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Review

Hormonal induction of sex reversal in fish

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Abstract

Hormonal induction of sex reversal is possible in 47 species (15 families) of gonochores (34 species, nine families) and hermaphrodites using one of the 31 (16 androgens, 15 estrogens) steroids. Intensity of endocrine treatment for sex reversal increases among the taxonomic groups in the following order: Cichlidae < Cyprinodondidae < Anabantidae < Poecilidae < Salmonidae < Cyprinidae. 17α-methyltestosterone and estradiol-17ß are the most preferred hormones for induction of masculinization and feminization, respectively. Dietary treatment and immersion are the most acceptable methods for administering steroids. The labile period is not always restricted to a specific life stage at least among poecilids, anabantids, cyprinodontids, cichlids and cyprinids. Androgen treatment of (castrated) sterile fish may restore masculinity but estrogen may fail to restore femininity. Hormonal induction of sex reversal may result in higher mortality among fish bearing homogamous (XX or ZZ) genotypes. However the sex reversed fish especially the cichlids and cyprinids may grow up to two to three times faster, when treated at optimal dose for sex reversal. Hormonally sex reversed fish may also suffer from poor reproductive performance.

Keywords: Sex reversal; Steroids

1. Introduction

The process of sex differentiation in teleosts is diverse and labile (Francis, 1992) rendering endocrine sex reversal possible in many gonochoristic and in a few hermaphroditic species. Hormonal induction of sex reversal may serve as a valuable tool to understand the process of sex differentiation, and to produce monosex populations for the aquaculture industry (Table 1). Hunter and Donaldson (1983) summarised relevant information in a comprehensive review. In 1983 Yamazaki claimed that functional endocrine sex reversal has been successfully achieved in 15 gonochoristic species (five families) using one or the other of 14 (eight androgens; six estrogens) steroids. At present we have treatment protocols

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Table 1
Major events in the history of hormonal induction of sex reversal in fish

Author(s)	Events
Padao (1937)	Use of synthetic hormones to sex reverse salmon
Yamamoto (1955)	Tracing heterogamous XY ♀♀ by progeny testing and production of YY ♂♂ medaka
Yamamoto (1958)	Induction of sterility in medaka
Hishida (1965)	Evidence for intraperitoneal administration reducing hormone requirement to the tenth of normal
Muller (1969)	Reports paradoxical endocrine sex reversal in tilapia
Fagerlund and Mcbride (1978)	Demonstration that methylation slows elimination of testosterone
Johnstone et al. (1983)	Caution pollution possibility due to excretion of 99% of the administered hormone within a few hours
Nandeesha et al. (1990)	Description of a bioassay for residual level of administered steroid
Piferrer and Donaldson (1991)	Evidence for aromatization of exogenous androgen as the cause for paradoxical sex reversal
Varadaraj and Pandian (1991)	Ensure ten times better solubilization of androgen (ET) using DMSO
Devlin et al. (1994)	Use of DNA Y probe to distinguish genotypic sex after hormonal masculinization
Varadaraj et al. (1994)	Demonstration of modifying effects of temperature, photoperiod and feeding regime on sex reversing potency of a steroid
George and Pandian (1995a)	Production of ZZ ♀♀ molly

Table 2
Advantages and disadvantages of using hormonal sex reversal techniques with fish

Advantages	Disadvantages
Hormone treatment ensures maximization of growth by diverting nutrients, which otherwise may be utilized for gonadal development[a] and ensures better meat quality[b]	The technique has to be used everytime production of a monosex population is wanted; hence it costs time and money; the residues of the administered steroids can be carcinogenic and/or can affect consumers
It enhances commercial value of edible fish and decreases production cost of ornamental fish	The hormonal induction of sex reversal can be a stressful process resulting in low survival of sex reversed males and females[c]
It eliminates precocious maturity of males (e.g. Salmonidae, Cichlidae)	The sex reversed fish have delayed sexual maturity and produce less eggs/sperm than their genetic counterparts[d]
The technique by itself (viviparous fish) or in combination with ploidy induction technique provides scope for broodstock development for all male[e], all-female[f] all-sterile[g] populations	Administeration at high dose leads to sterility and in some cases paradoxical sex reversal and may result in growth suppression (see Table 8)
Research in this area helps us to understand the mechanism of sex differentiation and determination processes	As over 99% of the administered hormone is metabolised and released within a few hours or days, large scale field application of the hormone may lead to synergic effect on sex reversal in aquafarms[h] but to environmental pollution[i]

Source:[a]Donaldson and Hunter (1982); [b]Ali and Rao (1989); [c]Pandian et al. (1994); [d]Kavumpurath and Pandian (1993a); [e]Varadaraj and Pandian (1989); [f]Pandian and Varadaraj (1990); [g]Varadaraj and Pandian (1990); [h]Gomelsky et al. (1994); [i]Johnstone et al. (1983)

for 47 species (15 families) of gonochores (34 species; nine families) and hermaphrodites using one of the 31 (16 androgens, 15 estrogens) steroids. The available publications have projected mostly the success or failure of hormonal induction of sex reversal in selected fish. For some reason, they have not given adequate attention to survival, growth and reproduction of the hormonally sex reversed fish. This report intends to take an integrated approach to problems and prospects of producing hormonally sex reversed fish.

In aquaculture the use of sex steroids especially androgens may not only induce faster growth (Donaldson et al., 1979) but also the desired sex reversal (e.g. Rao and Rao, 1983). Advantages and limitations of endocrine sex reversal techniques are summarised in Table 2. As the technique is practised in aquaculture industries of both developing (e.g. Pandian, 1988) and developed countries the benefits accruing from hormonal sex reversal appear to out-weigh the disadvantages. Perhaps this economic advantage also has been the main cause for supporting more and more research in this area.

2. A brief summary

In most teleosts, males grow faster than females; males of most ornamental fish are more colourful than females, and thus have a higher commercial value. Thus, protocols for masculinization are available for 47 species but for feminization with only 31 species. A careful analysis of the available publications indicates that first, protocols are required for hormonal induction of sex reversal of more species in which females are heterogametic, and for viviparous species. Second, the protocols are required to accelerate sex reversal in hermaphroditic species. However, there are problems in this context, (1) most of these hermaphrodites are coral-inhabiting species and cannot easily be reared in the laboratory (e.g. *Anthias squamipinnis*), (2) hermaphrodites such as the labrid (*Epinephelus fario*) are protogynous and function as females during the first 7 years of their life and subsequently, become males and the required treatment duration is fairly long (> 150 days; Kuo et al., 1988). Likewise the marine protandrous hermaphrodite *Acanthopagrus schlegeli* requires a treatment period of 150 days for hormonal induction of accelerated sex reversal (Chang et al., 1994; see also Tao et al., 1993). A possible reason for availability of information on limited numbers of gonochoristic species is that the availability of reliable protocols for hypophysation is restricted to a few commercially important food-fish. As endocrine treatment involves mostly the post-hatching and/or juvenile stages, it is not clear whether the non-availability of hypophysation protocol has been an impediment in the extension of these techniques to more gonochoristic species. Yet the number of teleosts, which are thus far subjected to hormonal induction of sex reversal, is too few to generalize for the entire group.

3. Endocrine treatment

3.1. Steroids

As many as 31 natural and synthetic steroids have been used for sex reversal in economically important species. In general the optimum dose required to induce complete sex reversal of all individuals is species-specific, and in some species, strain-specific (*Cyprinus carpio*: European carp strain; Nagy et al., 1981; Asian carp strain, Rao and Rao, 1983). Padao, 1937 was perhaps the first to use synthetic hormones to reverse sex of Oncorhynchus mykiss.

A survey of the relevant literature on endocrine treatment of fish indicates the following:

1. Among androgens, 17α-methyltestosterone is the most widely used hormone and has been tested in more than 25 species belonging to Salmonidae, Cichlidae, Cyprinidae, Anbantidae, Poecilidae and Cyprinodontidae (Table 3). From the view of potency, these androgens may be arranged in the following order: mibolerone > 19-nor-ethynyltestosterone > 17α-methyltestosterone > testosterone.
2. Among the estrogens, estradiol-17ß is the most preferred hormone and has been used for treatment of more than 15 species belonging to Salmonidae, Cichlidae, Cyprinidae, Anabantidae, Poecilidae and Ictaluridae (Table 4).
3. Brooding (viviparous) poecilids require the highest dose of 300–500 mg per kg diet, while bigger food-fish such as salmonids and cyprinids require 50–200 mg per kg diet and smaller oviparous cichlids and anabantids require the lowest dose of 5–50 mg per kg diet (Pandian, 1993). The trends observed for intensity of treatment for successful induction of masculinization or feminization are given in Fig. 1. With some food-fish belonging to Cyprinidae and Salmonidae, the success rate of hormonal induction is only about 60 and 95%, respectively.
4. As many as 11 synthetic androgens and 12 synthetic estrogens have been used for treatment but results for only five natural androgens and three natural estrogens have been reported. This is due to the fact that synthetic hormones are relatively cheaper and are subjected to relatively slower elimination.
5. For masculinization, synthetic 17α-methyltestosterone has been the choice of most workers, but for feminization the natural estradiol-17ß has been preferred by most authors.

3.2. Treatment methods

Table 5 lists advantages and limitations of the methods of hormone administration. Immersion techniques involving periodic or continuous exposure (e.g. Piferrer et al., 1994) of embryonic or juvenile stages in water containing steroid was first developed. The dietary supplementation method involves the homogeneous mixing of the steroid in the diet (e.g. Yamamoto, 1953). The most widely used of steroid application is the alcohol evaporation method (Guerrero, 1975). Recently Martin-Robinchand et al. (1994) ensured feminization in *Cyclopterus lumpus* using estradiol-17ß-enriched artemia as food.

Steroids administered through diet apparently suffer a greater loss than that administered through injection. Hishida (1965) is perhaps the first to show that for *Oryzias latipes* the required estrone dose, when administered intraperitoneally (or intramuscularly), can be reduced to a tenth of that required through diet. Over 99% of the hormone administered through the diet is released into water in less than 24 h (*Oreochromis mossambicus* and *O. mykiss*; Johnstone et al., 1983). Viviparous species require higher doses when brooding females are subjected to treatment. For example, *Poecilia reticulata* was masculinized by

Table 3
Androgens used for sex reversal of teleosts. Values indicate the number of species (Sp) and optimum dose (O.d, mg kg^{-1} diet, or $\mu g\ l^{-1}$ rearing water indicated by *) that induces complete sex reversal

Steroids	Salmonidae		Cichlidae		Cyprinidae		Anbantidae		Poecilidae		Cyprinodontidae	
	Sp	O.d.	Sp	O.d.	Sp	O.d.	Sp	O.d.	Sp	O.d.	Sp	O.d.
I. Natural												
1. Testosterone	1	55	1	1	-	-	-	-	-	-	-	-
2. 11-ketotestosterone	-	-	1	200*	1	350	1	60	-	-	-	-
3. 11-ßhydroxyandrostenedione	1	60	-	-	-	-	-	-	-	-	-	-
4. Androstenedione	-	-	-	-	-	-	1	90	1	400*	-	
5. Dehydroepiandrosterone	-	-	1	5	-	-	-	-	-	- -	-	-
II. Synthetic												
1. Mibolerone	-	-	2	2-15	1	15	-	-	-	-	-	-
2. 9(11)dimethyltestosterone	-	-	-	-	-	-	-	-	1	200	-	-
3. 19-nor-ethynyltestosterone	-	-	1	3	-	-	-	-	1	300	-	-
4. Fluoxymesterone	-	-	2	1	-	-	-	-	1	-	-	-
5. 17α-ethynyltestosterone	-	–	4	15-60	-	-	-	-	-	-	-	-
6. Methylandrostenediol	-	-	-	-	-	-	-	-	1	-	-	-
7. 17α-methyltestosterone	8	1-100	5	1-50	5	30-1200	1	140	3	400-480	1	20
8. Testosterone acetate	-	-	-	-	1	300	-	-	-	-	-	-
9. Testosteronepropionate	1	700	-	-	-	-	-	-	-	-	-	-
10. Chlorotestosterone	1	1-13[a]	-	-	-	-	-	-	-	-	-	-
11. Methyldihydrotestosterone	1	6	-	-	-	-	-	-	-	-	-	-

[a] Through injection

Table 4
Estrogens used for sex reversal of teleosts. Values indicate the number of species (Sp) and optimum dose (O.d. mg kg^{-1} diet $\mu g\ l^{-1}$ rearing water indicated by *) that induces complete sex reversal

Steroids	Salmonidae		Cichlidae		Cyprinidae		Anbantidae		Poecilidae		Cyprinodontidae	
	Sp	O.d.	Sp	O.d.	Sp	O.d.	Sp	O.d.	Sp	O.d.	Sp	O.d.
I. Natural												
1. Estradiol-17ß	7	2-50	3	30-120	1	200	1	125	2	400	-	-
2. Estrone	1	10-100	1	3-120	1	100	-	-	-	-	1	20-124
3. Estrial	-	-	1	30-120	-	-	-	-	-	-	1	130
II. Synthetic												
1. Diethylstilbesterol	1	10	4	25-125	-	-	1	40	-	-	-	-
2. Stilbesteroldiphosphate	-	-	-	-	-	-	-	-	-	-	1	7
3. Stilbesterol	-	-	-	-	-	-	-	-	1	50-1000*	-	-
4. Diethyldioxystilben	-	-	-	-	-	-	-	-	-	-	1	2
5. Diethylstilbesteroldiphosphate	-	-	-	-	-	-	-	-	-	-	-	-
6. Ethynylestradiol	-	-	4	60-100	-	-	1	100	-	-	1	1.7
7. Estradiol butnylacetate	-	-	1	250	-	-	-	-	-	-	-	-
8. Estradiol benzoate	-	-	-	-	1	800	-	-	-	-	1	1.2
9. Hexesterol	-	-	-	-	-	-	-	-	-	-	1	0.5
10.Euvastin	-	-	-	-	-	-	-	-	-	-	1	0.4
11.Ethylestradiol	-	-	-	-	-	-	-	-	-	-	1	0.8
12.Ethylesternol	-	-	-	-	1	-	-	-	-	-	-	-

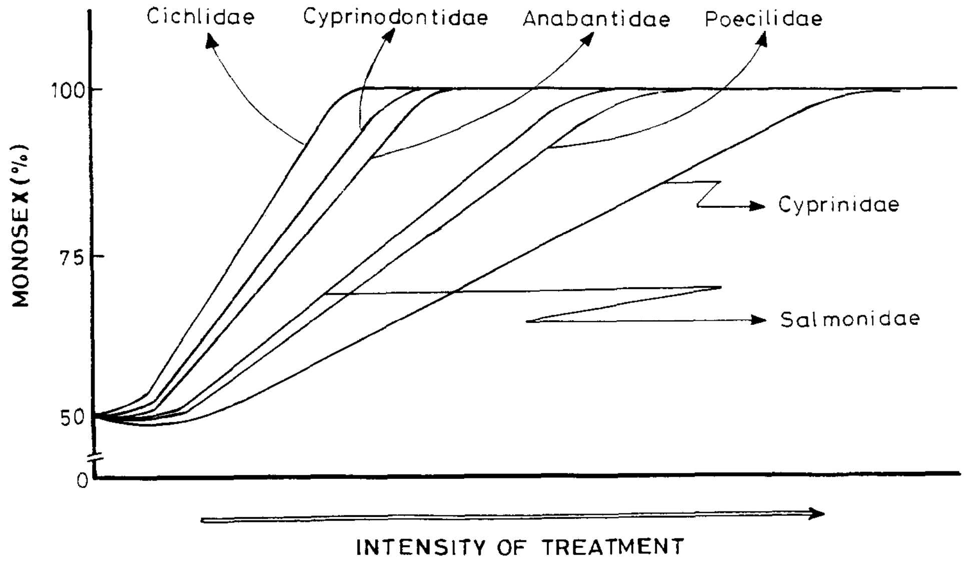

Fig. 1. Suggested trends of hormonal sex reversal as a function of treatment intensity for different teleost families

Table 5
Advantages and limitations of methods of hormone administration for sex reversal of fish

Advantages	Disadvantages
Dietary supplementation	
Most commonly used and cheapest method. Requires almost no skill	Hormone suffers degradation in digestive tract[a]. Its purity varies[b]; its solubility also varies with the solvent used[c]; uniformity of its distribution in feed may vary. Size hierarchy may lead to differential feed uptake and hence hormone intake. Intensive treatment can lead to sterility or paradoxical sex reversal
Immersion technique	
Frequently used method in cold water species. Ensures synergic induction. Cheaper than dietary treatment. Requires almost no skill	Mainly used for embryos and post-hatching stages alone. Almost not useful in field situation. Intensive treatment can lead to paradoxical sex reversal[d]
Systemic transfer	
By injection	
May require less hormone and ensure quicker reversal	Most laborious, expensive and skilled technique. Might lead to injury and infection. Not feasible for sex control in larvae
By silastic implantation	
Presumed to release uniform quantity of the implanted hormone. Ensures sex reversal in silver carp[e], which otherwise resist it	Costlier technique requiring skill for silastic implantation. Initial release of high dose. Can be used only for large fish with a late onset of sexual differentiation

Source: [a]Hishida (1965); [b]Pandian and Varadaraj (1991); [c]Varadaraj and Pandian (1991); [d]Muller (1969); [e]Mizra and Shelton (1988).

feeding gravid females a diet supplemented with 400 mg 17α-methyltestosterone per kg diet for 7–8 days preceding parturition (Pandian, 1993; Table 3). However, Dzwillo (1962), Dzwillo (1966) achieved 100% masculinization by immersing 8 day old fry for 24 h in water containing 3 mg 17α-methyltestosterone per litre. Likewise, brooding female *P. reticulata* required 300 mg estradiol-17ß per kg diet (Kavumpurath and Pandian, 1992) while *P. sphenops* fry required just 200 mg estradiol-17ß per kg diet for successful feminization (George and Pandian, 1995a).

The review by Hunter and Donaldson (1983) clearly indicates that endocrine treatment through diet is by far the most accepted mode of administering steroid. However, immersion treatment is preferred, especially with cold water species, as it permits earlier treatment of pre-feeding of eyed and alevin stages. Yet the treatment involving periodic immersion alone has not ensured 100% sex reversal with most salmonids (e.g. Johnstone et al., 1978). The highest value so far reported is 90% masculinization, when eyed-stage *O. kisutch* are immersed in water containing 1600 μg 17α-methyldihydrotestosterone (MDTH) per litre (Piferrer and Donaldson, 1991). However, Piferrer and Donaldson (1992) recently succeeded in inducing 100% females in *O. tshawystcha* by subjecting hatchlings to a single immersion for 8 h day^{-1} in 400 μg estradiol-17ß l^{-1} for 35 days. Juvenile salmonids, which had undergone periodic immersion in aqueous steroid solution during the pre-feeding stage responded more favourably to the dietary endocrine treatment than those fed on endocrine supplemented diet alone (Simpson, 1975; Johnstone et al., 1978; Hunter and Donaldson, 1983). For example, Goetz et al. (1979) ensured 60 and 100% sex reversal in *O. kisutch*, when the fish were treated with steroid supplemented diet alone and a steroid supplemented diet preceded by periodic immersion, respectively. Piferrer et al. (1994) have shown that periodic (2 h day^{-1}) immersion of embryonic stages of coho salmon yielded better results for inducing sterility than continuous immersion. An attempt by Rosenstein and Hulata (1992) to induce sex reversal in eggs and embryos of *Oreochromis* spp. by estrogen immersion proved ineffective. Hormonal sex control in tilapia may be achieved by the immersion technique (E.M. Donaldson, unpublished data) but dietary administration is more convenient.

Administration of steroid through either diet or immersion technique proved futile in some species with specialised feeding habits and relatively late, long-term gonadal differentiation (Shelton, 1986). Grass carp and silver carp are representatives of this group. Therefore, they are treated with an alternative to per os delivery by silastic implantation. This ensures the maintenance of critical androgen levels in the fish for a longer period, during which sex differentiation is known to occur (Mizra and Shelton, 1988). Thus, the use of immersion on implantation techniques is restricted to selected fish groups.

3.3. Labile period

Histological studies on sex differentiation have helped to delineate the labile period, during which the effect of administered steroid could be realized. A series of such histological studies undertaken by a number of workers on cichlids, salmonids and cyprinids has been comprehensively summarized by Hunter and Donaldson (1983). Broadly speaking two major groups are recognizable among teleosts; the first group includes species in which differentiation occurs just following hatching and lasts for a short period of 10–40 days; the

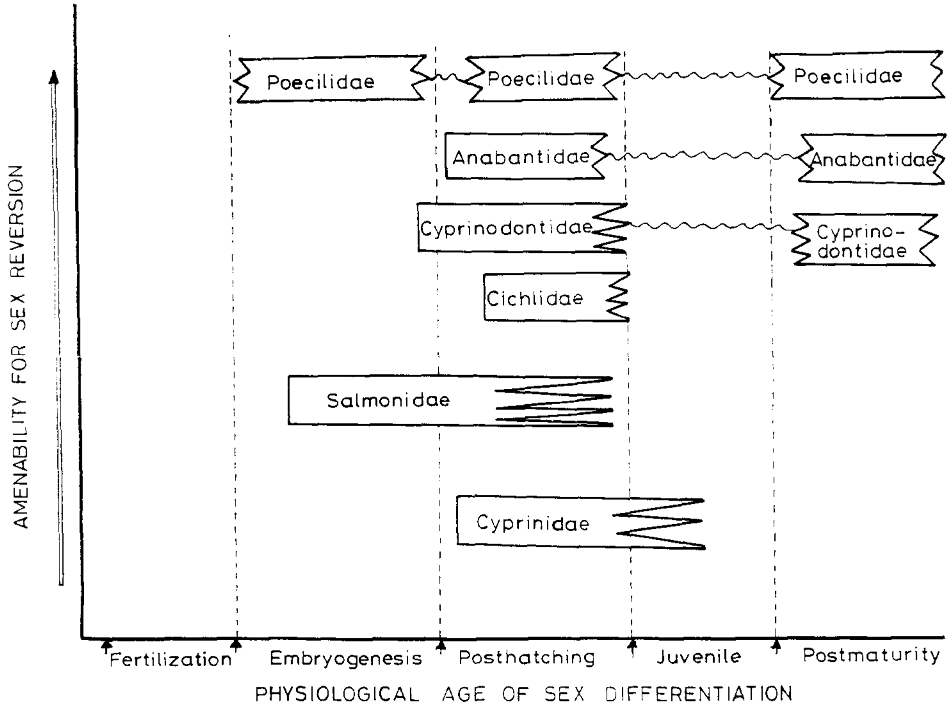

Fig. 2. Amenability of teleosts to sex reversal as a function of physiological age; wherever the period is clearly delineated,it is indicated by a straight line; in others it is indicated by a wavy line

second group is comprised of species in which differentiation occurs during the late juvenile stage and lasts for a period of 150–500 days (150 days for *O. mykiss*; Yamazaki, 1976; 500 days for *Ctenopharyngodon idella*; Shelton and Jensen, 1979). Differentiation occurs between Day 10 and 30 following hatching in cichlids and cyprinodontids, and between Day 3 and 40 following hatching in anabandtids. As a consequence of this difference in labile period, the intensity of treatment to ensure 100% sex reversal also is correspondingly increased (Fig. 1). Based on the data presented by Hunter and Donaldson (1983) and Yamazaki (1983), the amenability to hormonal induction of sex reversal varies not only from species to species but also from family to family. Many workers have designed experiments to precisely delineate the labile period (e.g. Pandian and Varadaraj, 1987; Piferrer and Donaldson, 1991; Kavumpurath and Pandian, 1993a).

Sex reversal has successfully been induced by surgical gonadectomy in adults of fighting fish (Noble and Kumpf, 1936; Lowe and Larkin, 1975), medaka (Shibata and Hamaguchi, 1988) and goldfish (Kobayashi et al., 1991). These surgical experiments have demonstrated that the germ cells of adult female fish, whose gonads have differentiated and matured, retain the sexual bipotentiality. The observations on poecilid species suggest that they are amenable to successful hormonal sex reversal during embryogenesis, i.e. treating the brooding female, post-hatching and post-maturity stages (Takahashi, 1975; Kavumpurath and Pandian, 1992; see also Howell et al., 1994 for *Gambusia affinis*). Efforts to induce sex reversal during the embryonic stage of cichlids have failed (Rosenstein and Hulata, 1992), but their amenability (to at least masculinization) is not restricted to a short post-hatching stage (Levy and Aronson, 1955). Therefore, the labile period, i.e. amenability for sex reversal, is not always restricted to a single physiological stage at least in the following families: Poecilidae, Anabantidae, Cyprinodontidae, Cichlidae and Cyprinidae. In fact, it is extended from embryogenesis to post-hatching and to adult stages (Fig. 2).

4. Intersexuality and sterility

4.1. Intersexuality

As treatment intensities for endocrine sex reversal is increased a known percentage of treated fish display intersexuality. At high dosages, some of the treated individuals become sterile; an analysis of the occurrence of intersex and sterility among steroid treated-fish suggests two patterns (Fig. 3). In the first one intersexes appear at a sub-optimum intensity of treatment (Table 6; e.g. anabantids). In the second one, intersex and sterile individuals simultaneously occur among the fish treated at super-optimal doses. Reports on simultaneous occurrences of intersex and sterile individuals are available for cyprinids, salmonids and cichlids.

Yamamoto (1975) introduced the term intersex and described the different kinds of gonads observed among intersexes of *O. latipes*. In a series of publications, Reinboth

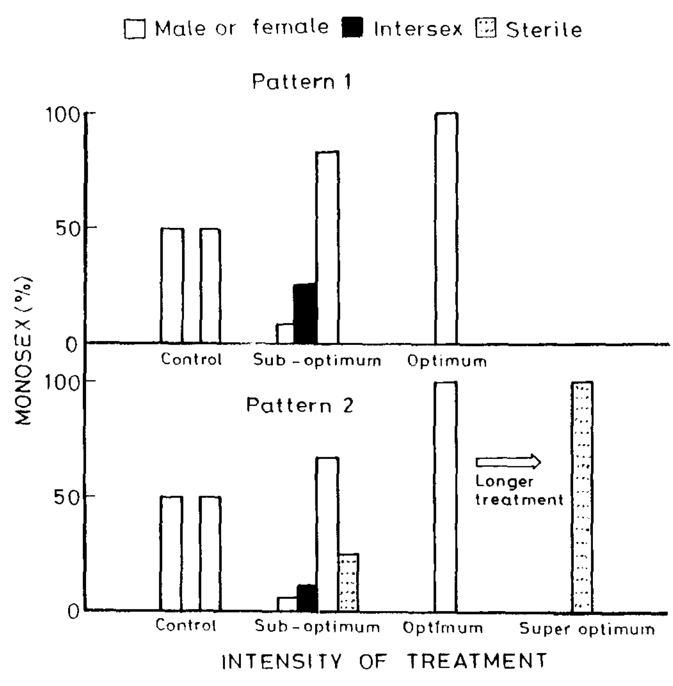

Fig. 3. Two patterns on appearence of intersexes and sterility for fish species subjected to hormonal sex reversal as a function of treatment intensity.

(1979), Reinboth (1980), Reinboth (1982) described intersexuality in seasonal and sequential hermaphrodites. In the 1940s and 1950s, gonadectomized fish were experimentally treated with steroids to understand the restoration of gonads and secondary sexual characteristics including behaviour. Such redifferentiation necessarily involves the same two but highly interactive and independent processes: gonadogenesis, the formation of the structural and supporting elements of the gonad and gametogenesis (see Kobayashi et al., 1991). Cichlids and anabantids have been the most preferred experimental animals for these endocrinological and ethological studies. Johns et al. (1969) observed that castrated male gourami *Trichogaster trichopterus*, on being treated with methyltestosterone, acquired secondary sexual characters but the level of sexual behaviour was not equal to that of the intact male. Castrated *Platypoecilus maculatus*, when treated with testosterone, developed gonopodia and performed lateral display, which is common to both males and females and represents the first phase of courtship. However, the treated fish failed to display the zigzag dance (phase 2) and mating activity (phase 3), typical of an intact male (Tavolga, 1949). In the viviparous sea perch (*Cymatogaster aggregata*) castrated males treated with 17α-methyltestosterone also acquired secondary sexual characteristics (Wiebe, 1967). Similar findings have been observed for the goby, *Bathygobius soporator*, which is known to brood infertile eggs (Tavolga, 1955). Experimental studies on cichlids indicate that castrated males tend to retain all elements of mating behaviour up to 42 (with *Aequidens latifrons*, (Aronson, 1951) and 202 days (with *Hemichromis bimaculatus*, (Noble and Kumpf, 1936) The castration effects on gonadogenesis were reversed by testosterone treatment (with *Tilapia macrocephalus*, (Levy and Aronson, 1955). Thus, treating the castrated males with one or other androgen, can restore gonads and/or secondary sexual features and may display part or the entire sequence of courtship activity.

However, female *Betta splendens* developed testes after ovariectomy and became functional males; these testes were considered to have developed from the ovarian wall, the tissue that was not removed by surgery (Lowe and Larkin, 1975). Similarly, the development of testis is reported after sham-ovariectomy of the medaka (Kasuga, 1973). Recently Kobayashi et al. (1991) showed that the ovariectomized adult female goldfish (*Carassius auratus*) developed testicular tissue from the ovarian fragments not removed by surgery; however, the implantation of 11-ketotestosterone capsule was required for the development of secondary sexual characters and testes (gonadogenesis) and to promote spermatogenesis. It appears that sham-ovariectomy in more plastic species such as the fighting fish and medaka, or ovariectomy followed by steroid administration in relatively less plastic species like the goldfish permits the development of testes (gonadogenesis) and formation of sperm (gametogenesis). Hence, the labile period was not restricted to the juvenile stage alone in these fish.

In contrast to their findings with castrated males, Noble and Kumpf (1936) found that ovariectomy of females *Hemichromis bimaculatus* resulted in total loss of reproductive behaviour. Ovariectomy of *T. macrocephalus* led to a reduction of the genital tube and appearance of immature operculum colour. Estradiol treatment of these females induced the growth of the genital tube but not the appearance of mature operculum colour (Aronson and Holz-Tucker, 1947). Even after treatment with estrone, ovariectomized females of *T. trichopterus* remained unattractive to males (Johns et al., 1969). Obviously estrone treatment failed to induce ovary development. However, estrogen treatment of ovariectomized

Table 6
Reported occurrence (indicated by $^+$) of intersex and sterility for endocrine treated fish

Species	Hormone	Intersex	Sterility	Reference
Cyprinidae				
Masculinization				
Ctenopharyngodon idella	MT	-	+	Shelton and Jensen (1979)
Cyprinus carpio	MT	+	+	Ali and Rao (1989)
C. carpio	MT	-	+	Rao and Rao (1983)
C. carpio	Mb	-	+	Das et al. (1990)
Hypophthalmichthys molitrix	MT-Sil	+	+	Mizra and Shelton (1988)
H. molitrix	DMSO-MT-sus	-	-	Mizra and Shelton (1988)
Feminization				
C. carpio	E-17ß	-	-	Rao and Rao (1983)
Salmonidae				
Masculinization				
Oncorhynchus mykiss	MT	-	+	Jalabert et al. (1975)
O. mykiss	MT	+	-	Olite and Brock (1991)
O. kisutch	MT	-	+	Goetz et al. (1979)
O. kisutch	MT	-	+	Hunter and Donaldson (1983)
O. kisutch	MT	-	+	Hunter et al. (1982)
O. kisutch	MT	-	+	Donaldson and Hunter (1982)
O. tshawytscha	MT	+	-	Baker et al. (1988)
Feminization				
O. mykiss	E-17ß	+	-	Johnstone et al. (1978)
O. mykiss	Est	+ + +	+	Jalabert et al. (1975)
S. salar	E-17ß	+ +	+	Sower et al. (1984)
S. salar	DES	-	+	Sower et al. (1984)
Poecilidae				
Masculinization				
Poecilia reticulata	And	+	-	Kavumpurath and Pandian (1993b)
P. reticulata	17α-ET	+	-	Kavumpurath and Pandian (1993b)
P. reticulata	19-nor-ET	+	-	Kavumpurath and Pandian (1993b)
P. reticulata (young ones)	17α-MT	+	+	Takahashi (1975)
Feminization				
P. reticulata	E-17ß	+	-	Kavumpurath and Pandian (1992)
P. reticulata	DES	+	-	Kavumpurath and Pandian (1992)
P. reticulata	17α-EE	+	-	Kavumpurath and Pandian (1992)
P. reticulata	EB	+	-	Kavumpurath and Pandian (1993c)
Anabantidae				
Masculinization				
Betta splendens	17α-MT	+	-	Kavumpurath and Pandian (1994)
B. splendens	19-nor-ET	+	-	Kavumpurath and Pandian (1994)
B. splendens	11-19-T	+	-	Kavumpurath and Pandian (1994)
B. splendens	And	+	-	Kavumpurath and Pandian (1994)
Feminization				
B. splendens	E-17ß	+	-	Kavumpurath and Pandian (1993a)
B. splendens	DES	+ + +	-	Kavumpurath and Pandian (1993a)
B. splendens	17α-EE	+	-	Kavumpurath and Pandian (1993a)
Cichlidae				

Species	Hormone	Intersex	Sterility	Reference
Masculinization				
Oreochromis mossambicus	Mb	-	-	Guerrero and Guerrero (1993)
O. mossambicus	MT	-	+	Nakamura (1981)
O. mossambicus	MT	+ +	-	Varadaraj (1990)
O. mossambicus	19-nor-ET	+ +	-	Varadaraj (1990)
O. mossambicus	17α-ET	+	-	Varadaraj (1990)
O. aureus	Mb	+	-	Meriwether and Torrans (1986)
O. macrochir	ET	-	+	Meriwether and Torrans (1986)
Feminization				
O. mossambicus	DES	+	-	Varadaraj (1990)
O. mossambicus	E-17ß	+	-	Varadaraj (1990)
O. mossambicus	EE	+	-	Varadaraj (1990)
O. aureus	Stilbestrol	-	+	Eckstein and Spira (1965)

And, androstenedione; EB, estradiolbenzoate; MT, methyltestosterone; DES, diethylstilbestrol; 17α-ET, 17α-ethylnyltestosterone; MT sil, MT silastic implant; Est, estrone; 19-nor-ET, 19-nor-ethylnyltestosterone; DMSO-MT-Sus, MT suspended in DMSO; E-17ß, estradiol; KT, ketoestosterone; Mb, mibolerene or 17ß-hydroxy 7α, 7-dimethyl estro-4 -en-3one; 17α-EE, 17α-Ethynylestradiol; 11-19-T, 11-(19)-testoterone.

females of *O. latipes* (Okado and Yamashita, 1994) and *B. splendens* (Noble and Kumpf, 1936) led to no restoration of gonadal tissue or secondary sexual characters. Likewise the treatment of *Colisa labiosa* females with testosterone proprionate induced male colouration but not the other male characters like nest building and defence (Forselius, 1957). Thus, surgical operation followed by androgen treatment may restore masculinity but castration followed by estrogen may fail to restore femininity. Indeed, George and Pandian (1995b) observed with *P. sphenops* that males were readily amenable to endocrine feminization but females totally resisted masculinization. It is not clear whether these observations have a bearing on the finding that homogametic females undergo a greater stress when treated with androgen (see Pandian et al., 1994).

4.2. Sterility

In teleosts sterility has been induced by intensive hormone treatment or triploidy induction (e.g. Kavumpurath and Pandian, 1990). However, this discussion is restricted to hormonal induction of sterility. It has not been possible to secure 100% sterile grass carp (*C. idella*), despite the fact that very intensive long-term treatments of more than 500 days (e.g. Shelton and Jensen, 1979) have been applied. It is likely a more potent synthetic hormone such as mibolerone may induce 100% sterility in *C. idella*. With *C. carpio*, a treatment involving less than 30 days of 50 mg mibolerone per kg diet ensured 100% sterility (Das et al., 1990) compared to 400 mg 17α-methyltestosterone per kg diet used by Nagaraj and Rao (1988).

Ali and Rao (1989) observed 1 year after treatment that the hormone-treated fish showed a higher survival (95%) and better growth (252 g) than the control (85% survival, 230 g weight). Most authors have reported similar observations for other species, treated with 17α-methyltestosterone (e.g. Baker et al., 1988) or mibolerone (e.g. Guerrero and Guerrero, 1993). However, long duration treatment and sterility can lead to stunted growth (Johnstone et al., 1978). Contradictory observations also are not uncommon regarding sterility induced by administering estrogens, e.g. administration of diethylstilbesterol is known to induce negative growth response with *C. carpio* (Nanjundappa and Varghese, 1988).

5. Survival, growth and reproduction

Information on survival, growth and reproduction of hormonally sex reversed fish is as important as information on achievement of hormonal induction of sex reversal. Very few studies have examined elimination rates of the administered steroids by fish. Johnstone et al. (1983) studied the elimination rate of methyltestosterone using labelled steroid. Nan-

Table 7
Genetic composition of fish, subjected to endocrine sex reversal and identified by progeny testing (modified from Pandian et al., 1994)

Species	Steroid	Effective minimum dose (mg kg^{-1} food)	Genotype composition (%) of treated individual	
Heterogametic male				
Masculinization			XY ♂♂	XX ♂♂
B. splendens	19-nor-ET	8	80	20
	17α-ET	15	70	30
	And	90	62	38
	11-KT	60	80	-
O. mykiss	17α-MT	1	85	15
P. reticulata	19-nor-ET	300	83	17
	17α-ET	500	90	10
	And	200	58	42
Mean sex composition (%)			76	24
Feminization			XX ♀♀	XY ♀♀
B. splendens	E-17ß	125	56	44
	E-17ß	50	70	30
	DES	20	63	37
O. mossambicus	DES	100	45	55
P. reticulata	E-17ß	400	69	31
	E-17ß	200	61	39
	DES	300	56	44
Mean sex composition (%)			60	40
Heterogametic female				
Masculinization			ZZ ♂♂	ZW ♂♂
T. aurea	17α-ET	30	63	27
	17α-MT	30	54	46
Mean sex composition (%)			59	37
Feminization			ZW ♀♀	ZZ ♀♀
P. sphenops	E-17ß	200	80	20

And, androstenedione; 17α-ET, 17α-Ethynyltestosterone; DES, diethylstilbestrol; 11-KT, 11-ketotestosterone; E-17ß, estradiol-17ß; 17α-MT, 17α-Methyltestosterone; 19-nor-ET, 19-nor-ethynyltestosterone. (Table reprinted with the permission of Current Science.)

Table 8
Growth response (+ positive; - negative) observed for hormonally sex reversed fish

Species	Growth	Reference
Salmonidae		
Oncorhynchus gorbuscha	-	Funk et al. (1973)
O. mykiss	+	Donaldson et al. (1979)
O. mykiss	-	Johnstone et al. (1978)
O. kisutch	+	Goetz et al. (1979)
O. kisutch	+	Piferrer et al. (1994)
O. tshawytscha	+	Baker et al. (1988)
O. tshawytscha	+	Piferrer and Donaldson (1992)
Cyprinidae		
Cyprinus carpio	+	Nagaraj and Rao (1988)
C. carpio	+	Rao and Rao (1983)
C. carpio	+	Lone and Matty (1983)
C. carpio	+	Nanjundappa and Varghese (1988)
C. carpio	+	Ali and Rao (1989)
C. carpio	+	Sehgal et al. (1995)
Cichlidae		
Oreochromis mossambicus	+	Guerrero and Guerrero (1993)
O. mossambicus	+	Macintosh et al. (1985)
O. niloticus	+	Tayamen and Shelton (1978)
O. niloticus	-	Cruz and Mair (1994)
O. niloticus	-	Green and Coddington (1994)
O. aurea	+	Shelton et al. (1981)
O. spirulurus	-	Lone and Ridha (1993)
Cyprinodontidae		
Oryzias latipes	-	Yamazaki (1976)

deesha et al. (1990) used bioassay procedures to quantify residual steroid level (dried carcass of tilapia which had been fed on a mibolerone-supplemented diet fed to castrated male rats) and observed no detectable structural development of male reproductive organs.

5.1. Survival

In general a treatment involving a synthetic steroid results in higher mortality of most species. Table 7 summarizes the available information on genotype composition of fish, subjected to endocrine sex reversal and subsequently progeny tested. As progeny testing involves long term experiments the kind of DNA probe developed by Devlin et al. (1994) may help to distinguish the genotype of the hormonally masculinized individuals. As the progeny testing involved sexually mature individuals, the percentage composition of genotypes of the sex reversed fish, that survived until breeding was estimated. This value is expected to be 50: 50 but almost all the values deviate considerably from this ratio and thereby indicate sex-dependent mortality among the sex reversed fish. Thus, masculinization with the male heterogametic species may lead to lower survival (24%) of XX males; likewise with feminization among the female heterogametic species may result in lower survival (20%) of ZZ females.

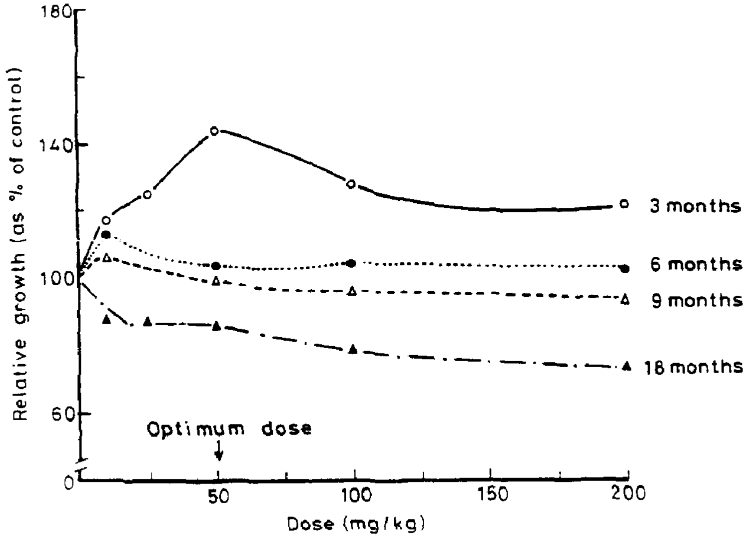

Fig. 4. Relative growth (as percentage of control) trends as function of increasing dose and age of *Poecilia sphenops* treated with 17α-methyltestosterone for 30 days from birth. Data obtained for the different dose groups at the ages of 12 and 15 months fell between those observed for the 9 and 18 month old individuals. Arrow indicates the optimum hormonal dose for sex reversal (from George and Pandian, 1995b)

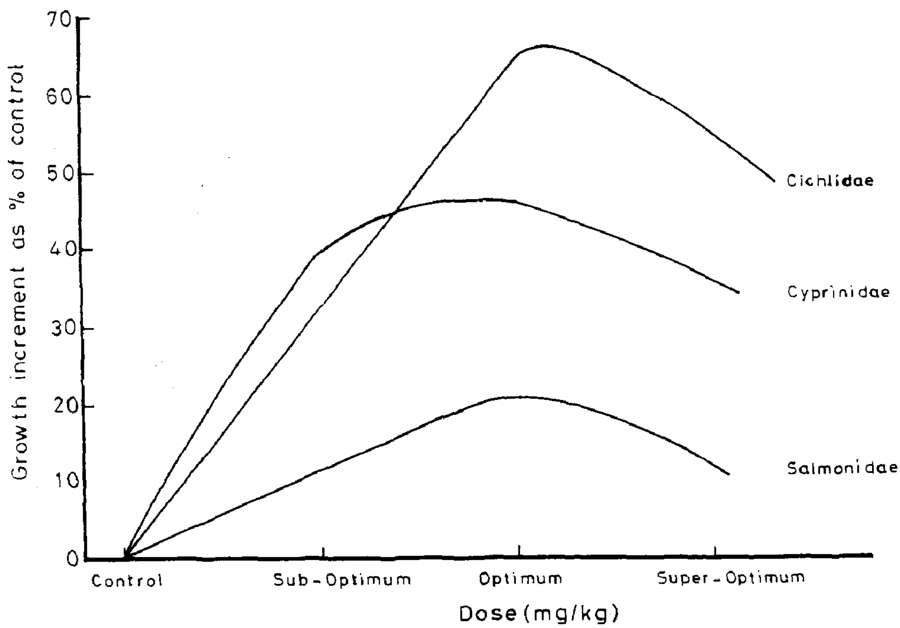

Fig. 5. Generalised growth trends observed for selected teleosts subjected to hormonal sex reversal. (Source: Salmonidae, Goetz et al. (1979); Cichlidae, Macintosh et al. (1985); Cyprinidae, Rao and Rao (1983).

Table 9
Comparative account of the functional equality of normal and endocrine reversed fish (modified from Kavumpurath and Pandian, 1993a)

Species	Steroid and genotype of treated parent		Age at puberty (day)	No. of young ones/ eggs released I time
Betta splendens	Control	XX	178	518
	Estradiol-17ß	XX	198	485
		XY	194	487
	Diethylstilbestrol	XY	219	405
		XY	241	425
Poecilia sphenops	Control	ZW	231	17
	Estradiol-17ß	ZW	188	9
		ZZ	190	7
	Diethylstilbestrol	ZW	191	10
		ZZ	+	+
Oncorhynchus tshawytsha	Control	XY	24[a]	0.38[b]
	17 α-methyl-testosterone	XX, XY	36[a]	0.25[b]

[a] Months.[b]Milt volume per replicate (ml). +, Did not survive.

5.2. Growth

One of the objectives of inducing hormonal sex reversal is to realise 100% growth potential. As many authors have not chosen to undertake long-term studies on the growth of sex reversed fish, available information on this aspect is scanty and inconsistent (Table 8). The long-term growth experiments undertaken by George and Pandian (1995b) on *P. sphenops*, treated with different doses of 17α-methyltestosterone are interesting. They estimated the weight gain as a measure of growth in treated (for a period of 1 month from birth) and control individuals at the age of 3, 6, 9, 12, 15 and 18 months. Relative growth was enhanced in 3 month old treated individuals with increasing steroid dose up to the pre-optimal level (for sex reversal) beyond which the increase in relative growth began to diminish (Fig. 4). Notably growth was consistently suppressed in 18 month old individuals irrespective of treatment intensity. Clearly, hormone treatment may accelerate growth in juveniles, and accelerated growth of juvenile stage may be an advantage in escaping from larger predators but with the penalty of suppressed growth of adults. These observations may explain some of the inconsistent reports on growth of the hormone treated fish. From the available information on growth of equally aged or sized treated individuals, generalised growth trends observed for hormonally sex reversed fish belonging to salmonids (*O. kisutch*: Goetz et al., 1979), cyprinids (*C. carpio*: Rao and Rao, 1983) and cichlids (e.g. *O. mossambicus*: Macintosh et al., 1985) are illustrated in Fig. 5. In general, the cyprinids show a positive growth response, of two to three times that of the control, while that for cichlids is one to two times faster. As the cichlids and cyprinids are warmwater fish, growth response of hormonally sex reversed fish may prove to be a greater advantages to tropical countries where more protein-hungry inhabitants are looking for fast growing fish.

5.3. Reproduction

Hormonally sex reversed females may not only suffer high mortality but also may not be functionally equal to genetic females. Table 9 summarizes the negative effects of hormonal sex reversal on age at puberty and fecundity of an oviparous and a viviparous species endocrine treatment postponed age at puberty with *B. splendens* but advanced it for *P. sphenops*. Fecundity is significantly reduced for both species, and females bearing homogamous and heterogamous genotypes suffered almost equally. The fact that females suffer the negative effects of the treatment long after the termination of treatment (nearly 160-180 days) deserves special attention. Indeed the male salmon (*O. tshawytsha*) exhibited delayed maturity and reduced sperm productivity even after a period of about 2 years after the termination of androgen treatment (Baker et al., 1988). Notably, Clemens et al. (1966) paired the methyltestosterone-treated male guppies with normal (genetic) females; only 14% of the males, including the sex reversed genetic females, sired young, although almost all the males displayed full male colouration and yielded viable sperm on stripping. The courtship behaviour and mating performance of castrated males, compensated by androgen administration or testis implantation, were not quantitatively comparable to those of intact males (see also Tavolga, 1949). These results pose some questions regarding the production of namely XX, or YY males and ZZ or WW female, however they are required for brood-stock development of monosex populations.

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