivable that during churning movements the likelihood of contact between eggs or fry and teeth would increase as the floor of the oral cavity is lowered. That fry are able to pass the pharyngeal tissue and come into contact with the teeth during oral rearing is evident by the presence of eggs and yolk-sac fry in the stomachs of brooding females (Liebman, 1933; Eyeson, 1983; Peters, 1983). For example, Peters (1983) observed 25% and 28% of O. mossambicus and S. melanotheron females, respectively,

to have eggs in the stomach. And Eyleson (1983) reports the presence of eggs and fry in 8%-15% of S. melanotheron brooders.

The dislodgement of yolk-sacs, which was the most common form of damage observed (Figs. 4.4b and c), may also be related to an increase in churning frequency. Earlier in the present study (Section 2.1) it was observed that during brooding the churning frequency decreases from 95-105/min to 20-30/min by the third day after spawning. And later it decreases even further to 3-8/min. In these trials the range in the numbers of damaged fry between individual O. niloticus females at six days post-spawning was high (Fig. 4.3a). This resulted from the inclusion of two spawnings from glass aquaria. In this confined environment it was observed that the brooding females were harassed by dominant fish. This resulted in a noticeable increase in her opercular and churning frequency at a time when the bodies of hatchlings were small and fragile, making them highly susceptible to damage.

In earlier studies on tilapias, decline in the numbers of fry during natural rearing has been attributed to limiting buccal volumes (Aronson, 1949; Lowe (McConnell), 1959; Riedel, 1965). Oral cavity volumes measured indirectly from foam casts showed that buccal volumes of both species increased exponentially with length (Fig. 4.7). The total volume of eggs and fry produced by various sizes of fish was below that of their buccal volume. In these studies eggs were stripped manually to avoid the low fecundity found to be associated with partial spawning during natural breeding in some females (personal observation). Also the maximum fry volume was

calculated on the assumption that every egg from the clutch developed into a swim-up fry.

It is important to note that the buccal casts made provided an estimate of the maximum buccal volume, i.e. with the hydroid arch distended as during natural rearing. However, one would expect the minimum volume to be the more critical measurement in assessing whether buccal volume is a limiting factor in oral incubation. Nevertheless, even assuming a 10%-20% difference between the maximum and minimum volumes, it is clear that the buccal cavity is considerably larger than the maximum fry volume and therefore is probably not limiting for fry rearing.

A further implication of Oreochromis breeding behaviour for hatchery fry production is that during the final phases of maternal rearing, the initial release of fry may be delayed. This, together with infrequent release, may reduce the feeding opportunity and result in smaller fry. A comparison made between the growth of artificially reared fry with their naturally reared 'siblings' showed that naturally reared fry were significantly ($P < 0.05$) lower in weight (Table 4.3). This was probably due to the limited feeding opportunities of orally reared fry.

In Section 3.2 it was found that onset of feeding commences at five to six days after hatching at 27°-28°C (i.e. 9-10 days post-spawning). In the present trial, however, the first observed time of release varied from 11-18 days post-spawning, i.e. first feeding opportunities were delayed for varying periods of time. Times of

first release reported by earlier workers for O. mossambicus also indicate a wide range. Hofstede and Botke (1950) observed rearing times ranging from 13-21 days post-spawning. Baerends and Baerends-Van Roon (1950) reported that fry were first released 10-12 days after spawning and Panikkar and Tampi (1954) and Russock and Schein (1977) observed rearing times of 10-14 days. The reasons for the variation in first release times from the buccal cavity between females are unclear but personal observations suggest that the ability of the female to secure and defend a territory may be a factor. In those cases where females could not defend an area into which fry could be released the first release of fry was delayed.

In the present trials the addition of excess powdered diet (<500µm) to the spawning tanks helped to ensure that food of the same quality as that fed to the artificially reared fry was available to their naturally reared 'siblings' during any unobserved release by the brooders. Later examination of the gastrointestinal tract of some naturally reared fry confirmed that feeding had occurred but it was not certain whether this had occurred within the oral cavity or during periods of unobserved release, e.g. at night. However, in both species higher moisture levels were recorded in fry of some naturally reared clutches, which indicated that the fry were starved (Rana, 1981).

Further, the differences in growth between the artificially and naturally reared 'siblings' increased as the first release of fry was delayed (Fig. 4.8). This trend was clearer for O. niloticus. These interspecific differences were probably due to the differences

between egg and fry sizes and the higher metabolic demand of O. niloticus fry. As found in Section 3.4 the rate of decline in SGR as a result of progressive delays in initial feeding was lower in O. mossambicus than in O. niloticus fry (Fig. 3.13). This suggests that under conditions of prolonged buccal rearing the difference between the weight of artificially and naturally reared fry may be smaller in O. mossambicus than in O. niloticus.

In summary, the reproductive behaviour of the two Oreochromis species may affect both fry production and quality. Fertility rates and hence egg viability of clutches can be reduced by increased male spawning frequency thus reducing fry production. In addition, fry damage during oral rearing may also reduce the number of fry reared successfully. And finally, a delay in the time to first release of fry from the buccal cavity by brooders reduces their feeding opportunity and hence their growth potential.

CHAPTER 5

GENERAL DISCUSSION : APPLICATION OF RESEARCH FINDINGS
TO OREOCHROMIS BROODFISH SELECTION AND HATCHERY PRODUCTION
OF FRY

5.1 Current Fry Production Methods and Their Constraints

Mass production of fry of high quality is an important prerequisite for the expansion or intensification of tilapia culture. In recent years attempts to increase the production of tilapias has been constrained by the low and often variable production of fry from pond spawnings. Due to low numbers of eggs per spawn, inappropriate sex ratios, incompatibility between sexes and between species and disturbances of brood fish during fry harvesting, fry production from traditional ponds has been as low as 10 fry/female/month (Coche, 1982). Also, Lovshin and Da Silva (1975) reported a large variation in fry production from different ponds of between 500%-3000%.

Management practices for the harvesting of fry from ponds vary and different methods variously affect the quality and quantity of fry produced. For example, Broussard and Reyes (1985) harvested fry firstly after 60 days and then at 30 day intervals. This method yielded fry of non-uniform age and size which required laborious grading. Rothbard and co-workers, on the other hand, reduced the harvesting interval to approximately 20 days which resulted in the production of "more of less uniform fry" but gave poor yields/female/month (Rothbard, Solnik, Shabbath, Amado and Grabie, 1983) (Table 5.1). This low productivity was attributed to fry losses associated with the expulsion of eggs and yolk-sac fry by brooders during harvesting. These authors estimated such losses to be about 30% for fry produced in each breeding cycle. Thus attempts to improve fry uniformity through frequent harvesting may adversely affect fry yield. Moreover, the unpredictability of the quality

TABLE 5.1

The effects of different spawning systems and their management on tilapia seed production

Seed production systems (size)	Species	Broodstock size (g) (♂:♀)	Stocking rate (no./m ²)	Sex ratio ♀:♂	Seed harvesting frequency and stage of seed	Mean seed production no./ /month (Trial period - mths)	Source
Traditional ponds (600 m ²)	<u>O. niloticus</u>	700 : 200	0.5	4-5 : 1	Every month; 3-4cm fry	10	Coche (1982)
Traditional ponds (1 ha)	<u>O. niloticus</u> <u>O. aureus</u> x	1500-3000	0.3-0.5	1.3 : 1	Every 20 days; yolk-sac fry	500 (3)	Rotabard <u>et al</u> (1983)
Traditional ponds (4500 m ²)	<u>O. niloticus</u>	62-356	0.16	3.1 : 1	60 and 30 day intervals; 4.3g fry	70 (8)	Broussard & Rayes (1985)
Concrete circular tanks (22 m ²)	<u>O. niloticus</u> <u>O. aureus</u> x	80-200:250-500	1.7-2.0	6 : 1	Daily; fry	117-124	Haller & Parker (1981)
Concrete circular tanks	<u>O. macrochir</u>	N.G.	2.0	7 : 1	Every 2 days; fry		
Plastic pools (7.3 m ²)	<u>O. aureus</u>	127-284:127-298	1.6	3 : 1	As seen at water surface; fry	559 (3)	Snow <u>et al</u> (1983)
Happas in ponds (1.5 m ²)	<u>O. niloticus</u>	90-135	5.3-8.0	3 : 1	Monthly; fry	407 (1.3)	Coche (1982)
Happas in lakes (20 m ²)	<u>O. niloticus</u>	215 : 275	4.0	3 : 1	Daily; fry	43 (1.3)	Guerrero & Garcia (1983)
Happas in tanks (3.34 m ²)	<u>O. niloticus</u>	46-185:58	5.0	2 : 1	10-18 day intervals; eggs, yolk sac fry and fry	607 (2.5)	Hughes & Behrends (1983)
Tanks (55 m ²)	<u>O. niloticus</u>	200-400	4.4-6.5	5 : 1	Daily; swim-up fry	189-278	Coche (1982)
(3.14 m ²)	<u>O. niloticus</u>	250-300:200	3.2	3 : 1	As spawned; eggs	1000-1500 (3)	Present study

NOTE: N.G. = Not given

of fry obtained from ponds may be increased by genetic contamination of the desirable species used.

To bridge the shortfall in the production of quality seed by traditional pond methods, the development of hatcheries for intensive tilapia seed production is now recognised as being critical to the growth of the industry (Pullin and Lowe-McConnell, 1982). At present the intensive production of fry from hatcheries based on spawning arenas, tanks and happas (suspended enclosures in tanks, ponds or lakes) is beset by problems related to variations in fecundity between individuals, differences in spawning frequencies of individual females and the temporal asynchrony of spawning cycles between individuals. Nevertheless, these systems have increased fry production from less than 100 fry/female/month from traditional spawning ponds to over 500-600 fry/female/month (Table 5.1). Further improvements in fry production can be achieved, as shown in the present study, by the removal of eggs from brooders for artificial rearing (Table 5.1). These more efficient fry production systems, however, require greater managerial and technical inputs than traditional pond methods.

Fry production and fry quality from any seed production system can be improved by the discernment of the reproductive capabilities and breeding behaviour of captive stocks to enable a more prudent selection of broodfish.

5.2 Implications of Parental Influences on Egg and Fry Production and Quality

5.2.1 Considerations in maternal selection for egg and fry production and quality

As yet there is no clear rationale for deciding the age and size of tilapia broodfish for use in the mass production of high quality seed. In species such as salmonids and carps where the brooders are manually stripped of their gametes for artificial propagation, criteria different to those used for the tilapias may be used to determine the size of broodstock. For example, in rainbow trout, since egg and fry viability and fry vigour were found not to be affected by egg size Springate and Bromage (1984) advocated the use of virgin females which have a higher relative fecundity than older broodfish, although most farmers use four and five year old broodstock (A. Stevens, personal communication). In carps, on the other hand, the size of broodfish utilized is often limited by difficulties experienced in handling large (10-15kg) fish during the administration of hormone injections used to hasten ovulation and during subsequent stripping of gametes. Therefore "first spawners" are usually used (Woynarovich and Horvath, 1980).

In the tilapias the age and size of broodfish currently used for commercial culture or experimental trials vary widely (Table 5.1). Data collated from the available literature indicate that size of broodfish used is largely related to the size of the fry production system; the bigger the system the larger the broodstock used (Table 5.1). For example, in Israel 1.5kg-3.0kg broodfish are

bred in 1 ha ponds to increase fry production (Rothbard et al., 1983), while in the Philippines 90g-200g broodstock are used in 1.5m² to 20m² happas (Broussard, Reyes and Raguindin, 1983; Guerrero and Garcia, 1983). Unfortunately, none of these authors states the age of broodstock used.

It is generally accepted that the fecundity of females increases with their age, length and weight (Bagenal and Tesch, 1978; Wootton, 1979; Mann and Mills, 1979; Hislop, 1984). For O. niloticus, Babiker and Ibrahim (1979a) and Payne and Collinson (1983) have shown the number of oocytes in 'mature ovaries' to increase with body weight or length. It is doubtful, however, if these relationships can predict the actual quantities of eggs or fry obtained from a given female. Hitherto, information on the size and number of eggs spawned which could be attributed solely to O. niloticus and O. mossambicus broodfish age and size was not available. Siraj et al. (1983) evaluated the fecundity and egg size of three age-classes of overwintered O. niloticus broodfish but feeding and rearing conditions were not stated, although these are known to affect the laying down of ovarian germinal epithelia (Nikolskii, 1969), recruitment of oocytes and the magnitude of atresia (Mironova, 1977; Scott, 1979).

The results of the present study with O. niloticus and O. mossambicus support the generally held view that egg size is predominantly influenced by the age of the spawner (Nikolskii, 1969), but also show that a female's egg laying capacity is more closely related to her weight and size than to her age (Tables 3.4, 3.5 and 3.6).

These results also confirm that for these two species the rate of increase in the number of eggs shed per spawning declines proportionally with increase in maternal size, or that smaller fish produce more eggs per unit weight. Thus based on the egg laying capacity of females alone, two management practices to increase egg production can be suggested:

- (a) selection of larger females from any given age-class of broodfish - giving more eggs per female or
- (b) selection of younger or smaller broodfish (e.g. yearlings) giving more eggs per unit body weight.

In addition to the above options for broodfish selection total egg production can be increased by selecting for individual females showing high fecundity. Evidence from the present study shows that even after accounting for the variations between successive spawns of individual females some females consistently yield significantly higher numbers of eggs (Table 3.2). It will require further investigation, however, to establish if high fecundity is a heritable trait.

A further consideration for broodstock selection may be the number of spawnings required to meet target fry production which will vary depending on female size. As shown in Table 5.2 to reach a target production of 85,000 eggs/month (= 1 million/yr) 87% more spawnings/month would be required from 100g O. niloticus females compared to 300g females. Similarly for O. mossambicus 56% more spawnings would be required (fecundities extrapolated from Table 3.6). The number of females that would be needed to meet the number

TABLE 5.2.

Predicted fecundities¹ of three sizes of O. niloticus and O. mossambicus females in relation to number of spawnings required for target production of 85,000 eggs/month (= 1 million/yr)

Female weight (g)	Total fecundity		Relative fecundity		Number of spawnings required/month	
	<u>O.niloticus</u> (no.eggs/spawning)	<u>O.mossambicus</u>	<u>O.niloticus</u> (no.eggs/Kg♀)	<u>O.mossambicus</u>	<u>O.niloticus</u>	<u>O.mossambicus</u>
100	529	621	5293	6211	161	137
200	782	818	3913	4089	109	104
300	983	960	3279	3202	86	88

¹ Fecundities determined from regression equations given in Table 3.6

of required spawnings would depend on their inter-spawning intervals (ISI). The ISI, however, is strongly influenced by the management of brooding females and the spawning system employed (Table 5.3). For example, the ISI of egg-robbed females from the present study was found to be approximately half that of natural brooders. Similarly, Dadzie (1970b) reported the ISI of egg-robbed O. aureus females to be between two to three weeks.

Although the results of the studies by Lee (1979) and Siraj et al. (1983) suggest that the ISI of yearling O. aureus and O. niloticus is slightly shorter than that of older females, to date there is no clear evidence relating the ISI of females to their age or size. Data available from the literature and the present study on the ISI of mouth-brooding species show that the seed production system affects the ISI rather than broodfish size (Table 5.3).

In addition to the influence of maternal age and size on egg production, maternal influence on the quality of eggs and fry are also important considerations in broodfish selection. There is, however, a paucity of published information for the tilapias and thus comparisons with other tilapia species are limited.

Lee (1979) and Siraj et al. (1983) reported that the hatchabilities of eggs from yearling O. aureus and O. niloticus females, respectively, were lower than those of eggs from older broodstock. These findings, however, were based on the incubation of up to three-day old naturally spawned egg clutches after enumeration without due consideration to the variation in fertility rates of the egg

TABLE 5.3

Comparison of representative methods of broodstock management on the inter-spawning intervals (ISI) for three widely cultured Oreochromis species

Species	Production system	Natural rearing (N) Egg or fry removal (R)	Broodstock weight (g)	Sex ratio :	ISI (days)	Source
<u>O. niloticus</u>	500 l glass aquaria	R	400 - 500	Individual pairs	30 - 50	Mires (1977)
	600 l glass aquaria	R	≈ 50	N.G.	26 - 45	Mires (1982)
	100 l glass aquaria	R	48 - 160	3 : 1	24 - 40	Fig. 3.2
	Plastic pools	R	49 - 157	3 : 1	7 - 12	Siraj <u>et al.</u> (1983)
	Plastic pools	R	293	3 : 1	10 - 20	Siraj <u>et al.</u> (1983)
	1 m ² × 2 m diameter fibre-glass tanks	R	50 - 400	3 : 1	12 - 24	Fig. 3.2
<u>O. mossambicus</u>	Ponds	N	N.G.		30 - 40	Vaas & Hoofstede (1952)
	100 l glass aquaria	R	50 - 100	4 : 1	35 - 49	MacIntosh (1985)
	100 l glass aquaria	R	30 - 98	3 : 1	28 - 40	Fig. 3.2
	1 m ² fibre-glass tanks	R	41 - 210	3 : 1	12 - 24	Fig. 3.2
<u>O. aureus</u>	Ponds	N	N.G.	N.G.	33 - 59	McBay (1961)
	Ponds	N	N.G.	N.G.	42 - 52	Bard <u>et al.</u> (1976)
	Glass aquaria	R	38 - 298	N.G.	30 - 56	Dadzie (1970a)
	Glass aquaria	R	N.G.	N.G.	13 - 30	Lee (1979)
	600 l aquaria	R	≈ 50	N.G.	30	Mires (1982)

NOTE: N.G. = Not given

clutches. The fertility rates of naturally spawned egg clutches from yearling females were found to be lower than those of older females (Table 5.4); and the findings of Lee (1979) and Siraj et al. (1983) probably also reflect this. In addition, it was found in the present study, that maternal age and hence egg size does not affect the viability of artificially reared eggs.

For rainbow trout (Springate and Bromage, 1985), Atlantic salmon (Thorpe et al., 1984) and carp (Zonova, 1973; Tomita et al., 1980) the survival of fry under conditions of equal care was not affected by maternal age and egg size. In the present studies, the overall survival of fry from yearling females was lower than in older broodfish (Sections 3.2 and 3.4). In addition to fry survival, the feeding success of fry is also higher in fry from the older broodfish (Fig. 3.8). Even though onset of exogenous feeding is not influenced by maternal age the maximum number of fry successfully feeding is improved by using older broodfish (Fig. 3.8). Further, under conditions of delayed initial feeding or reduced feeding opportunity, e.g. retention of fry by brooders in the buccal cavity during natural rearing, the larger fry from older females are able to sustain food deprivation for a longer period and maintain a better condition than fry from yearling mothers (Fig. 3.14). Overall early fry survival (up to 20 days) and feeding success of fry from both species would be improved by selecting 1+ and 2+ females in preference to 0+ females. In addition, the selection of older broodfish would also improve the size of first feeding fry which may result in larger juveniles (Section 3.4).

TABLE 5.4

Comparison between mean fertility rates of naturally spawned and manually stripped and fertilized egg clutches derived from three age-classes of *O. niloticus* and *O. mossambicus* female broodfish

Species	Broodstock age-class (months)	n	Naturally spawned egg clutches ¹				Manually stripped egg clutches ¹				Hatch rate (%) x̄ (SD)	
			Broodstock		Fertility rate of clutch (%)		Broodstock		Fertility rate of clutch (%)			
			Length(cm) x̄ (SD)	Weight(g) x̄ (SD)	x̄ (SD)	Range	Length(cm) x̄ (SD)	Weight(g) x̄ (SD)	x̄ (SD)	Range		
<i>O. niloticus</i>	0• (6-9)	21	10.5(1.8)	42.9(20.6)	56.4(34.3) ^{a1}	4-98	18	11.5(1.8)	58.2(30.1)	92.6(5.8) ^{a2}	83-100	91(6.1) ^a
	1• (14-17)	27	17.3(1.0)	180.1(28.9)	70.8(22.6) ^{a1}	8-100	10	17.2(2.1)	166.1(20.6)	92.4(7.4) ^{a2}	84-100	89(5.8) ^a
	2• (20-26)	15	19.1(2.1)	265.1(60.1)	72.3(27.8) ^{a1}	10-100	16	20.3(1.6)	280.1(40.2)	96.2(5.7) ^{a2}	86-100	93(6.6) ^a
Overall mean			15.6	162.7	66.5	7-99		16.3	168.1	93.7	84-100	91
<i>O. mossambicus</i>	0• (8-11)	18	8.9(1.2)	36.2(10.9)	63.0(17.2) ^{b1}	0-98	19	9.2(1.2)	48.1(11.6)	90.4(5.5) ^{b2}	88-100	88(6.2) ^b
	1• (14-17)	39	16.0(1.1)	138.2(21.1)	70.1(20.7) ^{b1}	0-98	15	15.9(1.9)	160.1(30.1)	94.9(3.4) ^{b2}	91-100	92(8.6) ^b
	2• (22-27)	37	18.5(1.7)	203.1(24.3)	71.3(22.6) ^{b1}	11-98	18	19.2(1.5)	250.1(36.1)	87.5(7.7) ^{b2}	78-100	83(10.5) ^b
Overall mean			14.5	125.8	68.1	4-98		14.8	152.8	90.9	85-100	88

¹ Mean values within a column and species having the same letter are insignificantly different (P > 0.05)

Mean values within a row and species having different numbers are significantly different (P < 0.05)

Clearly, on the basis of number of spawnings required to meet a target production of fry and to improve fry quality the selection of females from 1+ and 2+ broodfish is desirable. The selection of these older and larger broodstock may, however, prove difficult under some conditions. In countries such as Israel where fish need to be overwintered the extra space and costs incurred in on-growing and overwintering larger broodfish may be limiting.

5.2.2 Consequences of parental breeding behaviour on fry production and quality

Unlike salmonids (Craik and Harvey, 1984; Thorpe et al., 1984; Springate and Bromage, 1984, 1985) and carp (Woynarovich and Horvath, 1980; Shireman and Smith, 1983) where the gametes of broodstock are stripped manually and incubated artificially, thereby eliminating the effects of parental breeding behaviour, intensive tilapia seed production at present relies mainly on natural spawning in captivity. Therefore the egg laying capacity of the selected broodfish may be affected by behavioural factors associated with sex ratios and crowding (Allison, Smitherman and Cabrero, 1976; Mires, 1982; Hughes and Behrends, 1983; Macintosh, 1985) in fry production systems. Recently Hughes and Behrends (1983) have shown that O. niloticus seed (eggs, yolk-sac fry and swim-up fry) production in 3.34m² single net happa enclosures was depressed by 73% when the female to male sex ratio was increased from 2:1 to 3:1. Further, they also found that increase in stocking density decreased seed production. Similarly, in fry production from ponds, Mires (1982) observed that increasing the sex ratio from 1:1 to 3:1 depressed

fry production by 27%. The improved seed production using a low female to male sex ratio was attributed to a greater availability of males (Mires, 1982).

The results of the present study, however, suggest that parental breeding behaviour, especially in confined conditions of happas, pools and tanks, may also adversely affect fry production.

During the present study, examination of numerous females held in isolation from males revealed that the gonads of mature females, irrespective of age or size, were in a similar stage of maturation, the majority of oocytes being in the advanced vitellogenic phase. These observations suggest that social interactions within and between sexes in fry production systems may play a key role in oocyte maturation, ovulation and ultimately spawning. In cichlids, the frequency of gonadal cycling is increased by sensory stimulation from, e.g., visual stimuli, sound production, lateral line contacts and probably from chemical communication (Aronson, 1945, 1951; Polder, 1971; Marshall, 1972; Chien, 1973). By subjecting O. mossambicus females to different degrees of social contact Silverman (1978a, 1978b) showed that visual stimuli hastened mainly ovulation whilst unlimited social contact with either sex advanced oocyte development. Further, he suggested that regular spawning would most likely occur when males with ripe gonads are continuously available for new females.

How can the behaviour of males and females during spawning affect seed production? In mouth-brooders, where there is intimate contact

between a male and female during spawning and fertilization, factors such as disturbance during spawning, spawning experience and compatibility between the sexes may affect the spawning success of the breeding pair. Under crowded conditions (e.g. high density or biomass) attempts by the male and female to ward off persisting intruders during spawning may upset the synchrony of spawning between the pair thereby reducing the chances of the male fertilizing the eggs. Especially, as the eggs once laid are immediately picked up by the female (Trewavas, 1983). Under these conditions seed production will depend not only on the reproductive capacity of the selected females but also on the fertility rate of the clutch achieved during spawning. In addition, aggression by spawning males may chase off the female, which may then not spawn all her eggs.

More importantly, it has hitherto been conjectured that males can successfully spawn with numerous females in succession (Lowe (McConnell), 1959; Peters, 1971; Polder, 1971; Baylis, 1981). Evidence from this study shows that whilst a male can mate with several females his egg fertilizing capacity declines rapidly as the number of spawnings he milts over in any one day increases (Fig. 4.2). Dadzie (1969) provides histological evidence to show that in O. mossambicus the lobules of the testis, once discharged of most of its sperms are empty, and remains so until intensive spermatogenesis recommences to replenish the ripe sperms. In the cichlid Aequidens portalegrensis this period was four days (Polder, 1971). Consequently, depending on spawning history, repeated matings by males at short intervals may result in fewer spawned eggs being

successfully fertilized. This may have serious implications for seed production, especially in management systems where females are first fattened and then introduced into spawning systems in an attempt to synchronise spawnings between females. In the confined conditions of arenas, happas and tanks, which are frequently stocked with a sex ratio of 3-5 females to 1 male (Balarin and Haller, 1982; Broussard and Reyes, 1985), an inadequate number of males may therefore result in reduced seed production. Moreover, the availability of males to females is insufficient by itself to maximise the viability of the clutch. The testes of the males must also contain sufficient quantity of sperms to maximise the probability of egg fertilization.

Therefore to maximise the fertility rates of the clutches of the selected females in fry production systems the following conditions should be provided:

- (a) conditions which are conducive to minimal disturbance of the spawning pairs, e.g. lower stocking density, separation of spawning areas from shoaling areas;
- (b) availability of a suitable number of males to allow females to select compatible males;
- (c) adequate number of males to maintain maximal fertilization rates of clutches.

In addition, the fertility rates of naturally spawned O. niloticus and O. mossambicus clutches were found to be affected by maternal age, being lower for yearling clutches than those of older females (Table 5.4). These lower fertility rates, however, were not due

to poor egg quality (Table 5.4) and may therefore have been due to behavioural differences between broodfish of different age-classes. Overall, since the fertility rates of 1+ and 2+ females achieved during natural spawning was higher than those of yearling females (Table 5.4) their selection over other age-classes would improve fry production.

Fry productivity may also be influenced by the extent of injury to the delicate yolk-sac fry during buccal rearing (Fig. 4.3). The extent of injury to fry may be increased if broodstock are kept in confined systems such as glass aquaria, irrespective of broodfish age and size. Aronson (1949) and Baerends and Baerends-Van Roon (1950) attributed the differences between fecundity and fry production of females to a disparity between total clutch volume and buccal volume of the brooder. Evidence from the present study, however, suggests that the buccal cavity of females is considerably larger than the total clutch volume and that increases in churning frequency of the clutch by the brooder may be implicated in the extent of damage to fry and hence reduction in the numbers of fry released (Section 4.3.2). Predation of fry by parents in fry production systems may also reduce seed production (Berrios-Hernandez and Snow, 1983).

In summary, the main reason for the poor yields of naturally produced fry from spawning systems appear to be:

- (a) the low egg laying capacity of broodfish and partial spawning of eggs by females;

- (b) sub-optimal fertility rates of egg clutches achieved during spawning;
- (c) reduction of fry numbers due to fatal injury during buccal rearing and other losses such as swallowing of young by brooders;
- (d) the predation of fry during release from buccal rearing by parents, larger fry, insects, etc.

The probability of reduced fertility rates of clutches and the risk to fry of mechanical injury, however, would increase if target seed production depended on a large number of spawning acts. The greater numbers of spawnings required from yearlings (Table 5.2) together with the lower mean fertility rates of their naturally spawned clutches (Table 5.4) would suggest that yearling broodstock may be less satisfactory than older females even though they produce more eggs per unit body weight.

5.2.3 Considerations for the renewal of broodfish

For the tilapia seed producer the question of how often to renew broodfish is as yet unresolved. At present broodfish are replaced whenever fry production is observed to decline. Roberts (see discussion in Mires, 1982) mentioned that at Stirling, Scotland, O. niloticus, O. mossambicus and O. spilurus broodstock bred in aquaria are replaced after an 18 month period while Lovshin (in Mires, 1982) reported that in Brazil fry production in ponds falls by about 50% after one year and therefore broodstock are replaced after this time. In the Philippines brooders are usually changed

every 21 months. In larger farms (5,000-10,000m²), however, brooders are changed every 15 months (Yater and Smith, 1985). The reasons for the temporal decline in fry production, commonly observed in ponds is as yet unclear. Lovshin (see discussion in Mires, 1982) suggested that this trend may be caused by the lower spawning frequency of older broodstock. The decline in fry production, however, may be related to different broodstock management practices. For example, Mires (1982) stated that periodic water exchange (once every one or two months) in ponds by commercial fry producers in Israel renews spawning activity in their large broodstock.

On a practical basis, the effect of spawning frequency on egg viability and the egg laying capacity of mouth-brooding tilapias would be of interest in deciding on when to renew broodstock. In the present study a maximum of nine consecutive spawnings was obtained from 1+ and 2+ O. niloticus and O. mossambicus females robbed of their eggs, at intervals of 12-20 days. In these females there was no evidence of a decline in fecundity or egg viability, although there were fluctuations in egg numbers between spawns. Overall, the fecundity increased, an observation also made by Dadzie (1970b), Lee (1979), Mires (1982) and Siraj et al. (1983). Therefore, females could be used for at least nine spawnings without loss in fecundity or egg viability. Mires (1982) reports that the viability of eggs from six-year old Oreochromis broodstock was just as good as that from younger broodstock.

The egg laying capacity of individual females eventually becomes affected by their spawning frequency and by the senescence of their

ovary, which occurs at a faster rate than somatic tissue senescence (Woodhead, 1979). Studies on guppies, Poecilia reticulata showed that the ovaries of young females consist of closely packed primary oogonia. With ageing and spawning, however, the numbers of ripening eggs in the ovary were observed to decrease and the number of atretic eggs increase (Woodhead and Ellet, 1969, cited by Woodhead, 1979). Further, Woodhead (1979) reported that with ageing there was a noticeable infiltration of the ovary by connective tissue and thickening of ovary walls.

To date there is insufficient information on the effects of ageing and spawning frequency on the egg laying capacity of tilapias to help provide a rationale for the renewal of broodfish. At present the decision to renew broodstock on many farms appears to be dictated by availability of sufficient numbers of broodfish (D. J. Macintosh, personal communication). The influence of factors such as nutrition (Mironova, 1977; Scott, 1979), female age and spawning frequency (Woodhead, 1979) and their interaction on ovarian changes need to be resolved to establish if indeed the temporal decline in fry production in ponds is related to change in the egg laying capacity of individuals in a breeding population and/or to the current broodstock management practices.

5.3 Hatchery Production of Fry

5.3.1 The need, advantages and feasibility of artificial incubation of eggs and fry

At present, since tilapias spawn readily in captivity, induced breeding techniques which would enable the entire life cycle to be controlled, have been neglected. Attempts to artificially propagate tilapia seed have been restricted to the stripping and incubation of naturally ovulated eggs and incubation of seed after removal from brooders. Unlike species such as carps, mullets, milkfish and catfishes (Harvey and Hoar, 1979) the success of induced spawning of tilapias has been poor (Dadzie, 1970a; Babiker and Ibrahim, 1978b). The successful development of such techniques would, in addition to enabling the production of seed more precisely according to need, would also improve the overall fertility rates of clutches (Table 5.4) and would eliminate the possibility of partial spawnings, adverse behavioural influences on spawning and behavioural differences associated with species incompatibility, as with hybrid production (Mires, 1982).

Among the methods currently used to increase the production of fry of uniform size, frequent harvesting of seed is gaining popularity (Rothbard et al., 1983; Hughes and Behrends, 1983; Berrios-Hernandez and Snow, 1983) (Table 5.1). Such fry production techniques, however, inevitably result in the collection of eggs, yolk-sac fry and swim-up fry. Indeed, seed harvested at 10-18 day intervals from happas consisted of 50% eggs and 17% sac-fry (Hughes

and Behrends, 1983). If this method and manual stripping of broodfish is to be widely accepted, however, the development of efficient yet simple artificial incubation systems that give consistently high hatch and survival rates, together with a successful rearing method of fry are required.

There are many advantages in artificially rearing tilapia eggs and fry. The removal of eggs from brooding females for artificial rearing decreases the ISI of females (Fig. 5.1; Table 5.3) thereby improving their fry productivity. In addition, a seed production system based on the removal of eggs from tagged fish would facilitate closer monitoring of their reproductive performance. Unproductive females could be culled or removed, while selection of females for desirable traits such as higher fecundity and larger egg size would become possible. And selection programmes on farms for genetic traits such as body form, colour, growth efficiency and sex ratio would be more effective under controlled hatchery conditions.

In addition to improving the efficiency of fry production, artificial rearing could substantially improve the desired qualities of the fry. For example, the efficacy of sex reversal techniques used in all male fry production could be increased by the use of extra-orally reared fry. The early feeding of these fry is predictable, whereas with natural rearing the sex of the fry may become fixed during the prolonged period of parental care. For this reason, in Taiwan fry reared artificially from the egg stage are commonly used for sex reversal treatment (B. Hopher, personal communication). Moreover, fry reared from the egg stage can be fed continuously

from the time they are first capable of exogenous feeding and may therefore be larger than the naturally reared fry where early feeding opportunities may be reduced due to prolonged parental care (Section 3.4; Table 4.3).

To artificially rear tilapia eggs and fry various workers have used conical upwelling containers (Valenti, 1975; Rothbard and Hulata, 1980) and shaking tables (Rothbard and Pruginin, 1975; Lee, 1979; Siraj et al., 1983; Snow et al., 1983) for continuous agitation of eggs. However, losses of up to 20% and 40% of egg batches, respectively, have been reported (Rothbard and Hulata, 1980). These losses have been attributed to bacterial and fungal infection, but as yet it is uncertain that egg infection is the primary cause of egg and fry mortality. Evidence from the present study suggests that mechanical injury caused by continuous agitation of eggs may play an important role in reducing egg viability (Section 2.1). Using the technique of partial agitation the overall survival rates to the swim-up fry stage of artificially reared eggs using round-bottomed containers and UV treated water were consistently high (Table 2.2). In the absence of UV sterilization, survival rates can still be improved by removing eggs from females for incubation after the pigmentation stage (48-72h at 28°C) (Shaw and Aronson, 1954; Lee, 1979; Rothbard and Hulata, 1980; Siraj et al., 1983) and by chemotherapy (Valenti, 1975; Lee, 1979; Siraj et al., 1983; Subasinghe and Sommerville, 1985).

Evidence from the present study suggests that the artificial incubation of tilapia eggs and fry for mass seed production could be

practicable. In the present study round-bottomed containers (volume 0.75 l) could adequately incubate up to 10g of eggs (1,000-2,500 depending on egg size) with a success rate of 85% to swim-up fry. Since fry should remain in the incubators for six days (to swim-up stage at 28°C) 10 incubators occupying an area of only 0.015m² potentially could accommodate 25,500-63,700 fry/month. Since dead eggs and fry are automatically flushed out of the system labour requirements for rearing eggs and yolk-sac fry would be minimal. In overall terms the production cost of installing an artificial system would be negligible. The incubators used in these studies were made from disposable soft drinks bottles and a UV irradiation unit which was capable of sterilizing 820 litres of water/hour was purchased for about £70.00 (\$100.00 - Aquatic Services Ltd., England). In the absence of UV sterilization chemotherapy may be used (Subasinghe and Sommerville, 1985). Clearly additional space, labour and capital and running costs for artificial incubation systems are small. The success or failure of such systems however, will be determined by factors such as the reliability of electricity, and the availability of suitably trained personnel.

5.3.2 Some considerations for the hatchery rearing of fry

The fry of Oreochromis species, unlike marine larvae, are amenable to hatchery rearing. Nash (1977), in his review of the culture of marine species, pointed out that the failure to cultivate marine species was due to the difficulties with the small size and fragility of the larvae. In addition, the failure to meet the daily needs of an adequate live food was a major factor limiting mass production

(Houde, 1973; Girin and Person-Le Ruyet, 1977; Kuronuma and Fukusho, 1984). Consequently the survival rates of successfully weaned hatchery reared larvae of commercially important marine fish such as sea bass, Dicentrarchus labrax (Caubère, 1984; SIME, 1984), grouper, Epinephelus tauvina (Chen et al., 1977), turbot, Scophthalmus maximus and gilthead sea bream, Chrysophrys aurata (Girin, 1979) are less than 5%. In contrast, Oreochromis fry lack a larval stage (Noakes and Balon, 1982) and have well developed fins and a large mouth at first feeding (Fig. 2.11b). And since these fry are omnivorous (Noakes and Balon, 1982) and feed readily on particulate matter they can be reared successfully on powdered diets without the need for weaning.

The inflation of the swimbladder to achieve neutral buoyancy is an important prerequisite for successful feeding (Aronovich et al., 1975; Doroshev et al., 1981). On achieving neutral buoyancy the energetic cost of swimming becomes reduced and feeding efficiency is improved (Hunter, 1972). Therefore if the swimbladder fails to inflate or its inflation is delayed, the growth of fry or larvae may be depressed (Von Ledeber and Wunder, 1938, cited by Doroshev et al., 1981) or they may die (Johnson and Katavic, 1984; Kolbeinshavn and Wallace, 1985). Abnormal inflation of swimbladders or their failure to inflate has been observed in artificially cultured pelagic larvae. For example, nearly an entire population of Black Sea turbot, Scophthalmus maeoticus either failed to inflate or developed excessive inflation (Spectorova and Doroshev, 1976). Similar observations were also recorded for the larvae of grey mullet, Mugil cephalus (Nash, Kuo, Madden and Paulsen, 1977). In

striped bass, Roccus saxatilis, the percentage of larvae with uninflated swimbladders may be as high as 90% (Doroshev, 1970). In contrast, cases of uninflated swimbladders in tilapias under hatchery conditions are rare (Doroshev et al., 1981). General fry abnormalities were below 2% in hatchery reared fry (Section 2.1).

Information on the timing of the transition to exogenous feeding and feeding success of Oreochromis fry under hatchery conditions is lacking. Based on observation of the morphological development of O. aureus and O. mossambicus fry, Hallerman et al. (1983) reported that swim-up stage and exogenous feeding occurs at nine days after hatching at 29°C. In the present study the feeding ability of fry was determined by presenting food to previously unfed yolk-sac fry (Sections 2.2 and 3.2). The onset of exogenous feeding which coincides with the swim-up stage is temperature dependent (Section 2.2). The time sequence of fry development at 28°C is presented in Fig. 5.1. At higher rearing temperatures each event occurs earlier. For example, feeding commences at five to six days post-hatching at 28°C (Fig. 5.1) and at four days post-hatching (i.e. 8-9 days post-spawning) at 30°C (Section 2.2). Similarly the point-of-no-return, though related to egg size (Section 3.2) occurs earlier under conditions of higher rearing temperature (Section 2.2). Further, evidence from the present study shows that the yolk reserves of swim-up fry may not be adequate to meet all their metabolic and nutritional demands if initial feeding is delayed (Sections 2.2 and 3.2). Consequently their growth in the absence of suitable food at this stage may be depressed. Therefore for the successful hatchery rearing of fry the temperature

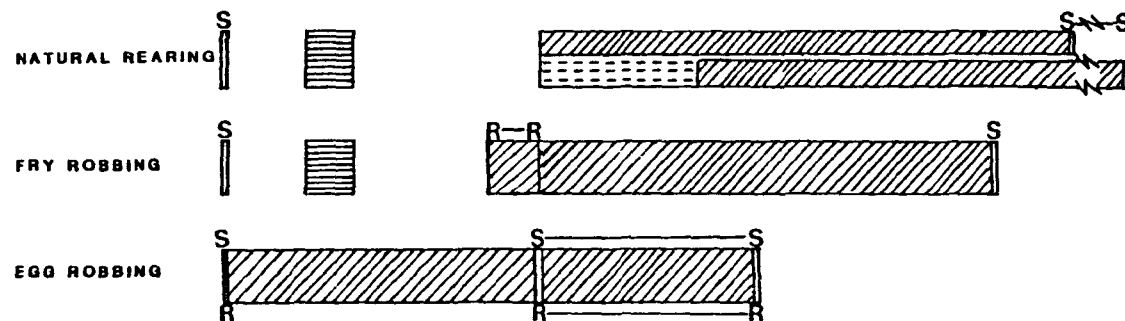
of the rearing water should be recorded to enable hatchery operators to commence feeding of fry at the optimal time.

In view of the above, the quality of tilapia seed in terms of their survival and growth rates would be influenced by the hatchery management practice (Fig. 5.1). A comparison of the time period leading to the onset of feeding suggests that since the naturally reared fry are capable of exogenous feeding before the time at which they are initially released from the buccal cavity by the brooder (Fig. 5.1), they may begin to lose condition whilst being naturally reared. Although the survival times (times to 50% survival) may not be approached during this period fry mortalities may commence. Indeed, the high moisture content recorded for fry from some naturally reared clutches (Table 4.3) may indicate that fry experience starvation even during buccal rearing. If yearling broodfish are selected for seed production the fry from their small eggs may be further disadvantaged by their small yolk reserves especially at high rearing temperatures (Section 2.2). Thus, if naturally reared and released fry are collected for on-growing their weight may be sub-optimal for their age (Table 4.3). Furthermore if yearling females are used for fry production the loss in growth opportunity would be high (Table 3.14; Figs. 3.12 and 3.13). Similarly, in other fish species, for example sockeye salmon (Bilton and Robins, 1973) and grunion (May, 1971), a delay in initial feeding resulted in sub-optimal growth of fry. If the initial feeding of tilapia fry is delayed, even by a day, such weakened fry could easily become victims of predation (Berrios-Hernandez and Snow, 1983), cannibalism (Uchida and King, 1962; Macintosh and De Silva,

FIGURE 5.1

Interpretation of O. niloticus and O. mossambicus fry production by three hatchery management methods with respect to the events of feeding, growth and survival of fry. Responses of both species based on data from the present study. Ranges given for feeding capabilities, growth and survival were due to differences in their mean egg size. Broodstock and fry reared at 27°-28°C

Management of brooders



KEY

- S SPAWNING
- R REMOVAL OF EGGS OR FRY
- HATCHING
- INITIAL RELEASE OF FRY BY BROODERS
- OVARIAN DEVELOPMENT

Fry performance

O. niloticus

FEEDING CAPABILITIES



GROWTH & SURVIVAL



O. mossambicus

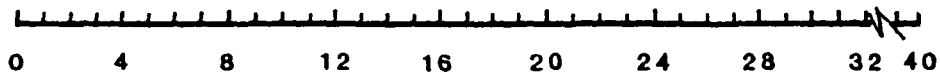
FEEDING CAPABILITIES



GROWTH & SURVIVAL



- △ △ ONSET OF EXOGENOUS FEEDING
- ■ MAXIMUM NUMBER OF FRY FEEDING
- ○ END OF YOLK-SAC-STAGE
- ● POINT-OF-NO-RETURN
- ▲ ▲ MAXIMUM BODY WEIGHT OF FRY
- □ SURVIVAL TIME OF 50% OF FRY



DAYS AFTER SPAWNING

1984), or disease (Roberts and Sommerville, 1982; Rothbard et al., 1983). Therefore, to maximise the growth potential of tilapia fry the seed producer should remove or encourage the release of fry from brooders prior to the swim-up stage or remove the eggs or sac-fry from brooders for artificial rearing. Fry that approach swim-up stage could then be fed from this point onwards.

The nutritional quality and the presentation of diets would also affect the survival and growth of hatchery reared fry. Tilapia fry are said to have a high requirement for dietary protein (Jauncey and Ross, 1982). Maximum growth of first feeding O. mossambicus fry was attained with a diet containing 50% protein (as fishmeal) (Jauncey, 1982). It should be mentioned, however, that tilapia fry are omnivorous and need to feed continuously. In many species an increase in feeding frequency has been one of the major factors improving the survival and growth of fry. In common carp (Szlaminska and Przybyl, 1986), turbot (Bromley and Howell, 1983) and sole (Gatesoupe, 1983) feeding throughout the day also substantially improved survival and growth. In the present study it was found that for O. niloticus and O. mossambicus fry, a feeding rate of six times a day in excess reduced fish size variation thus minimising fry losses through cannibalism.

If tilapia fry are inadequately fed fry losses through cannibalism can be high. Macintosh and De Silva (1984) have shown that a reduction of food ration from 24%-12% bodyweight/day increased cannibalism significantly. In their investigation 10%-35% of fry mortalities were due to size-dependent cannibalism. Uchida and King (1962)

reported losses of up to 17% which they attributed to predation by aquatic insects and cannibalism.

The presentation of the correct particle size to fry also plays an important role in feeding success. In fishes such as common and Chinese carps where the mouth aperture is small the size of first feed of 50-300µm is acceptable (Horvath and Lukowicz, 1982). In O. niloticus and O. mossambicus where the mouth aperture is larger a 300-500µm particle size was found to be readily acceptable to first feeding fry.

In summary, for any given genetic strain the quality of hatchery reared O. niloticus and O. mossambicus fry produced under optimal environmental conditions is dependent on the interaction between rearing temperature, the size of eggs and hence the broodstock age and size, source of seed, that is from naturally reared and released or artificially reared clutches, and the feeding regime adopted.

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APPENDICES

APPENDIX 1 Mean reproductive traits of individual *O. niloticus* females from 0+, 1+ and 2+ age-classes

Age (months)	Female No.	REPRODUCTIVE TRAITS ¹				
		Mean dry egg weight (mg)	Total Fecundity No. of eggs per clutch	Relative Fecundity No. of eggs per Kg ♀	Clutch wet weight (g)	Egg:Body weight ratio (%)
0+ (5-7)	1	1.71 ^{a1}	353 ^{a1}	9,977 ^{b1}	1.33 ^{a1}	3.72 ^{a1}
	2	2.03 ^{a1}	139 ^{a1}	5,152 ^{a1}	0.62 ^{a1}	2.20 ^{a1}
	3	1.57 ^{a1}	262 ^{a1}	8,507 ^{b1}	0.89 ^{a1}	2.89 ^{a1}
	4	1.75 ^{a1}	348 ^{a1}	10,070 ^{b1}	1.31 ^{a1}	3.80 ^{a1}
	5	1.89 ^{a1}	373 ^{a1}	8,017 ^{b1}	1.55 ^{a1}	3.30 ^{a1}
	6	1.57 ^{a1}	378 ^{a1}	9,912 ^{b1}	1.38 ^{a1}	3.61 ^{a1}
1+ (11-13)	1	3.54 ^{d2}	716 ^{b2}	3,705 ^{c2}	5.28 ^{b2}	2.74 ^{b1}
	2	2.78 ^{c2}	970 ^{bc2}	4,888 ^{cd2}	5.76 ^{b2}	2.90 ^{b1}
	3	2.00 ^{b2}	1,313 ^{c2}	6,865 ^{d2}	5.53 ^{b2}	2.89 ^{b1}
	4	2.73 ^{c2}	919 ^{bc2}	5,444 ^{cd2}	5.36 ^{b2}	3.18 ^{b1}
	5	2.75 ^{c2}	793 ^{b2}	4,190 ^{c2}	4.85 ^{b2}	2.57 ^{b1}
	6	2.54 ^{c2}	780 ^{b2}	3,824 ^{c2}	4.48 ^{b2}	2.20 ^{b1}
2+ (23-26)	1	4.32 ^{fg3}	1,229 ^{e2}	2,727 ^{e2}	11.38 ^{ef3}	2.53 ^{c1}
	2	3.44 ^{f3}	747 ^{d2}	2,915 ^{e2}	5.87 ^{c3}	2.29 ^{c1}
	3	3.51 ^{f3}	1,178 ^{e2}	3,688 ^{e2}	9.04 ^{de3}	2.83 ^{c1}
	4	3.89 ^{fg3}	772 ^{d2}	4,322 ^{e2}	7.37 ^{cd3}	4.12 ^{c1}
	5	2.91 ^{e3}	1,256 ^{e2}	2,643 ^{e2}	8.05 ^{cd3}	1.69 ^{c1}
	6	3.57 ^{f3}	1,771 ^{f2}	3,741 ^{e2}	13.21 ^{f3}	2.79 ^{c1}

¹ Values in the same column with different letters and numbers are significantly different (P < 0.05) for females within and between ages, respectively. Each value is the mean of three consecutive spawnings

APPENDIX 2 Mean reproductive traits of individual *O. mossambicus* females from 0+, 1+ and 2+ age-classes

Age (months)	Female No.	REPRODUCTIVE TRAITS ¹				
		Mean dry egg weight (mg)	Total Fecundity No. of eggs per clutch	Relative Fecundity No. of eggs per Kg ♀	Clutch wet weight (g)	Egg:Body weight ratio (%)
0+ (5-8)	1	1.53 ^{b₁}	792 ^{cd₁}	12,282 ^{b₁}	2.70 ^{b₁}	4.18 ^{c₁}
	2	1.65 ^{b₁}	584 ^{bc₁}	10,134 ^{ab₁}	2.26 ^{b₁}	3.93 ^{bc₁}
	3	0.96 ^{a₁}	370 ^{ab₁}	7,353 ^{a₁}	0.95 ^{a₁}	1.89 ^{a₁}
	4	1.64 ^{b₁}	254 ^{a₁}	9,186 ^{b₁}	0.87 ^{a₁}	3.12 ^{bc₁}
	5	1.44 ^{b₁}	1,017 ^{d₁}	12,125 ^{b₁}	3.32 ^{b₁}	3.85 ^{bc₁}
1+ (15-16)	1	2.50 ^{d₂}	414 ^{e₁}	3,706 ^{c₂}	2.31 ^{c₂}	2.07 ^{d₁₂}
	2	2.27 ^{cd₂}	928 ^{f₁}	8,056 ^{d₂}	4.64 ^{d₂}	4.03 ^{e₁₂}
	3	2.17 ^{cd₂}	814 ^{f₁}	8,356 ^{d₂}	4.00 ^{d₂}	4.11 ^{e₁₂}
	4	1.95 ^{c₂}	534 ^{e₁}	4,137 ^{c₂}	2.37 ^{c₂}	1.84 ^{d₁₂}
	5	1.92 ^{c₂}	537 ^{e₁}	3,576 ^{c₂}	2.47 ^{c₂}	1.64 ^{d₁₂}
2+ (18-23)	1	2.48 ^{g₂}	646 ^{g₁}	3,558 ^{e₂}	3.51 ^{e₂}	1.93 ^{f₂}
	2	2.87 ^{h₂}	790 ^{g₁}	5,068 ^{e₂}	5.16 ^{f₂}	3.31 ^{g₂}
	3	2.21 ^{fg₂}	677 ^{g₁}	3,890 ^{e₂}	3.23 ^{e₂}	1.86 ^{f₂}
	4	1.79 ^{e₂}	802 ^{g₁}	5,619 ^{e₂}	3.28 ^{e₂}	2.30 ^{fg₂}
	5	2.02 ^{ef₂}	1,064 ^{h₁}	5,460 ^{e₂}	6.32 ^{f₂}	2.53 ^{fg₂}

¹ Values in the same column with different letters and numbers are significantly different (P < 0.05) for females within and between ages, respectively. Each value is the mean of three consecutive spawnings.

APPENDIX 3 Correlation (r) matrix of the growth and reproductive traits of *O. niloticus* females^{1,2}

	GROWTH TRAITS		REPRODUCTIVE TRAITS				
	Weight (g)	Length (cm)	Mean dry egg weight (mg)	Total fecundity (no. eggs/ spawn)	Relative fecundity (no. eggs/ Kg ♀)	Clutch wet weight (g)	Egg : Body weight ratio (%)
<u>Traits:</u>							
Age (months)	0.940***	0.928***	0.861***	0.707***	-0.795***	0.853***	-0.383***
Weight (g)		0.997***	0.844***	0.829***	-0.753***	0.930***	-0.350***
Length (cm)			0.830***	0.847***	-0.728***	0.937***	-0.325**
Mean dry egg weight (mg)				0.586***	-0.769***	0.815***	-0.217*
Total fecundity (no. eggs/spawn)					-0.258*	0.945***	0.151
Relative fecundity (no. eggs/Kg ♀)						-0.496***	0.784***
Clutch wet weight (g)							0.018

¹ Data from spawnings of 36 individual females ² Levels of significance (with df = 91)

*** P < 0.001

** P < 0.01

* P < 0.05

APPENDIX 4 Correlation (r) matrix of the growth and reproductive traits of *O. mossambicus* females^{1,2}

	GROWTH TRAITS		REPRODUCTIVE TRAITS				
	Weight (g)	Length (cm)	Mean dry egg weight (mg)	Total fecundity (no. eggs/spawn)	Relative fecundity (no. eggs/Kg ♀)	Clutch wet weight (g)	Egg : Body weight ratio (%)
<u>Traits:</u>							
Age (months)	0.906***	0.930***	0.714***	0.324**	-0.622***	0.580***	-0.337**
Weight (g)		0.990***	0.599***	0.448***	-0.606***	0.643***	-0.368***
Length (cm)			0.620***	0.430***	-0.613***	0.637***	-0.365**
Mean dry egg weight (mg)				0.217	-0.409***	0.585***	0.022
Total fecundity (no. eggs/spawn)					0.439***	0.913***	0.594***
Relative fecundity (no. eggs/Kg ♀)						0.166	0.899***
Clutch wet weight (g)							0.475***

¹ Data from spawnings of 29 individual females ² Levels of significance (with df = 72)

*** P < 0.001

** P < 0.01

* P < 0.05