Aspects of the Reproductive Biology and Endocrinology of the Substrate-Spawning Cichlid *Tilapia zillii*.

A thesis submitted for the degree of Doctor of Philosophy

by

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DECLARATION.

The work presented in this thesis was carried out between 1991 and 1994 at the Institute of Aquaculture, University of Stirling. Except where specifically acknowledged, the work presented herein has been conducted independently and has neither been accepted nor submitted for any other degree.

CANDIDATE.....................................

SUPERVISOR...................................

DATE...

Abstract.

This study investigated several, previously little-known, aspects of reproductive physiology and endocrinology in the substrate-spawning cichlid *Tilapia zillii*; a tilapia that is becoming increasingly popular in world aquaculture. Studies were undertaken in controlled laboratory aquaria, thereby reducing the potential influence of environmental variation evident in many previous field studies of this species. Analysis involved two strains of *T. zillii:* strain 'A' (*T. zillii*) and strain 'B' (formerly known as *Tilapia tholloni*).

Spawning periodicity and total fecundity generally increased with fish size. Egg size varied within a narrow window and did not generally increase with fish size though fish weighing 100 - 200g tended to produce the largest eggs. The best estimate of spawning periodicity was considered to be 'mean days elapsed/spawn' as this figure was based upon both spawning and non-spawning fish in an experimental group. Mean days elapsed/spawn increased with increasing fish size and averaged 61.4 days and 37.5 days in strains 'A' and 'B' respectively. The shortest reproductive cycles observed were just 7 days and 6 days for strains 'A' and 'B' respectively. Total fecundity ranged from 461 - 11640 eggs/clutch. Mean total fecundity was 3606±280 in strain 'A' and 3560±243 in strain 'B'. Mean egg diameter was 1.5±0.04mm and 1.4±0.08mm in strains 'A' and 'B' respectively. Fecundity and egg size also varied over successive spawns in serial-spawning females but these variations did not appear to be related to spawning periodicity.

Regression analysis revealed strong relationships between fish size (weight and length) and total fecundity, relative fecundity and total egg volume. Relationships between fish size and egg size were generally much weaker. Fecundity and egg size were related to the length of the preceding inter-spawn-interval (ISI) in fish of certain weight categories but not others, providing limited evidence that length of ISI may in part, control fecundity and egg size in this species.

Ovarian recrudescence was classified into ten distinct developmental stages based upon oocyte size, biochemical properties and structure. This classification

scheme was comparable to classification schemes developed for other teleosts but represents the first detailed description of oocyte growth in a substrate-spawning tilapia.

Radioimmunoassay and stereological analysis provided valuable and novel data concerning the dynamics of ovarian development in this species. Levels of 17ß-oestradiol (E_2) and testosterone (T) peaked within 6 days of spawning, suggesting that vitellogenesis began as early as day 2 or 3 post-spawning. By day 8, ovaries were dominated by large late-vitellogenic/maturing oocytes (stages 6 & 7) occupying 60 - 70% of the ovary. Gonadosomatic index (GSI) reached maximal levels by day 14. Since the proportion of stage 6/7 oocytes exhibited little change from day 8 onwards, it is suggested that pre-vitellogenic oocytes are recruited into vitellogenic growth immediately after spawning and complete vitellogenesis as early as day 8 post-spawning. Analysis of serial-spawning fish found that initial post-spawn E_2 and T peaks (on days 2 - 6) were much lower after the second spawning.

Sex steroid levels were also found to be suppressed in confined *T. zillii* (i.e. where stocking densities were > $10kg/m^3$). Confined females failed to spawn but displayed a marked tendency to do so after transfer to individual aquaria. Serum E_2 and T were suppressed during confinement but increased rapidly following transfer to individual aquaria (coincident with resumed spawning activity). It is suggested that levels of E_2 and T under confinement are not sufficient to allow completion of vitellogenic growth and are most probably suppressed via a pheromonal mechanism.

Finally, the present study investigated the effect of prolonged food restriction on various aspects of reproduction. *T. zillii* were rationed from first feeding and throughout the following 17 months. Despite very large differences in fish size, no significant differences were detected in total fecundity, egg diameter nor total egg volume once data had been adjusted for differences in fish size. These data suggest that despite very large differences in food availability throughout the periods of sexual differentiation and on-growing, investment in reproduction remained relatively consistent. It appeared that during food restriction, *T. zillii* sacrificed body weight and growth so as to maintain reproductive investment.

In summary, this study provides valuable and novel information regarding the reproductive physiology and endocrinology of female *T. zillii* and suggests that this species may be a suitable 'model' species for future work on fecundity and ovarian development.

KEYWORDS: Tilapia, *T. zillii*, fecundity, spawning periodicity, ovarian development, food restriction, confinement.

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Glossary of scientific and common names of fish mentioned in this thesis.

Note: some species of fish (especially tilapia) do not have widely recognised common names. These species are indicated here by a dash (-) in place of the common name.

SCIENTIFIC NAME.	COMMON NAME.
Acanthobrama terrae-sanctae	-
Acipenser transmontanus	White sturgeon
Anguilla anguilla	European eel
Brachydanio rerio	Zebrafish
Carassius auratus	Goldfish
Carassius carassius	Crucian carp
Chelidonichtys kumu	Red gurnard
Chromis dispilus	Damselfish
Cichlasoma citrinellum	Midas cichlid
Cichlasoma nigrofasciatus	Convict cichlid
Clarias gariepinus	African catfish
Clupea harengus	Atlantic herring
Clupea harengus pallasi	Pacific herring
Colisa lalia	Dwarf gourami
Cottomephorus grewingki	Yellowfish Baikal sculpin
Cymatogaster aggregata	Sea perch
Cynoscion nebulosus	Spotted sea trout
Cyprinus carpio	Common carp
Dicentrachus labrax	Sea bass
Esox lucius	Pike
Etroplus suratensis	Pearlspot
Engraulis japonica	Japanese anchovy
Fundulus confluentus	Marsh killifish
Fundulus heteroclitus	Killifish
Gadus morhua	Cod
Gasterosteus aculeatus	Three-spined stickleback
Gobius jozo	Black goby
Haplochromis 'argens'	-
Heteropneustes fossilis	Indian catfish
Hippoglossus hippoglossus	Atlantic halibut
Lates niloticus	Nile perch
Leuciscus leuciscus	Dace
Lepomis gibbosus	Pumpkinseed sunfish
Leptocottus armatus	Staghorn sculpin
Limanda limanda	Dab
Lutjanus campechanus	Red snapper
Maccullochella macquariensis	Australian freshwater trout cod
Melanogrammus aeglefinus	Haddock
Merlangus merlangus	Whiting
Micropogonias undulatus	Atlantic croaker
Micropterus salmoides floridanus	Largemouth bass
Mugil cephalus	Grey mullet
Mycteroperca olfax	Galapagos bacalao
Oncorhynchus keta	Chum salmon
Oncorhynchus kisutch	Coho salmon
Oncorhynchus mykiss	Rainbow trout
Oncorhynchus nerka	Kokanee salmon
Oncorhynchus rhodurus	Amago salmon
Oncorhynchus tschawytscha	Chinook salmon
Oreochromis aureus	Blue tilapia

Oreochromis esculenta	-
Oreochromis leucosticta	-
Oreochromis macrocephala	-
Oreochromis mossambicus	Mozambique mouthbrooder
Oreochromis niloticus	Nile tilapia
Oreochromis spilurus niger	-
Oryzias latipes	Medaka
Pagrus auratus	Snapper
Pagrus major	Red sea bream
Perca flavescens	Yellow perch
Petromyzon marinus	Sea lamprey
Pimophales promelas	Fathead minnow
Pleuronectes platessa	Plaice
Pseudoplueronectes americanus	Winter flounder
Poecilia monacha	Top minnow
Poecilia reticulata	Guppy
Pterophyllum scalare	Angelfish
Rutilus rutilus	Roach
Salmo salar	Atlantic salmon
Salmo trutta	Brown trout
Salvelinus alpinus	Arctic charr
Salvelinus fontinalis	Brook trout
Sardinops melanostictus	-
Sarotherodon galileus	-
Sarotherodon leucostictus	-
Sarotherodon melanotheron	Black-chinned tilapia
Scomber scombrus	Atlantic mackerel
Scopthalmus maximus	Turbot
Seriola dumerilii	Mediterranean yellowtail
Solea solea	Dover sole
Sparus auratus	Gilthead sea bream
Symphysodon aequi fasciata axelrodi	-
Thunnus albacares	Yellowfin tuna
Tilapia guineensis	-
Tilapia macrocephala	-
Tilapia mariae	-
Tilapia rendalli	-
Tilapia tholloni	-
Tilapia zillii	Redbelly tilapia
Trichogoita trichopterus	Blue gourami

Glossary of commonly-used abbreviations.

ABBREVIATION	FULL TERM
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
E_1	Oestrone
E_2	17ß-oestradiol
E_3	Oestriol
FSH	Follicle stimulating hormone
GnRH	Gonadotropin releasing hormone
GnRIH	Gonadotropin release inhibitory factor
GSI	Gonadosomatic index
GTH	Gonadotropin
hCG	Human chorionic gonadotropin
HSI	Hepatosomatic index
LH	Luteinizing hormone
MIS	Maturation-inducing steroid
ND	Numerical density
POF	Post-ovulatory follicle
PRL	Prolactin
SE	Standard error
T	Testosterone
VF	Volume fraction
VTG	Vitellogenin
3ß-HSD	3ß-hydroxysteroid dehydrogenase
11-KT	11-ketotestosterone
17α-OH-P	17α-hydroxy-progesterone
17α-20ß-P	17α-20ß-dihydroxy-pregnen-3-one

Chapter 1

General introduction, literature overview and aims of research.

(1) General introduction, literature overview & aims of research.

Over recent years, tilapiine fish (Family: Cichlidae) have steadily grown to become one of the most commercially important groups of freshwater fish species in tropical aquaculture and represent a major protein source in many developing countries. Whilst tilapia are indigenous to African countries, their distribution has been widened through artificial introduction to over 100 tropical and sub-tropical countries including the Americas, the Middle East and Asia (Balarin & Hatton, 1979; Pullin & Lowe-McConnell, 1982; Pullin, 1983).

Tilapias offer immense potential to aquaculture due to several beneficial physiological attributes. Firstly, tilapias tolerate a variety of environmental conditions and can adapt to wide ranges of salinity, oxygen tension and overcrowding. Growth rates are swift even when maintained on various natural or cheap artificial foods. Tilapias have relatively short reproductive cycles, breed prolifically under culture conditions, are strongly resistant to disease and infection and are relatively amenable to handling. Most importantly, tilapias are valued by humans for their palatability as a food source (Pullin & Lowe-McConnell, 1982). An excellent example of the tilapias suitability to aquaculture was provided by Edwards *et al.* (1988), who claimed yields of 5 - 6 tonnes/hectare/year of the Nile tilapia *Oreochromis niloticus* (L.) raised in ponds solely fertilized with sewage over a period of 5 to 7 months.

Despite the current widespread distribution and development of tilapia culture, world production of tilapia from aquaculture totalled just 391,000 tonnes in 1990 (Source: Food and Agricultural Organization, 1992; cited by Macintosh & Little (1995)). This production figure forms just over 5% of the annual total freshwater fish production for 1990.

Over 70 species of fish are referred to by the common name 'tilapia'. Of these, Huet (1971) listed 16 species that had been subject to either experimental or commercial culture; a more recent study by Balarin and Hatton (1979) quoted a total of 23 separate species. Realistically, just eight or nine species of tilapia remain of significance in terms of aquaculture (Schoenen, 1982; Pullin, 1983).

Culture of tilapia has progressed swiftly over recent years, particularly in certain parts of Asia, e.g. Thailand, Taiwan, the Phillipines and notably as part of integrated culture schemes in China (Chen, 1990). Culture has remained relatively poorly developed in African and other regions.

The potential for future expansion of commercial tilapia culture is extremely high. An estimated shortfall of approximately 20 million tonnes is expected between market demand and expected marine fisheries production (100 million tonnes) by the year 2000. In order to address this shortfall, output by inland fisheries will need to rise proportionally (Manzi, 1989; Haight, 1992; cited by Macintosh & Little, 1994). Clearly then, the further development of tilapia culture operations not only shows huge potential but will become a necessity within the next decade.

As consumer demand for tilapias increases it becomes vital that culture operations strive to meet such market demand. Optimisation of hatchery effiency is of paramount importance if production is to be maximised and maintained. Mass production of high quality fry remains the most important prerequisite in the expansion and intensification of tilapia culture. Broodfish productivity remains one of the most significant constraints on commercial production costs and thus knowledge of the factors affecting broodstock productivity is of great importance to the futher development of tilapia culture.

Sexually mature Cichlidae are generally able to undergo successive reproductive cycles at intervals of 4 - 6 weeks. Theoretically, this should lead to an almost continous production of fry assuming that seasonal environmental variation remained minimal (Moreau, 1979). Under intensive farming conditions however, the asynchronous nature of the reproductive cycles of individual broodstock may lead to spasmodic fry production to the detriment of total farm output (Jalabert & Zohar, 1982). Low fecundity and asynchronous spawning patterns by female broodstock inevitably lead to the need for extensive fish holding facilities to even approach satisfactory output and often requires careful, time-consuming management of very large numbers of fish.