

Contribution of research in reproductive physiology to the culture of tilapias

Jean-François Baroiller⁽¹⁾ and Bernard Jalabert⁽²⁾

⁽¹⁾ CTFT/CIRAD, Département Pêche et Pisciculture, 45 bis, avenue de la Belle-Gabrielle, 91736 Nogent-sur-Marne cedex, France.

⁽²⁾ INRA, Laboratoire de Physiologie des Poissons, Campus de Beaulieu, 35042 Rennes cedex, France.

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Abstract

In confined environments, the precocious reproductive efficiency of "tilapias" (various cichlid species belonging to the genera *Oreochromis*, *Sarotherodon* and *Tilapia*) can rapidly lead to overcrowding and nanism. Therefore, the artificial control of reproduction in these species is very important for success in their culture. Such a control may consist either in completely preventing fry production in a population, in order to improve growth, or in favouring synchronous massive productions of alevins, in order to set up cultures with homogeneous populations.

At present, some of the most intensive techniques are based on the separate rearing of male fish, either after manual sorting or following hormonal inversion treatments leading to monosex populations. New ways of obtaining a male monosex progeny are suggested, based on the viability and fertility of a new genotype YY. Recent data on the sex differentiation of tilapia gonad suggest the possible use of steroids which have been identified *in vitro* during the early stages of development of the testis and which exhibit high masculinizing potentialities; these studies also suggest the tentative use of specific inhibitors of the activity of some of the enzymes involved in the synthesis of the above mentioned steroids.

The occurrence of parental care is an important factor in the reproductive efficiency of tilapias. Recent data show close relationships between the chronology of the mouth-brooding behaviour in *Oreochromis*, and that of the ovarian cycle. A better knowledge of the underlying physiological factors may facilitate the development of new techniques for the inhibition and/or the synchronization of reproduction.

Keywords : Physiology, reproduction, sexuality, parental care, tilapia culture.

Apport des recherches sur la physiologie de la reproduction à la pisciculture des tilapias.

Résumé

En milieu confiné, l'efficacité précoce de la reproduction des «tilapias» (diverses espèces de la famille des cichlidés appartenant principalement aux genres *Oreochromis*, *Sarotherodon* et *Tilapia*), conduit à une rapide surpopulation avec une tendance au nanisme. C'est pourquoi le contrôle artificiel de leur reproduction est très important pour la réussite de l'élevage. Selon les besoins, un tel contrôle peut consister soit à empêcher complètement la production d'alevins dans une population pour améliorer la croissance, soit à favoriser leur production massive pour la mise en élevage de populations homogènes.

Actuellement, les techniques les plus intensives sont fondées sur l'élevage séparé des mâles, dont le potentiel de croissance est plus important, soit après un tri manuel, soit après traitement hormonal conduisant à une population monosex mâle. De nouvelles voies d'obtention de telles descendance monosexes mâles sont proposées, fondées sur la viabilité et la fertilité d'un génotype nouveau YY. Des travaux récents sur la différenciation du sexe de la gonade de tilapia permettent de suggérer l'utilisation éventuelle de stéroïdes identifiés *in vitro* pendant le développement testiculaire précoce, et

présentant de fortes potentialités masculinisantes, ainsi que l'essai d'inhibiteurs spécifiques de certaines activités enzymatiques conduisant à leur synthèse.

L'existence d'un comportement parental est un facteur important de l'efficacité de reproduction des tilapias. Des travaux récents montrent une étroite relation entre la chronologie du comportement d'incubation buccale chez *Oreochromis* et celle du cycle ovarien. Une meilleure connaissance des facteurs physiologiques sous-jacents devrait permettre la mise au point de méthodes originales pour inhiber la reproduction et/ou pour la synchroniser.

Mots-clés : Physiologie, reproduction, sexualité, soins parentaux, pisciculture des tilapias.

INTRODUCTION

With already more than 20 cultured species (Guerrero, 1982), tilapias belong to a group of Cichlid fish, genera *Oreochromis*, *Sarotherodon* and *Tilapia*, which is particularly appreciated in the fish-farming activity for its robustness, its wide distribution, its significant growth rate and ease of reproduction. This reproductive efficiency directly results from several biological or ethological characteristics:

- The making of a nest combined with a nest protecting behaviour (Lowe-McConnell, 1959; McBay, 1961; Bruton and Boltz, 1975; Ruwet *et al.*, 1976; Philippart and Ruwet, 1982).

- A sequential oviposition immediately followed by the fertilization of each group of ova (Shaw and Aronson, 1954; Philippart and Ruwet, 1982; Mélard, 1986).

- The occurrence of parental care provided to the eggs immediately after they have been fertilized. In mouth-brooding species (*Sarotherodon* and *Oreochromis*), the eggs are brooded in the buccopharyngeal cavity; this behaviour is often combined with a migration of the breeding fish to a planted and thus protected area. Substrate-spawning species (*Tilapia*) ventilate their eggs by constantly stirring the water with their caudal fins (McBay, 1961; Fishelson, 1966; Arrignon, 1969; Rothbard and Pruginin, 1975; Rothbard, 1979; Ruwet *et al.*, 1976).

- Parental care is also provided after hatching (Ruwet, 1962; Ruwet *et al.*, 1976; Perrone and Zaret, 1979; Owusu-Frimpong, 1987), and lasts at least until the resorption of the yolk vesicle.

- Reproduction may begin quite precociously for small-sized animals (Lowe-McConnell, 1958; 1983; Ruwet *et al.*, 1976). Therefore, under certain conditions, maturation has been observed for 3.8 cm long *Sarotherodon melanotheron* (Eyeson, 1983), 4.2 cm long *Oreochromis mossambicus* (Arrignon, 1969) and 6 cm long *Tilapia zillii* (Dadzie and Wangila, 1980).

- Successive reproduction cycles enable a female to produce a new batch of fry every 4 to 6 weeks (Arrignon, 1969; Rothbard and Pruginin, 1975; Ruwet *et al.*, 1976; Mélard and Philippart, 1981), except in environments exposed to significant seasonal variations (Moreau, 1979). Under optimal conditions,

the relative lack of synchronization between individual breeding females from the same population leads to a continuous fry production.

All these biological characteristics may be responsible for a rapid evolution towards overcrowding and nanism in a confined environment and in a situation of competition for food (Hickling, 1960; McBay, 1961; Loya and Fishelson, 1969; Hyder, 1970; Fryer and Iles, 1972; Bard *et al.*, 1974; Bruton and Allanson, 1974; Eyeson, 1983; Lazard, 1984). Therefore, controlling reproduction should improve the profitability of tilapia culture. As a general trend in fish, methods usually are expected to act on the gonads, by either modulating their development (stimulation or inhibition, temporary or definitive), or preferentially biasing the sex-ratio in favour of the sex which has the best aquacultural potentialities.

For tilapias, these approaches are facilitated by specific characteristics of this group: first, the precocious sexual dimorphism of the urogenital papilla (2-3 months after fertilization) makes it possible to separate male from female fish just before the very first reproduction. Second, hybridization between certain species results in male monosex progenies.

Thus, all the techniques presented above depend either on the control of gonadal development in order to prevent the production of gametes, or on the separation of the sexes so as to impede fertilization. However, the importance of parental care behaviour in these Cichlids can also suggest new approaches to the practical artificial control of reproduction.

This article reviews the present knowledges regarding the physiological mechanisms of gonadal differentiation and maturation, reproductive behaviour and parental care in tilapias, in relation to practical techniques for the control of reproduction.

HORMONE INVERSION AND DIFFERENTIATION OF THE GONAD'S SEX

The easiest and most radical means of avoiding reproduction consists in separating male from female fish. For tilapias, it is an accepted fact that male growth is faster than that of female (Pruginin, 1967; Shell, 1967; Hickling, 1968; Lazard, 1980; Hanson *et*

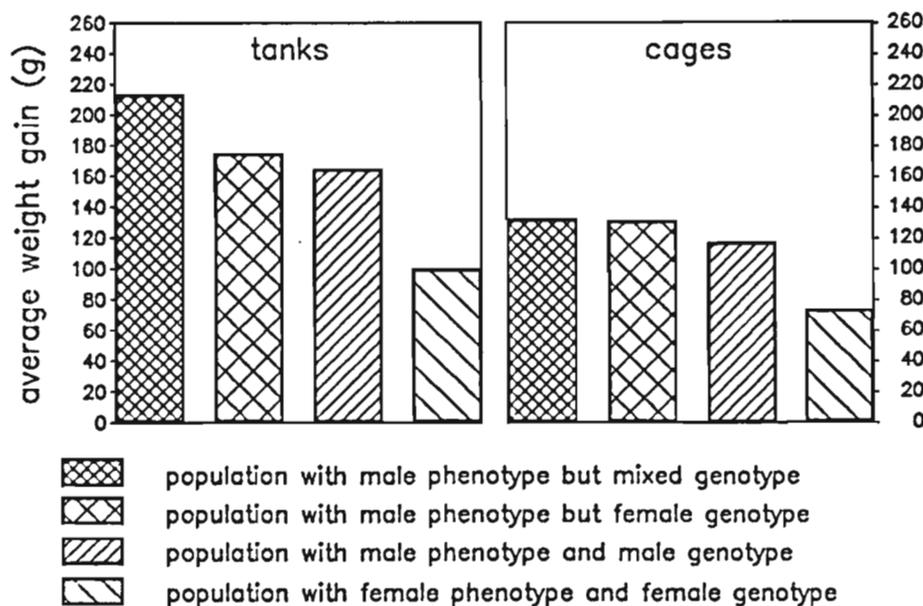


Figure 1. — Comparative growth in tanks or in cages of monosex populations of tilapia (*Oreochromis niloticus*), phenotypically male or female, but having various kinds of sexual genotype (drawn from data reported by Hanson *et al.*, 1983).

al., 1983; Legendre, 1986). As a matter of fact, Hanson *et al.* (1983) showed that male populations always have a better growth than female populations (*fig. 1*), by using some of the techniques mentioned below. Besides, they observed that the growth potential is equal for male individuals which are genetically female and for animals having a male phenotype and genotype. In practice, male monosex populations can be obtained by manual sexing, hybridization and/or by hormone inversion. This manual sexing according to the urogenital papilla consists in getting rid of all females, *i.e.* about half of the population. However, this biomass has indeed growth potential which seem equal to those of the male fish population when its phenotype is imposed. Moreover, this sexing can only be carried out for animals at least 20 to 50 g weight (Lazard, 1980; Hephher and Pruginin, 1982; Mélard, 1986). On the other hand, it takes time and labour and diagnosis mistakes can range from 2.7 to 10% (Lazard, 1980; Chervinski and Rothbard, 1981/1982). As far as hybridization is concerned, it appears very difficult in common practice to maintain very high percentages of male first generation hybrids for commercial production (Pruginin *et al.*, 1975; Lovshin, 1982; Guerrero, 1982). This evolution seems to be due to a progressive contamination, which appears almost unavoidable in commercial fish farm conditions, of domestic brood stocks from each parental species one by the other. On the other hand, possible interspecific incompatibilities seem to account for low reproduction and fertility rates (Lovshin, 1982; Guerrero, 1982; Mires, 1982). On the contrary, the masculinizing hormonal inversion proved to be an efficient and reliable tool.

Hormonal inversion of the sex

After Yamamoto's experiments (1953, 1958 and 1969) on the medaka, *Oryzias latipes*, functional sex inversions have been carried out in numerous species by means of hormone treatments given during early developmental stages (review by Hunter and Donaldson, 1983).

Whatever the species, the easiest method consists of masculinizing a population of tilapia fry by the incorporation of a synthetic steroid in the diet. Although experimental conditions were very heterogeneous (*table 1*), a 100% efficiency is obtained by numerous authors using this kind of treatment. However, even if this technique appears reliable and simple, there still exists problems linked to the use of hormones on growing animals which are directly intended for marketing (Shelton *et al.*, 1978; Johnstone *et al.*, 1983; Goudie *et al.*, 1986*a* and *b*). The experimental use of radioactively labelled steroids in *Oreochromis aureus* shows that the viscera contain 90% of the total radioactivity present in the fry during the treatment (*fig. 2*); after this treatment had been stopped, the total radioactivity measured in the whole animal decreased by 90% in 24 hours and by 99% in 3 weeks (Goudie *et al.*, 1986*a*). Nevertheless, if the metabolism of synthetic steroids in higher vertebrates follows the same pathways as those taken by equivalent endogenous hormones (Fotherby and James, 1972), the fate of such molecules in fish and the possible effects of their degradation products on the environment are not very well known yet. It is therefore possible to envisage an indirect approach which would avoid these problems.

Table 1. — Reports of masculinizing treatments (synthetic steroids administered in the food of fry) leading to 100% male population in tilapia (genus *Oreochromis*).

Species	Age or Size	Dose (mg, kg) (in food)	Duration (days)	Mortality (%)	Temperature (°C)	Percent. of ♂ in controls	Steroid treatment	Authors
<i>O. mossambicus</i>	7-10 days	10, 30 40	69	36-92	27 then 20	62-77	MT	Clemens and Isnee, 1968
	2 months	40	60	47	32 to 30	56	MT	Jalabert <i>et al.</i> , 1974
<i>O. niloticus</i>	9-12 mm	30 60	35, 49, 23 35, 59	4 to 33	20 to 26	50 to 54	ET, MT ET, MT	Tayamen and Shelton, 1978
	8, 5-12 mm	50, 100,	30		27 to 30	60 to 72	MT	Nakamura and Iwahashi, 1982
	13, 2-20, 2 mm	100	30					Owusu-Frimpong and Nijjhar, 1981
	10 mm	50	28 to 42	2 to 53		78	MT	
<i>O. aureus</i>	9-11 mm	60	21	6-22	21	56	ET	Guerrero, 1975
	9, 5 to	60	21, 23,	<10	25 to 32	45 to 56	ET	Shelton <i>et al.</i> , 1981
	10, 5mm		21		21			
<i>O. hornorum</i>	9-11 mm	60, 90 120, 240	22	28 to 44	27 to 32	45	ET	McGeachin <i>et al.</i> , 1987
	9-11 mm	30	21 or 28	0 to 18	27	53	MT	Obi and Shelton, 1983

MT : 17 α -Methyltestosterone.ET : 17 α -Ethinyltestosterone.

Species with a WZ female heterogamety such as *O. aureus* (Guerrero, 1975; Liu, 1977) or *O. hornorum* (Hickling, 1960; Chen, 1969) represent the easiest case (fig. 3): after inversion by means of feminizing hormones, the neo-female fish can be identified rapidly according to the sex ratio of their progeny. As a matter of fact, a neo-female ZZ bred with a non-treated male ZZ (a) leads to the production of 100% animals ZZ of male phenotype; the normal crossbreeding (b) of a female WZ with a non-treated male ZZ produces 50% animals of each sex. Inversed individuals ZZ can thus be isolated in order to be used as breeding fish; when crossed with non-treated male fish of the same species, they shall thus produce male monosex populations.

Concerning the species with a female homogamety XX (fig. 4), such as *O. niloticus* (Jalabert *et al.*, 1974) or *O. mossambicus* (Hickling, 1960; Chen, 1969), an additional step is necessary to reach an equivalent result. The progeny of female fish which had been submitted to hormonal treatment during early development can present two types of sex ratios: the first one (a) is normal, composed of 50% of each sex, coming from animals of female genotype XX. The second one (b) which comprises 75% male fish and 25% female fish results from the crossbreeding of the neo-female XY with a non-treated male fish XY. Among these 75% male fish, there are some YY individuals which can be identified by their male monosex progeny during a new test (c). These animals of a new genotype which are viable and fertile (Baroiller *et al.*, unpublished data) can be used as breeding fish producing 100% male fish populations at each reproduction with non-treated female fish. Sex inversion by means of hormones thus remains an

essential and necessary tool in such indirect approaches of monosexing.

However, the extension of this technique can meet with some difficulties, such as the occurrence of variable results as regards the monosex percentage obtained (Hunter and Donaldson, 1983) and a lesser efficiency of the feminizing treatments when compared to the masculinizing ones (Tayamen and Shelton, 1978; Hopkins *et al.*, 1979). The latter difficulties may suggest either that the hormones which are used may not correspond to those involved *in vivo* in the differentiation of the gonad's sex (this puts into question the nature of the steroid or even the molecule which must be administered), or that the moment and duration of the treatments may have been chosen inadequately. In fact, no determining physiological proof has been brought so far to support Yamamoto's hypothesis (1969) according to which steroids are natural inducers of the gonadic differentiation. The modification of the natural process of sexual differentiation by exogenous steroids could be due to a pharmacological action, as suggested by Reinboth (1970). If steroids really are the endogenous inducers, which ones are involved?

Gonadal sex differentiation

Very few works have been performed in gonochoristic fish in order to characterize early steroidogenic pathways in the gonad during its differentiation (*Salmo gairdneri*: Van der Hurk *et al.*, 1982; tilapias: Rothbard *et al.*, 1987, and Baroiller *et al.*, 1988). In *O. niloticus* (Baroiller, 1988) steroidogenic potentialities have been examined by histochemistry and by *in vitro* studies of metabolism in gonads which

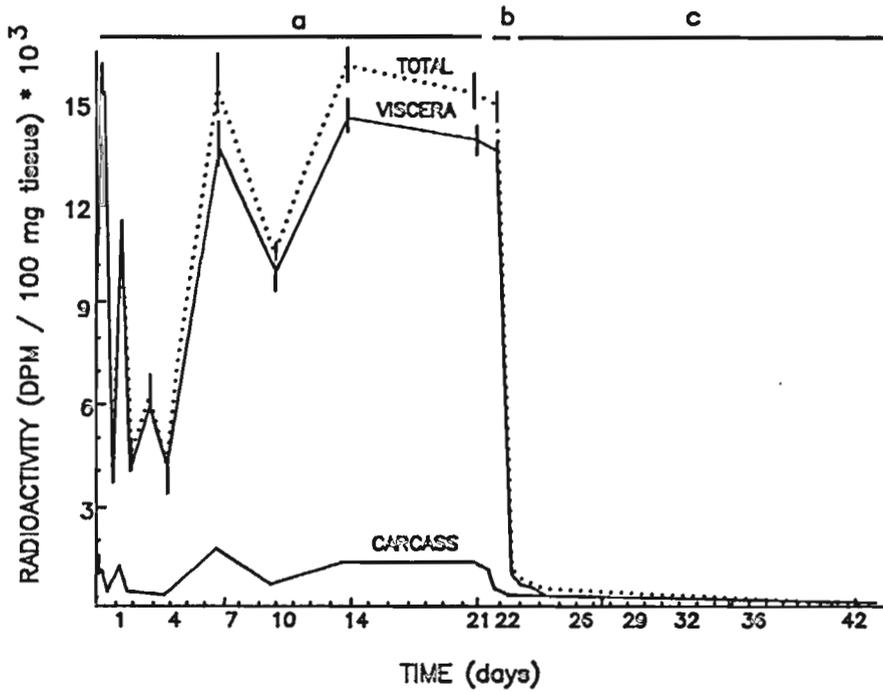


Figure 2. — Radioactivity (DPM/100 mg) present in carcass, viscera and whole body of sexually undifferentiated *Oreochromis aureus* fed a diet containing radiolabelled methyltestosterone for 21 days (redrawn from Goudie *et al.*, 1986).

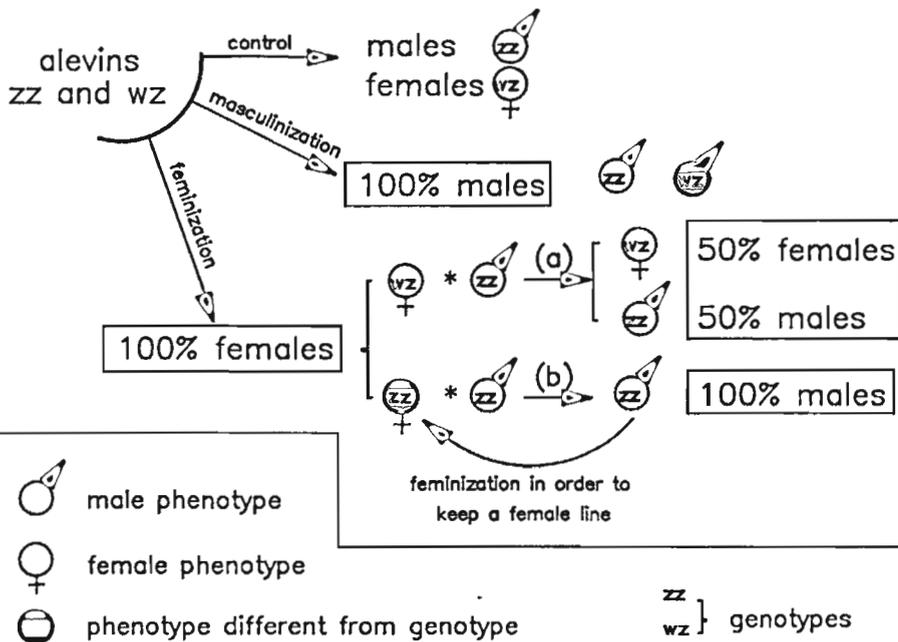


Figure 3. — Theoretical protocol in order to obtain monosex populations in species with male homogamety, such as *Oreochromis aureus* and *O. hornorum* (redrawn from Baroiller, 1988).

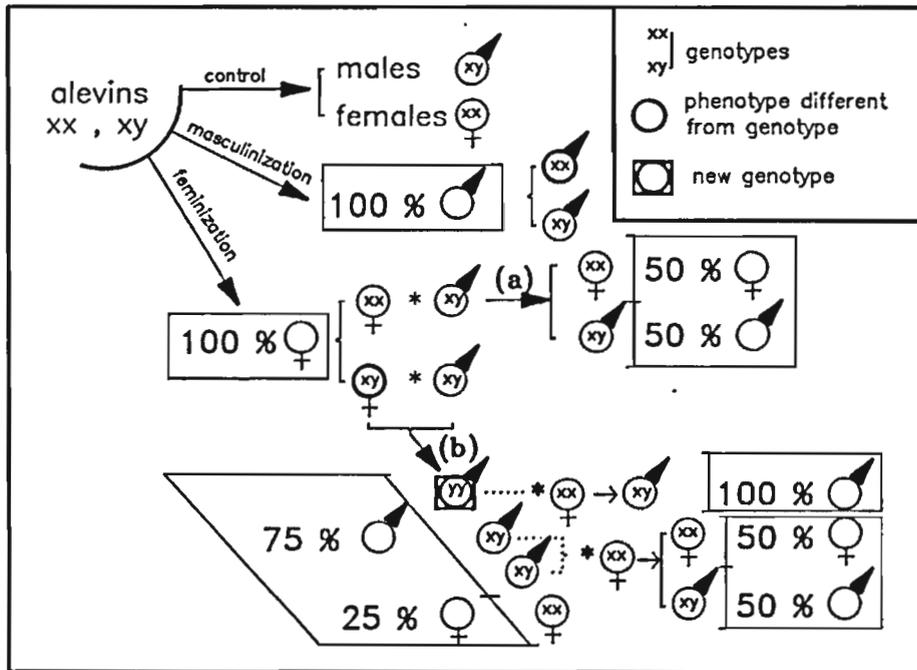


Figure 4. — Theoretical protocol in order to obtain monosex male populations in species with female homogamety, such as *Oreochromis niloticus* and *O. mossambicus* (redrawn from Baroiller, 1988).

were still histologically undifferentiated. When differentiation starts and while it is in progress, metabolites which are specific for each sex are identified. These do not systematically correspond to steroids which are used in the traditional treatments of hormone sex inversion; moreover, an androgen (testosterone) responsible for the male differentiation and of oestrogens involved in that of the ovarian organization has not always been confirmed by these results. For example, testosterone can be identified early in male as well as in female gonads. It can be expected therefore, that a better knowledge of the gonad endocrinal environment at these key moments could bring some improvements in the present sex control techniques and suggest new kinds of treatments. Particularly for *O. niloticus* as well as for *Salmo gairdneri*, a derivative of androstenedione ($11\beta\text{OH}\Delta_4$) seems to be specific to the early stages of the testicular ontogenesis. Yet, this steroid is capable of masculinizing a normal fry population with great efficiency in rainbow trout (Van der Hurk and Slof, 1981; Van den Hurk and Van Oordt, 1985); steroid allows to obtain a 100% male population from a set of *O. niloticus* which are genetically female XX (Baroiller *et al.*, unpublished data). Compounds inhibiting the synthesis of some of the above mentioned hormones could be used to perform *in vitro* experiments in order to verify the hypothesis according to which female differentiation would result from the lack of key steroids, specific of the testicular ontogenesis. They also could be used to carry out *in vivo* research on new and more reliable feminization techniques in such

species as *O. aurea* with the aim of obtaining neofemale ZZ broodstocks. The latter would give 100% male offsprings without result from direct hormonal treatment.

GAMETOGENESIS

For tilapias, gametogenesis has only been partially described (Dadzie, 1969; Hyder, 1970 *a* and *b*; Polder, 1971; Von Kraft and Peters, 1963; Hodgkins and Man, 1978; Babiker and Ibrahim, 1979; Moreau, 1979 *b*; Grier and Abraham, 1983; Smith and Haley, 1987; 1988). It seems to take place in the same way as for the majority of teleosts which have been studied. For this species, since the classical stages of oogenesis and spermatogenesis have been the subject of excellent reviews (Hoar, 1969; Dodd, 1972; Billard, 1986; also see Jalabert and Zohar, 1982), only the characteristics which are specific to tilapias will be mentioned.

The eggs of Tilapias can be classified in two categories according to the presence or absence of secretion of a sticky substance secreted by the granulosa layer around specialized filaments. This characteristic is linked to the parental behaviour of tilapias. In general, substrate spawning fish (genus *Tilapia*) are the only fish which have this type of secretion, contrary to mouth-brooding species (genus *Oreochromis* and *Sarotherodon*). However, two intermediate cases

are particularly interesting as regards the study of the possible evolution of the substrate spawning group towards that of mouth-brooding fish: *Sarotherodon galilaeus* which does not immediately take the fertilized eggs back into its mouth (Fishelson, 1966b) and *Tilapia discolor* which transports the eggs layed in the nest in its mouth (participation of the male and female fish) and then lays and ventilates them on the substrate of a more protected zone (Owusu-Frimpong, 1987): both *Sarotherodon galilaeus* and *Tilapia discolor* have such adhesive structures.

As for other species having a well developed parental care behaviour, mouth-brooding *Sarotherodon* and *Oreochromis* spawn eggs which are less numerous (several hundreds per spawning) than those layed by substrate spawning fish (several thousands). The size, weight and number of eggs generally increase according to the size of female fish. Yet, for the same total spawning weight, the number of eggs which constitutes the spawning can vary in a significant way (Peters, 1963).

REPRODUCTION RATE AND SOCIAL FACTORS

It has already been mentioned in the introduction that the age at first maturity can vary to a great extent. Although genetic as well as environmental factors have been implicated (Hyder, 1970; Siddiqui, 1979), the lack of experiments does not allow yet to draw any conclusions on the nature of the determinism of this puberty.

Once they have reached sexual maturity, male and female fish are capable of undergoing successive reproduction cycles throughout the whole year, under optimal conditions. The role of external factors on the reproduction rhythms and on the intensity of gametogenic production are still very poorly known; however, various works have clearly demonstrated the importance of social factors in the reproduction of these cichlids.

In the absence of any social stimulus, an isolated *Sarotherodon melanotheron* female fish still repeats the sequence oviposition—mouth-brooding initiation at a few month intervals (Aronson, 1945; 1951). Three types of stimuli are generally considered to be involved:

— Breeding male and breeding female fish can emit sounds; however these sounds seem to be linked to reproduction only for the male fish which emit them from his nest (Rodman, 1966; Lanzing, 1974); female fish apparently only emit sounds when they eat (Lanzing, 1974). Male sound stimuli appear sufficient to increase the oviposition frequency of *O. mossambicus* females which are isolated from other stimuli (Marshall, 1972).

— Visual stimuli from congeneric fish also affect the ovulation of mature females since for *O. mossambicus*, ovulation occurs more frequently for stimulated fish than for totally isolated animals. Such stimuli are

equally efficient when they come from male or from female fish (Silverman, 1978a and b).

— The mature female fish could emit a pheromone type substance, thus activating the courting behaviour of the male fish which is already visually stimulated. Pheromonal substances released by ovulated females have been identified in other fish species (for review, see Stacey *et al.*, 1988 and 1987, and Stacey, 1987). In tilapias however, the likely involvement of pheromones is only supported by the observation that the courting activity of a male fish significantly increases when the reproduction period for female fish approaches, if these are living in the same aquarium, but not if only visual and sound stimulus can be exchanged between male and females through adjacent aquarium (Silverman, 1978a and b). These stimuli are all the more efficient since for *Sarotherodon melanotheron* (Aronson, 1945) as well as for *O. mossambicus* (Silverman, 1978a and b), the reproductive activity is significantly increased for animals having a non-limited contact with one another than for those having only a visual contact. A more detailed study (Silverman, 1978a and b) which links these different social factors to a histological analysis of gonads, shows that visual contacts mostly seem to hasten ovulation of full-grown oocytes and that they have no clear influence on the oocyte development pace. On the contrary, non-visual stimuli appear to accelerate not only ovulation, but also the onset of vitellogenesis, thus affecting the overall duration of oocyte development through various steps of oogenesis. A better knowledge of the pheromones involved during reproduction of tilapias could allow to synchronize, or at least to group spawnings of female breeders.

Social factors linked to the reproductive behaviour can even have an indirect influence on growth. The aggressive behaviour linked to the choice of the nest location, on which depends the future efficiency of courting, is strongly correlated with the relative size of male fish *O. mossambicus* placed together in a same large-sized aquarium. After 24 weeks, the initial and final sizes of male fish are negatively correlated; however, the hierarchy which has been initially acquired as regards the aggressivity and location of the nest has not been put into question. The most aggressive male fish which choose the best locations spend most of their time defending their nest and reproducing at the expense of their diet. The less aggressive male fish thus have more food which is easily accessible (Turner, 1986). Since it was established that steroid hormone treatments (specially androgen masculinizing treatments) administered during development influence behavioral differentiation in *S. mossambicus* (Billy and Liley, 1985), it would be interesting to evaluate the effect of this kind of treatments on the relative aggressivity of males and growth variability in fish farm conditions.

HORMONAL DETERMINISM OF THE PARENTAL BEHAVIOR

The reproductive efficiency of tilapias is greatly due to the parental care lavished on the progeny.

Knowledge of the hormone determination of parental behaviours could allow to elaborate new techniques for the artificial control of reproduction. For fish as for other vertebrates, reproduction is controlled through the hypothalamus-hypophysis-gonad axis. The possible implication of a pituitary hormone, prolactin (PRL), in the regulation of parental behaviour has been suggested. Surprisingly, however, tilapias have still not been chosen for such analysis, in spite of their wide range of parental care behaviour, from substrate spawning to mouth-brooding. Indeed, for numerous Cichlids, fertilization is quickly followed up by a ventilation of the spawned eggs by means of alternative movements of the pectoral fin which is precisely oriented towards the eggs. Ovine prolactin is capable of inducing as well as maintaining such behaviour when normal external stimulus (such as the presence of eggs or of a nest) are lacking. The intensity of this behaviour which is linked to a clear decrease in the fish aggressivity and appetite depends on the prolactin dose which has been administrated. From a minimum threshold dose up to an average value, the duration and intensity of ventilation increase significantly; then, when doses are further increased, this behaviour is progressively inhibited and eventually stops completely. At least four Cichlids react this way to the injection of this ovine hypophysis hormone: *Symphysodon aequifasciata axelrodi*, *Pterophyllum scalare* and *Aequidens pulcher* (Blüm and Fiedler, 1965; Blüm, 1966) as well as *Chromis chromis* (non-published data quoted by Blüm, 1968). A Labrid fish, *Crenilabus ocellatus* had already be reported to react in such a way to prolactin (Fiedler, 1962). Although no similar experiment in tilapias has been reported so far, a tilapia prolactin has been purified (Blüm, 1973) by using bioassays performed in the Discus (*Symphysodon* sp.) based on the above mentioned behavioural effects and also on histiotropic effects, *i.e.* an increase in the number of mucus cells and mucus production by the brooders' skin (this mucus appears to be used as a food by the fry of this species).

Other "prolactins" from tilapias, now recently isolated and purified on the basis of biochemical criteria, should thus be tested within the same species to look for a possible effect on the behaviour of substrate spawning or mouth-brooding fish.

Further knowledge of the hormone determinism of this behaviour could allow to try and delay, or even stop the ovarian development or inhibit any parental care so as to avoid fry proliferation.

As a matter of fact, a recent study in *O. mossambicus* (Smith and Haley, 1987) established some relationship between the duration of the successive eggs and fry mouth-brooding periods and the duration of the ovarian cycle (*fig. 5*). In that species, a continuous mouth-brooding period is normally observed till the 15th day post-fertilization (d.p.f.) at about 27°C; then, during an additional 10 day period (till the 25th d.p.f.), mouth-brooding is discontinuous: the fry swims near their mother up until the slightest

signal on her part which immediately gathers them back into its buccopharyngeal cavity. In a few cases, the mother rejects the eggs the day after fecundation. This latter situation can also be provoked by removing the eggs from the mothers mouth 1 d.p.f. A histological study shows that a post ovulatory follicular tissue is persisting differently in these two kinds of situation. Ten days after the egg removal or loss, this structure begins to degenerate and quickly disappears; on the contrary, such a structure persists up until the 25th day after fecundation for mouth-brooding female fish. This cellular mass can be compared to some extent to the *corpus luteum* of mammals, since it exhibits the main usual ultrastructural and histochemical characteristics of steroid producing cells, at this moment of the cycle. These steroidogenic potentialities can be observed as long as this tissue persists: however, the developing follicles of the following wave quickly show a potential activity which is first equal to—around the 6th d.p.f.—, and then even superior to that of post-ovulatory follicles. All these gonadal changes result in a rapid ovarian development for non-brooding female fish, which leads to ovulation taking place 25 d.p.f.; on the contrary, they result in a slower cycle with an ovulation taking place 40 d.p.f. for female fish having a normal behaviour. Parental care is thus associated with a 15 day inhibition of the oocyte development. The presence of eggs and then fry in the buccopharyngeal cavity seems to determine the persistence of the post ovulatory follicles and its secretion as well as the progress of oogenesis. These authors (Smith and Haley, 1988) measured the concentrations of plasma steroids for both categories of animals and showed some difference between the respective endocrinological profiles, as far as oestradiol-17 β and testosterone are concerned: quantitatively comparable levels were only reached after a 5 day delay for brooding female fish compared to non-brooding females.

The use of appropriate steroids in order to mimic such physiological situations should be explored as a possible way either to inhibit the ovarian development or to disrupt the parental behaviour. Even if attempts are made to avoid reproduction of cultured tilapias in order to eliminate overcrowding and nanism, massive productions of fry, homogeneous in age and size, are also quite desirable for a better management of fish farms. This should be particularly interesting, as far as two practical problems of commercial fish farming are concerned:

— When the production of monosex populations by means of hormonal inversion is required, the efficiency of the treatment depends, among other necessary conditions, on the initial age of the fry which should be as low and homogeneous as possible. If reproduction is not synchronized, it is presently indispensable to keep very numerous brooders and then to sort out the fry according to their size.

— In certain types of fish farms, the lack of synchronization can lead fish farmers to carry out the first alevin harvesting only after 1.5 to 2 months,

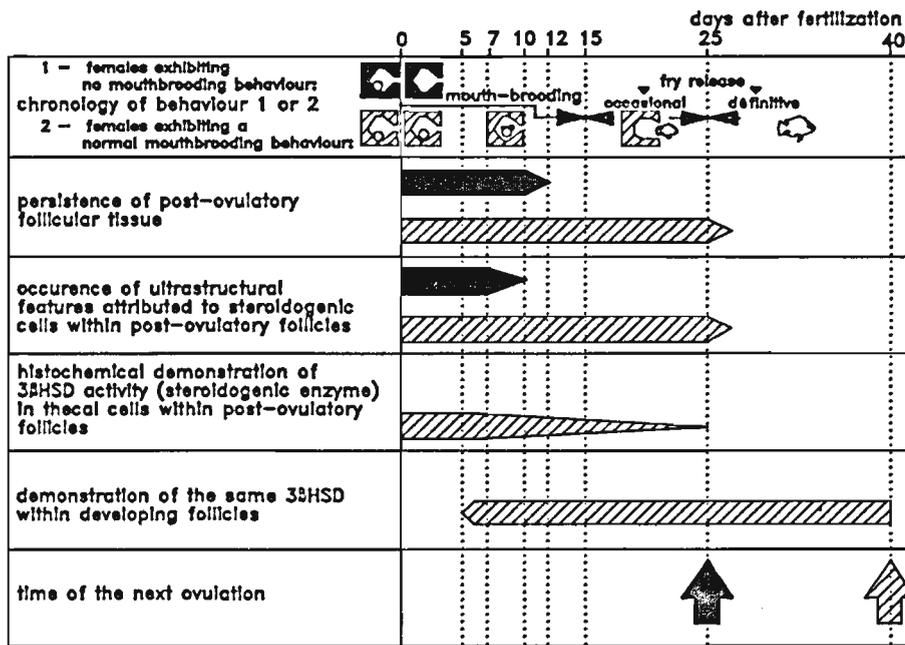


Figure 5. — Evolution of cytological and histochemical characteristics of ovarian follicles, and duration of the ovarian cycle, in relationship with either normal or abnormal mouthbrooding behaviour in *Oreochromis mossambicus* (schematic representation of data reported by Smith and Haley, 1987).

when the greatest number of alevins can usually be obtained. However, the surface swimming behaviour of fry and the harvesting methods allow the fish which were born first (1 to 2 weeks after the introduction of breeding fish) to escape this first catch. Still, juve-

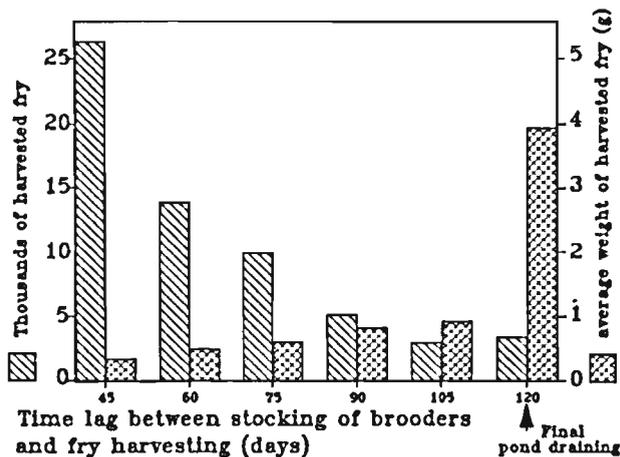


Figure 6. — Evolution of the number and average weight of harvested *Oreochromis niloticus* fry as a function of successive harvests. Mean data out of ten 350 m² ponds, each containing 60 male and 180 female brooders (drawn from data reported by Lazard *et al.*, 1988).

niles are efficient predators for younger fish, even more than breeding fish. A progressive yield decrease (fig. 6) can thus be noticed for the following catches performed in the same production structure (Lazard *et al.*, 1987). The simultaneous increase in the average weight of alevins which have been caught reflects the partial harvest of predator juveniles which have finally all been caught when the final draining of the pond was made (6th sample).

CONCLUSION

The analysis of specific features of tilapia's reproductive physiology brings to the fore two main paths of research which are to be studied for a better reproduction control.

The modification of normal sex differentiation by exogenous steroids presently remains the best way to avoid reproduction and to benefit by a growth potential of all individuals which are directly or indirectly oriented towards a male phenotype. The relatively empirical approach which has been carried out until now allowed to quickly lead up to efficient masculinizing treatments. However, it is necessary to make a detailed study of the endocrinal determinism of differentiation as regards the possible indirect use of hormones, since the feminization process—which represents one of the main tools to be used to this

end —, is still very difficult to implement. Moreover, a better knowledge of the gonad hormone environment during its differentiation should allow to use specific inhibitors of the enzymatic activities as new types of treatments. Finally such a study seems to be necessary in order to make a detailed analysis of the influence of epigenetic factors on differentiation.

The reproductive efficiency of tilapias essentially results from the importance of the behaviours before and after spawning. New tools for the control of reproduction can be expected to be developed from a better knowledge of the determinism of these different behaviours. The natural inhibition of the ovarian development during the mouth-brooding period and the likely involvement of pheromones in the regulation of reproductive behaviour during spawning suggest possible research fields aimed at controlling tilapia reproduction by inhibiting disorderly fry production and favoring synchronous spawning for mass production of homogeneous fry.

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