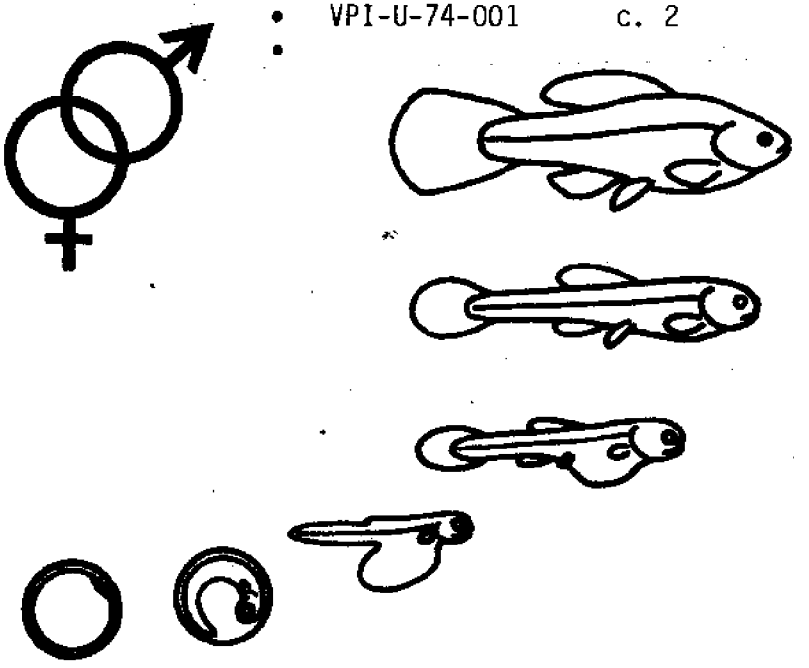

Control of Sex in Fishes

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Contents and Contributors

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Introduction

Carl B. Schreck

An up-to-date understanding of the scope and limitations of our knowledge basic to the art of controlling the sex of fishes, in addition to its philosophical scientific interest, has pragmatic implications for fisheries management. How does a fish become male or female? How does it come to function as a male or female, or change its function from one to the other? What factors intrinsic to the fish and what factors extrinsic to the fish regulate the observed events of its reproductive processes? How can the sex of a fish be determined at will, and, after it has already become differentiated as to sex, how can its reproductive function be manipulated for the benefit of human society?

The following three chapters attempt to provide a concise account of the progress already made toward answering these questions, while revealing some pitfalls of oversimplification and some already obvious tasks that remain to be carried out before further progress in the areas of research concerned can be accelerated. If these accounts fall short of providing a multidimensional model of sex regulation in teleost fishes, perhaps they will at least give the researchers in various specialities a synoptic appreciation of the complex of problems of which their immediate concerns are only a part.

R. W. Harrington's discussion of "Sex determination and differentiation in fishes" lays the foundation on which the other two papers build. V. L. de Vlaming considers "Environmental and endocrine control of teleost reproduction" in fish in which the sex has already been established. Finally, I briefly review "Hormonal treatment and sex manipulation in fishes," emphasizing influences on junvenile teleosts, *viz.* hormonally-induced sex reversal, or the experimentally-controlled production of fish of the sex (phenotypic sex) that is the opposite of their inherited sex (genotypic sex).

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SEX DETERMINATION AND DIFFERENTIATION IN FISHES

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Abstract

In the chains of causes from chromosome level to earliest definitive female or male elements in differentiating gonads, few links have been described. Of these, some are newly open to question or reinterpretation. The conceptual framework which, for many years, has guided research on sex determination and differentiation rests upon theories that still retain potent explanatory or didactic utility at more abstract levels of problem formulation but that have become increasingly difficult to reconcile with empirical data at more concrete levels.

Generalizations of reviewers relying on secondary sources or uncritical acceptance of authors' conclusions are not necessarily valid. Researchers on fishes are well advised to scrutinize *in toto*, themselves, both older and current literature. The diversity among the 20,000 species of teleosts and our small uneven knowledge of their sexuality do not warrant generalization.

Sex determination and differentiation are virtually inseparable. In a short account, bias cannot altogether be avoided and it seems best to emphasize wide-open questions presently confronting those seeking to find out how the genetically-prescribed sex is actualized as the phenotypic sex and how such actualization can be deflected to produce the opposite sex.

The present review concerns the, mostly invisible, causal chains and the chronology of observable events between the outset of genotypic sex and the outset of phenotypic sex. This causal span extends from fertilization to the earliest definitive expression of phenotypic sex. In a brief account one can do no more than stress how few links of these chains we know and point out links that are conventionally accepted as closed but which, in fact, are open to question.

Fertilization, in restoring the diploid condition, determines the genotypic sex by bringing together the pair of sex chromosomes and the other chromosome pairs, with their male and female sex-determining factors, chromosomal or genic. In teleosts, as in other animals, virtually nothing is known at present of sex determination at the molecular level of transcription and translation, except possibly its approximate timing along the growth-time axis of embryonic and larval development. Sex-gene activity during gonadogenesis is sometimes spoken of as the "turning on of the sex genes." This interval of sex-gene

activity has been equated with the interval of primary sex differentiation found to be hormone-labile in *Oryzias latipes* (Yamamoto, 1953, 1958, 1968) and temperature-labile in *Rivulus marmoratus* (Harrington, 1968, 1971). Such a hormone-labile interval has since been delimited in *Hemihaplochromis multicolor* (Hackmann and Reinboth, 1974). Implicit in all of these cases, however, is the reservation of Stern (1955) that "genic action may have immediately preceded the observable event or it may have taken place much earlier, invisibly preparing a situation which is the prerequisite for later events."

In some fishes the phenotypic sex is recognizable first in the somatic tissue of the gonads, in others, in the germ cells. In as yet somatically undifferentiated gonads, primary oogonia and spermatogonia are called *protogonia*, while secondary oogonia and spermatogonia are called *deutogonia* (or deuterogonia), following Italian usage, because oogonia and spermatogonia can usually be recognized as such only in somatically identifiable ovaries or testes. As Reinboth (1972) phrased it, "the distinction between spermatogonia, oogonia, and primary oocytes before meiotic prophase becomes somewhat arbitrary and frequently relies on somatic features of the gonad." Even among gonocytes in *early* first meiotic prophase, I have been unable so far to distinguish between primary oocytes and primary spermatocytes despite unusually favorable material, *viz.* gonads of the hermaphroditic fish *Rivulus marmoratus*. Classical cytologists seem to have overlooked or evaded this refinement of discrimination, but cytochemical studies on a hermaphroditic mollusc led Vitagliano (1950) to consider germ cells undifferentiated up to the pachytene stage of the first meiotic prophase. The generalization dismissed as premature or false by Atz (1964) and Harrintgon (1971), *viz.* that a female phase occurs in the gonadogenesis of all fishes regardless of ultimate sex, is plausibly attributed to protogonia or deutogonia wrongly identified as oogonia. In some rare cases it is a matter of arbitrary formal definition. In gonads of cyclostomes and anguillids, on the other hand, true male and female sex cells coexist in the same gonad long before the gonadal (phenotypic) sex is established, after which one or the other sex-cell line degenerates (Hardisty, 1965, and references therein). In the same paper, Hardisty reported that in *Lampetra planeri* protogonia as well as deutogonia may enter the first meiotic prophase, and cited literature reporting such "direct oogenesis" in the teleostean genera *Salmo, Gambusia,* and *Poecilia* (Girardinus). Further confusion is added by testis-ova, those oviform cells occurring sporadically even in mature testes, under conditions reviewed by Atz (1964) and Yamamoto (1969). Testis-ova cannot be taken as evidence either of intersexuality or of a transitory female phase in testicular development. Despite these cautionary facts, germ cells usually express the phenotypic sex dependably from the outset of rapid meiotic divisions by spermatocytes and of primary growth and vitellogenesis in oocytes.

Claims that higher numbers of primordial germ cells are diagnostic of female fish before somatic gonadal differentiation were ably reviewed by Hardisty

(1967). Satoh and Egami (1972) reported increasing numbers of these cells in presumed future females of *Oryzias latipes*, from just before hatching, and a cessation of their mitoses in presumed future males, directly after hatching. Quirk and Hamilton (1973), using known XX and XY genotypes of the same species, however, reported the same number in both male and female genotypes, at and just before hatching, but later, further mitoses yielded more germ cells in females than in males. The value of this count as a precocious indicator of phenotypic sex remains problematical.

At the morphogenetic level, the critical interval of sex differentiation extends from the earliest formation of the gonadal primordia to the recognition of gonadal sex. Comparatively few embryological studies describe fish gonadogenesis, and few, if any, of these adequately cover this critical period. Therefore, we cannot ignore any embryological studies on fishes, before or after the outset, in 1900, of genetics as a continuing discipline. Some studies by 19th century embryologists equal or surpass in quality many of the 20th century. In the first three decades of our century, attention focussed on the origin and migration of the germ cells. The critical interval of gonadogenesis was described only incidentally or not at all, and its incidental description sometimes later proved the more important contribution. Descriptions of gonadogenesis in live-bearers are disproportionately numerous, but fusion of right and left gonads in these tends to obscure significant events within this critical period. Although conspicuously few in recent years, more of such studies are needed, especially on oviparous forms, with special attention to the above emphasized critical interval and to broader phylogenetic sampling.

A list of the titles referring, explicitly or by possible implication, to descriptions of gonadal differentiation in fishes is too long for this report. Such a list can be compiled largely from the bibliographies of recent symposia or reviews concerning reproduction and sexuality, e. g. Ball, 1960, Brambell, 1956, Dodd, 1960, Franchi, 1962, Franchi *et al.*, 1962, Hoar, 1969, Marshall, 1960, Rauther, 1954, and other relevant works cited elsewhere in this report. However, it will be found that unfortunately the vast majority of the investigations concerned lack data on the critical interval of gonadal differentiation, some because the context for recognizing its significance had not developed, others because the data were incomplete for one reason or another.

In the first decade of this century, sex was found in general to be inherited as a Mendelian trait and cytologists discovered sex chromosomes, an identical pair (XX) in the sex called homogametic and an odd pair (XY) in the sex called heterogametic. Sex linkage was demonstrated in *Oryzias latipes* (Aida, 1921) seven years after the term was coined (Morgan, 1914), proving the existence of sex chromosomes in fishes genetically over three decades before they could be demonstrated cytologically (Nogusa, 1955, 1960). Although it is held that fishes in general are male heterogametic, *viz.* that the males carry the odd sex chromosome, conditions in most species are unknown. In

one species, both homogametic and heterogametic males and homogametic and heterogametic females occur (Kallman *et al.*, 1973: references & notes, 2). The only hermaphroditic species studied genetically is evidently homogametic (Kallman and Harrington, 1964; Harrington and Kallman, 1968).

Since the second decade of this century, two theories have dominated the conceptual framework within which sex determination and differentiation have been studied in vertebrates. The *balance theory of sex determination* began with the notion of dosage effect (Goldschmidt, 1911) and received a final summing up over 40 years later (Goldschmidt, 1955). According to this abstract genetic theory, sex is determined by the dominance of male (M) over female (F) factors, or the reverse. These are symbolized, according to male or female heterogamety, by the letters M and F for the factors themselves or for the sex chromosomes presumably carrying these factors. Yamamoto (1969) has briefly reviewed the attempts of Kosswig and his colleagues to use this theory to explain the results that have been obtained in studies of sex inheritance in laboratory strains of the medaka and various live-bearing poeciliid fishes. None of these attempts has proved satisfactory and, in fact, Goldschmidt (1937) himself was unable to reconcile data and theory in any coherent fashion.

The *inductor theory of sex differentiation,* called also the *theory of cortico-medullary antagonism,* contrived in 1914 by Witschi to particularize the balance theory at the level of gonadal morphogenesis, was defended and elaborated *ad hoc* by him during many subsequent years (e. g. Witschi, 1957) and stimulated much research. Witschi concluded from embryological studies that the amphibian gonad has a double primordium. The outer covering or *cortex* of the gonad derives directly from the peritoneum lining the body cavity, as a fold receiving from above not only the germ cells but also the gonadal *medulla,* a mass of cells that migrate from a blastema, or primitive anlage, that gives rise also to renal and interrenal elements. The blastema itself also derives from the peritoneum ultimately. Bacci (1965) has summarized disputed details. The genotypic sex, by the imbalance between M and F factors, decides whether cortex evolves at the expense of medulla, or the reverse. According to which of the two prevails, the initially bipotential germ cells are sexualized, in the male direction in the medulla by an embryonic inductor, *medullarin,* or in the female direction in the cortex, by *cortexin.* Witschi considered these hypothetical sex inductors nonsteroid. Whether they are steroid or nonsteroid remains in dispute (Yamamoto, 1969; Reinboth, 1972). A double primordium is affirmed also for amniotes and selachians.

Of special interest to us is the assertion of D'Ancona, first made in 1941 and repeated in a long series of papers, that the gonads of cyclostomes and teleosts have a unitary primordium formed wholly and directly from the peritoneal lining of the body cavity. The assertion of this gulf between cyclostomes and teleosts on the one hand and the rest of the vertebrates on the other con-

tinues to be repeated and emphasized in reviews and symposia but its importance may be exaggerated. In an exhaustive and judicious reexamination of the facts, Hardisty (1965) considered the distinction to be blurred by the close spatial relationship between interrenal and gonadal elements and by the succession of nephric, adrenocortical, and gonadal elements, derived from various differentiations of the peritoneum. Moreover, Gropp and Ohno (1966) claim for cattle a unitary gonadal primordium, which occupies the main, central part of the gonadal fold. Ohno (1967) believes that the fate of the indifferent gonad is decided by the direction taken by its unitary somatic blastema rather than by competition between hypothetically antagonistic cortical and medullary primordia. D'Ancona (1949) had difficulty in reconciling his assertion of a unitary gonadal primordium in teleosts with Witschi's theory. On the one hand, he was compelled to postulate hypothetical sex inductors with uncertain production sites, *androgenine* and *gynogenine*. On the other hand, he explained the discrete male and female territories in gonads of hermaphroditic fishes by assuming that such fishes are confined to phylogenetically advanced families. He denied male and female somatic territorial localization to phylogenetically primitive families, among which he listed the Cyprinodontidae (D'Ancona, 1955). However, distinct male and female territories have now been verified in the ovotestes of the cyprinodont *Rivulus marmoratus*, both histologically (Harrington, 1967, 1971) and embryologically (unpublished). Localization of male and female somatic territories in ovotestes strengthens at least one tenet of Witschi's theory, *viz.* the sexualization of originally bipotential germ cells by somatic tissues.

As Yamamoto (1969) reminds us, we should not rely on negative results. Proving the negative is a dubious undertaking. Merely in being inferential, it is subject to the logician's *fallacy of selection,* in the present case: incomplete serial sections per fish throughout the target area, gaps in the age-length gradation of developmental stages within the already much emphasized critical period, and too narrow a phylogenetic sampling. Clearly, new studies of fish gonadogenesis are needed, properly focussed and with attention to these pitfalls.

The concept of Mittwoch and her associates (Mittwoch, 1969, 1971, 1973; Mittwoch *et al.*, 1971) that sex determination is mediated at the level of the gonadal rudiment by a genetically-controlled mitotic rate, earlier and more ample in the heterogametic sex, could apply to either a unitary or a dual gonadal rudiment. Quirk and Hamilton (1973) ruled out its application to the germ cells of *Oryzias latipes,* in which the males have the odd sex chromosome but have neither earlier nor more ample germ-cell mitoses, but it still might apply to the somatic component of the gonad. Another temporal aspect of the genetic control of sex in fishes is the unequivocal demonstration (Kallman *et al.*, 1973) of a pair of sex-linked alleles in the platyfish that control the age at which males become sexually mature by controlling the age at which gonadotrops

differentiate in the pituitary. Males homozygous for early differentiation mature in 10-16 weeks, those homozygous for late differentiation, in 22-40 weeks. The heterozygotes are intermediate.

The whole subject of sex determination and differentiation in fishes has been profoundly affected by the recently expanded knowledge of hermaphroditic fishes, which have proved to be far commoner than formerly suspected and to exhibit several different modes of hermaphroditism that undoubtedly are genetically-prescribed. Hermaphroditism is not a subject of this report. Nevertheless some terms applicable to sex determination or differentiation have become ambiguous in their usage for gonochoristic versus hermaphroditic fishes and need clarification. Such a clarification was attempted by the present writer (Harrington, 1971, especially pp. 412-428) without coining any new terms, and need not be repeated here. The following sources (and references therein) provide up-to-date reviews and critiques of the present status of hermaphroditism in fishes, *viz.* Atz, 1964, Chan, 1970, Reinboth, 1970, Harrington, 1971.

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ENVIRONMENTAL AND ENDOCRINE CONTROL OF TELEOST REPRODUCTION

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Abstract

Both environmental and hormonal factors are extremely important in regulating reproductive cycling in teleosts. Various central mechanisms translate environmental cues into chemical messengers which function to activate and maintain the reproductive organs. In this regard, the functional relationship between the hypothalamus and pituitary gland is important, and the pineal gland plays a possible role in regulating sexual maturation. The pituitary has a central role in controlling gonadal activity, and there are recent developments concerning identification, morphological characteristics and functional changes in pituitary gonadotropic cells. Recent research on purification of teleost pituitary gonadotropic hormones is important in implicating these hormones in gametogenesis and gonadal sex steroid production. The sex steroids also play a possible role in gametogenesis and are involved in development of secondary sexual characters. Other endocrine glands and hormones have also been implicated in influencing gonadal function.

Introduction

The survival of any species in a seasonally unstable environment is dependent on the development of mechanisms that permit it to adjust physiological functions to changes in that environment. Successful reproduction is an essential factor for species survival, and in most animals breeding periods are adjusted in time so that they are suitable for rearing offspring. In most teleost species the process of spawning is limited to a relatively brief time span (Breder and Rosen, 1966). Gonadal development (recrudescence), however, is a complicated physiological process requiring some time. These internal physiological processes prepare animals for spawning at the most appropriate time.

Baker (1938) suggested that animal breeding cycles are subject to *ultimate* and *proximate* control mechanisms. The *ultimate* determination of a reproductive cycle depends on progeny survival; thus, natural selection favors the gene complexes of individuals producing their young during the most favorable season. Generally, the *ultimate* control of breeding seasons can be accounted for in terms of available food supply. Many teleosts have evolved response mechanisms to various environmental stimuli, the *proximate* factors, which function as advance signals of an approaching season suitable for reproduction.

These environmental synchronizers function to coordinate endogenous events leading to reproduction while *ultimate* factors provide the cause.

Annual cycles of temperature and daylength are characteristic of the temperate latitudes. In the tropics relatively dry seasons often alternate with rainy seasons which can change aquatic environments. Changes in these physical factors frequently lead to changes in food availability. Due to the energy requirements of reproduction, especially ovarian maturation, it is essential that fishes integrate physiological function with these environmental cycles. The endocrine system serves as the link between the environment and reproductive organs. Reproduction thus depends on a series of coordinated events involving several endocrine organs which will be discussed below. Photoperiod and temperature are presumed to be the most important *proximate* factors influencing the neuroendocrine centers which control gonadotropin secretion in teleosts. De Vlaming (1972a) has recently reviewed the literature dealing with environmental control of teleost reproductive cycles. It was concluded that photoperiod may exert the dominant regulatory role in the salmonids and gasterosteids. Temperature may be extremely important in regulating sexual cycling in the cyprinodontiform fishes. Both photoperiod and temperature may be important controlling factors in the cyprinid and perciform fishes. Furthermore, de Vlaming (1972a) suggested that short term laboratory studies can lead to erroneous statements concerning the importance of exogenous factors in controlling annual reproductive cycles. Responsiveness to exogenous factors appears to depend on the gonadal condition of the beginning controls (effects vary with season). Considerable variation is seen in the reproductive timing mechanisms among the teleosts, and few generalizations can be made with regard to phylogenetic, geographic or ecological relationships because so few species have been studied.

Before discussing the recent studies dealing with environmental control of reproduction, a brief summary of cyclicty in teleost gonadal development seems appropriate.

THE OVARY

In most teleosts the ovary is a hollow organ (cystovarian) which is covered by a germinal epithelium, the mesovarium, which is an extension of the peritoneum. Internally, many branched connective tissue septa arise; these ovigerous folds or lamellae project into the lumen of the ovary. Germinal epithelium also covers these ovigerous lamellae. Oocytes are generated from oogonia which are located within the germinal epithelium. A thin connective tissue (i. e., the theca) usually envelopes the oogonium. In some species the theca may consist of an internal cell layer or theca interna and an external cell layer, the theca externa; in other teleosts the theca consists of only one cell layer (cf., Barr, 1968; Hoar, 1969). Inside the theca and surrounding the ooganium is the follicular epithelium or zona granulosa which usually con-

sists of a single layer of cells. There is some debate as to whether the granulosa cells migrate through the theca after oocyte development begins or whether it is present throughout development; although there may be variability as to when the granulosa develops, once formed these cells remain in close contact with the oocyte.

The transformation of oogonia into oocytes is referred to as oogenesis. Oogonia multipy in the ovary by mitotic division. Transformation of oogonia into primary oocytes is made when the oogonium enters the prophase of the first meiotic division. Completion of the first meiotic division yields a secondary oocyte and a polar body. The second meiotic division of the secondary oocyte results in the formation of the definitive ovum. The two meiotic divisions normally occur outside the ovary in teleosts. Within the ovary, however, the primary oocyte undergoes the process of yolk accumulation or vitellogenesis.

Although not common among the teleosts, in some species oocytes in the ovary develop synchronously. Many teleosts in the temperate latitudes are characterized by group synchrony of oocytes. In this case two groups of oocytes can be distinguished in the developing ovary—one group consists of large yolky oocytes and the other of small yolkless oocytes. Species which are characterized by this type of ovarian maturation usually spawn once during a year over a relatively short period. Yet in other teleost species oocyte development is asynchronous and oocytes in various stages of maturity are present in the ovary. Fishes with this type of development characteristically have prolonged breeding seasons and individuals spawn more than once each year.

In annually spawning oviparous teleosts ovarian development consists of several phases. The first phase is a mitotic increase in the number of oogonia. In some species oogonial proliferation continues throughout the year, in other species oogonial mitoses are restricted to the spawning season, and in yet other species mitotic division of oogonia occurs mainly in the postspawning season (c.f., Barr, 1968). Oogonia transform into primary oocytes, as mentioned above, and enter into a primary growth phase in which the cytoplasm increases in size. The secondary growth phase of the primary oocyte is characterized by yolk accumulation. Yolk vesicles begin to appear in the periphery of the ooplasm early in this phase and when this phase is completed the oocyte is filled with yolk globules. At the end of the secondary growth phase, mature oocytes may be ovulated almost immediately or may remain within the follicle for some time depending on the species. The time of ovulation and oviposition also varies from species to species. In some species ovulation and oviposition occur essentially at the same time, whereas in other fishes mature oocytes are first ovulated into the ovarian cavity or oviduct with oviposition occurring at a later time.

In many species of teleosts "corpus luteum-like" structures are formed in the ovary from cells of the follicle after invasion and reabsorption of degenerating oocytes. These structures, which are more properly termed corpora

atretica are formed at two different stages in the ovarian cycle—from primary oocytes at any time during the secondary growth phase or from unovulated mature oocytes which remain in the ovary after spawning (c.f., Barr, 1968). The cells which invade the oocyte may have different origins in various teleosts, but thecal and granulosa cells are probably involved as well as phagocytic cells derived from the ovarian stroma. During follicular atresia in *Gillichthys mirabilis* the ooplasm takes on a lumpy appearance and the nucleus shows signs of degeneration (de Vlaming, 1972b). The zona pellucida is thrown into folds and sinks into the decomposing ooplasm. The granulosa hypertrophies and invades the area formerly occupied by the ooplasm. In the later stages of atresia the entire follicle, or a large portion of its interior, becomes a solid mass of cells which appear to stem from the granulosa; these corpora atretica are yellowish-green and become vascularized.

Thus, the corpora atretica in *Gillichthys,* as well as in various other teleost species (c.f., Barr, 1968), resemble the mammalian corpus luteum. One must take care, however, not to assume that these "corpus luteum-like" structures have an endocrine function solely on the basis of their appearance; they may, in fact, be related only to reabsorption. Application of a histochemical technique for the demonstration of 3β-hydroxysteroid dehydrogenase (3β-HSDH), an enzyme involved in steroid hormone synthesis, to the ovaries of *Scomber scomber* and *Poecilia reticulata* failed to reveal any activity in corpora atretica (Bara, 1960, 1965a, b; Lambert and van Oordt, 1965; Lambert, 1966, 1970a,b; Blanc-Livni *et al.*, 1969; Yaron, 1971).

In a majority of the oviparous teleosts examined the post-ovulatory follicle does not become reorganized into a "corpus luteum-like" structure, but rather collapses and is rapidly absorbed. In some species, however, the post-ovulatory follicle does develop into a glandular appearing structure (c.f., Barr, 1968). Bara (1965a) in *Scomber* and Lambert *et al.* (1972) in *Branchiodanio rerio* reported 3β-HSDH activity in post-ovulatory follicles and suggested that they may be involved in steriod hormone production.

Ovariectomy and replacement therapy with various sex steroids indicate that the teleost ovary possesses endocrine functions (Hoar, 1957, 1969; Pickford and Atz, 1957; Barr, 1968; Liley, 1969, 1972; Reinboth, 1972). For many years it has been known that ovarian extracts from fish contain estrogenic substances as determined in mammalian bioassay systems. The effects of exogenous sex steriods (mammalian and synthetic) on sex accessory organs, secondary sex characters and sexual behavior in female teleosts have been examined many times. The results of these experiments combined with the effects of ovariectomy leave little doubt that development of secondary sex characters and sexual behavior is controlled by gonadal steroids. The reader is referred to the reviews of Pickford and Atz (1957), Bern and Nandi (1964), Hoar (1957, 1965), Ball (1960), Nandi (1967), Baggerman (1968), Barr (1968),

Liley (1969, 1972) and Reinboth (1972) for a summary of this voluminous literature.

In higher vertebrates estradiol-17β is presumed to be the most active estrogenic steriod. Estradiol-17β, esterone and estriol have been isolated and quantified from the ovaries of several teleosts (Galzigna, 1961; Gottfried *et al.*, 1962; Lupo and Chieffi, 1963a, 1965; Penso and Galzigna, 1963; Botticelli and Hisaw, 1964; Chieffi, 1966; Eleftheriou *et al.*, 1966; Katz *et al.*, 1971. Estradiol-17β and esterone have also been quantified in the plasma of some teleosts (Cèdard *et al.*, 1961; Cèdard and Nomura, 1961; Eleftheriou *et al.*, 1966). Using labelled precursors with ovarian tissue *in vitro* several investigators (Chan and Phillips, 1969; Lambert and van Oordt, 1969; Eckstein, 1970; Eckstein and Eylath, 1970; Eckstein and Katz, 1971; Lambert *et al.*, 1971) have shown the capacity of the teleost ovary to synthesize estrogens. Estrogen biosynthetic pathways in teleosts have recently been discussed in detail (Ozon, 1972b).

Very little data is available on the site(s) of estrogen synthesis in the teleost ovary. In *Poecilia reticulatua* and *Xiphophorus helleri* 3β-HSDH activity is confined to the granulosa cells (Lambert and van Oordt, 1965; Lambert, 1966, 1970a, b). The same holds true for *Mugil capito* (Blanc-Livni *et al.*, 1969). Lambert *et al.* (1972) reported 3β-HSDH activity in the granulosa cells and in the post-ovulatory "corpus luteum" of *Branchiodanio rerio* whereas Yamamoto and Onozato (1968) suggested that in the same species 3β-HSDH activity is localized in the thecal cells and absent in the granulosa cells. 3β-HSDH activity in the ovary of *Scomber scomber* is presumably restricted to the thecal cells (Bara 1965). In the ovaries of *Tilapia nilotica* and *Acanthobrama terrae*, 3β-HSDH activity is found in both the theca and granulosa (Yaron, 1966, 1971).

THE TESTIS

Testicular structure in the teleosts is more variable than in any other group of vertebrates. In most teleosts the testes are paired elongated structures attached to the dorsal body wall. The testes in some teleost species consist of an extensive system of branching seminiferous tubules embedded in a connective tissue stroma, while in other species this organ is made of simple thin-walled lobules. Tubules or lobules open into the sperm duct which leads to the urogenital papilla. The testes in a majority of teleosts lack a permanent seminiferous epithelium lining. Germ cells proliferate in coordinated clusters enclosed in membranous cysts. Primary spermatogonia lie singularly or in small groups at scattered sites along the tubule or lobule walls. These are stem cells which, in most teleosts, are present during all seasons; clones of secondary spermatogonia arise from these stem cells by mitoses. Secondary spermatognia divide synchronously increasing the cell population within the cyst. These then transform all at the same time into primary spermatocytes. Meiotic division

of primary spermatocytes produces secondary spermatocytes and spermatids all within the cyst membrane. Spermatids undergo a metamorphosis into spermatozoa (spermiogenesis), the cyst expands and ruptures, liberating the gametic contents into the tubule lumina. Prior to spawning the lobules or tubules become packed with sperm. The testes usually become hydrated at the time of spawning and sperm is ejected into the sperm duct (spermiation).

In most north temperate zone cyprinoid fishes the males overwinter with only spermatogonia or primary spermatocytes in the testes. In contrast to the cyprinoids, spermatogenesis in the percoids is normally completed in fall, and winter is passed with spermatids or spermatozoa already present in the gonads (Nikolsky, 1963).

In teleosts it has been established by experiments involving injections of testicular extracts and castration followed by replacement therapy that the testes are a source of androgenic sex hormones upon which the state of development and functional activity of the secondary sexual characteristics and sexual behavior depend (c.f, Hoar, 1957, 1965; Pickford and Atz, 1957; Dodd, 1960; Bern and Nandi, 1964; Baggerman, 1968; Liley, 1969, 1972).

Testosterone, androstenedione and dehydroepiandrosterone have been extracted and quantified in the testes of several telosts (*Salmo gairdneri* and *Cyprinus carpio*-Galzigna, 1961; *Morone labrax*-Chieffi, 1962; *Serranus scriba*-Lupo di Prisco and Chieffi, 1965; *Gasterosteus aculeatus*-van Mullem and Gottfried, 1966; Gottfried and van Mullem, 1967; *Mugil cephalus*-Eckstein and Eyelath, 1968). Incubation of testicular homogenates or pieces from a number of different teleosts with various steroid precursors frequently yields testosterone and androstenedione (Arai *et al.*, 1964; Eckstein and Eyelath, 1968; Chan and Phillips, 1969; Colombo *et al.*, 1970, 1972a, b; Lupo di Prisco *et al.*, 1970).

Using a competitive protein-binding assay (Schreck *et al.*, 1972a), a direct relationship of plasma androgen concentration to gonadal development has been observed in *Salmo gairdneri;* furthermore, plasma androgen levels were not found to vary diurnally (Schreck *et al.*, 1972b).

Androgens in the salmonids may differ from those in other vertebrate groups in that testosterone may have a hydroxyl or keto group at the 11 position in the steroid nucleus. Idler *et al.* (1960, 1961a, b, 1964; Schmidt and Idler, 1962) were the first to identify 11-ketotestosterone as a natural product and subsequently show its androgenic activity in stimulating secondary sex characters in salmon. *In vitro* studies with testicular homogenates of *Salmo gairdneri* yielded 11-ketotestosterone and 11β-hydroxytestosterone (Idler and MacNab, 1967; Idler *et al.*, 1968). Testosterone has also been isolated and quantified in the testes (Idler and Tsuyuki, 1959) and plasma of *O. nerka* (Grajcer and Idler, 1961, 1963). The biosynthetic pathways and metabolism of teleost androgenic steroids has recently been reviewed (Ozon, 1972a).

The site of androgen synthesis in the teleost testis is somewhat debatable.

Two distinct types of ararngement of sex steroid producing cells in the teleost testis have been described (Marshall and Lofts, 1956; Lofts, 1968). The first is the typical vertebrate interstitial gland (Leydig cells) characteristically composed of modified connective tissue cells which occupy the interstices between seminiferous tubules or lobules. Using the 3β-HSDH technique it has been suggested that in several species of teleosts the interstitial cells are the site of androgen production (Della Corte *et al.*, 1961; Lupo di Prisco and Chieffi, 1963b; Stanley *et al.*, 1965; Bara, 1966, 1969; Delrio *et al.*, 1967). There is a direct correlation between histological signs of activity in interstitial tissue and androgens extractable from the testes in *Gasterosteus aculeatus* (Gottfried and van Mullem, 1967). Ultrastructural examination of the interstitial cells of some teleosts have shown them to have the same characteristic features of abundant agranular or smooth endoplasmic reticulum and tubular mitochrondrial cristae which typify steriod producing cells (*Gasterosteus aculeatus* and *Poecilia reticulata*-Follenius and Porte, 1960; Follenius, 1964, 1968a; *Salvelinus fontinalis*-Oota and Yamamoto, 1966; *Trichogaster leeri*-Horstmann and Breucker, 1972). Delrio *et al.* (1965) were unable to demonstrate 3β-HSDH activity in the interstitial tissue of several teleosts. Ultrastructural evidence suggests that the interstitial cells in *Oryzias latipes* are steriodogenic, but there is an absence of 3β-HSDH activity in these cells (Gresik *et al.*, 1973). This latter study indicates that caution must be applied in interpretation of histochemical studies.

In the second type of arrangement, the Leydig cells arise not in the interstices, but in the lobule or tubule walls and have been named lobule-boundry cells. In *Belone belone* the lobule-boundry cells bear histochemical features of steroid secretory cells whereas the interstitial tissue did not (Upadhyay and Gurara, 1971). O'Halloran and Idler (1970) showed that 3β-HSDH and Δ 5-3β-ol-dehydrogenase are located in the lobule-boundry cells in the testes of *Salmo salar*. The testes of *Tilapia mossambica* and *Ciclasoma nigrofasciatum* contain both lobule-boundry cells and interstitial Leydig cells. In *Tilapia* 3β-HSDH activity is found in both interstitial and lobule-boundry cells (Yaron, 1966) and in *Ciclasoma* both have ultrastructural characteristics of steroid secretory cells (Nicholls and Graham, 1972).

Sertoli cells are prominent features of the testis in some teleosts (Lofts, 1968, 1972; Hoar, 1969). The spermatogenetic cyst is bounded and supported by the connective tissue Sertoli cell. Functionally, the Sertoli cell may be nutritive, contractile, supportive and/or a steroid producing endocrine cell (cf., Lofts, 1968, 1972; Lofts and Bern, 1972). The Sertoli cells take on a glandular appearance as the testicular germinal content matures in *Fundulus* (Lofts *et al.*, 1966; Bara, 1969). Sertoli cells also give a strong 3β-HSDH reaction in *Cymatogaster aggregata* (Wiebe, 1969a). Ultrastructural evidence also suggests that the Sertoli cells in *Poecilia reticulata* may be steroidogenic (Billard, 1970). Stanley *et al.* (1965) suggested that lobule-boundry cells in *Gobius*

paganellus may actually be Sertoli cell homologues and that the reports of activity cycles in these cells may be explained by assuming they have a nutritive role. Nicholls and Graham (1972) presented ultrastructural evidence of an association of developing spermatids with lobule-boundry cells in *Cichlasoma* suggesting that they may indeed be homologous with Sertoli cells.

ENVIRONMENTAL CONTROL OF REPRODUCTIVE CYCLES

Previous investigations (see de Vlaming, 1972a for review) suggest that changes in daylength are important in regualting sexual cycling in the stickleback family, Gasterostidae. Recently, Baggerman (1972) reported that *Gasterosteus aculeatus* obtained in November, December or January and exposed to a short photoperiod (8L/16D) at 20° C do not reach sexual maturity wthin 65 days as determined by nest building in males and oviposition in females. Maintaining fish, collected during the same months, on a long photoperiod of (16L/8D) at 20° C promoted sexual maturation. If sticklebacks were exposed to a short photoperiod at 20° C during February, March or April essentially all fish matured. These data imply that responsiveness to photoperiod in this species varies with season and/or the condition of the hypothalmo-hypophyseal-gonadal axis at the onset of photoperiod treatment. Whether a long photoperiod at a low temperature would have been effective during the winter months was not considered. Histological criteria were not used to assess gonadal development in this study so we do not know whether a short photoperiod can accelerate the early phases of gametogenesis. Since fish experience low temperatures and short photoperiods in nature during fall and winter it would be interesting to know the effects of these factors on reproductive processes. Craig-Bennett (1931), Ahsan and Hoar (1963) and Schneider (1969), in fact, showed that spermatogenesis in *Gasterosteus* begins in late summer and is completed before January. In the experiments of Schneider (1969) sticklebacks were expoesd to both long and short photoperiods at either warm or low temperatures during the winter. At low temperatures gonadal development occurred at both photoperiods, but at a slow rate. Long photoperiod in combination with a warm temperature stimulated rapid sexual maturation, whereas a short photoperiod-warm temperature regime caused cessation of gametogenesis.

Baggerman (1972) also found that sticklebacks collected in June or July could not be brought to maturity by exposing them to a 16L/8D photo-regime at 20° C. These data suggest a refractoryness to these conditions at this time of year. Whether other photoperiod and temperature regimes would have resulted in gonadal development during this period was not examined. Possibly these fish require a "preconditioning" with low temperatures and/or a short photoperiod before they are capable of responding to a long photoperiod and warm temperatures.

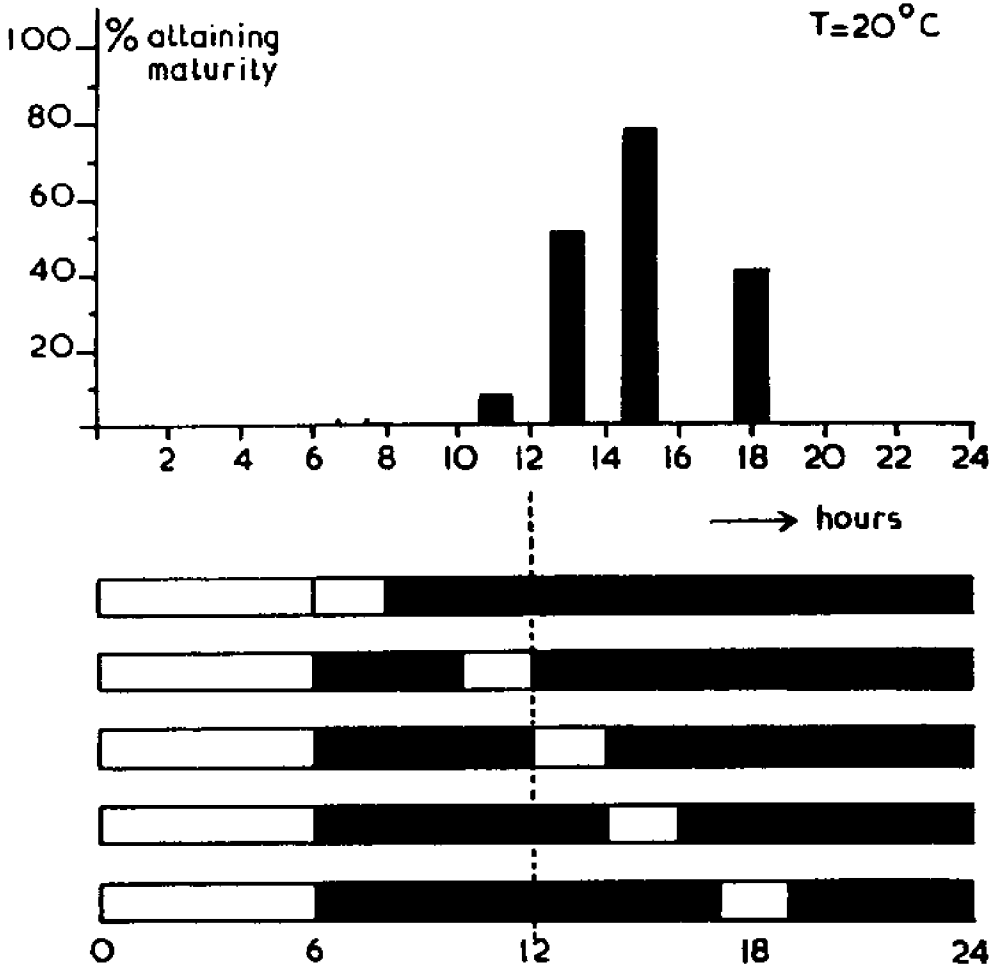

Figure 1. Percentage of sticklebacks attaining maturity when exposed daily to six hours of light followed by an additional two hours given at various times in the ensuing dark period (Reproduced by permission of Academic Press and Dr. B. Baggerman).

Considerable evidence is available which maintains that a circadian rhythm in sensitivity to light is the basis of gonadal responsiveness to photoperiods of differing lengths in various animal groups. Baggerman (1969, 1972) has demonstrated that such a rhythm may explain the photosexual response in *Gasterosteus*. In her experiments, Baggerman acclimated animals collected in fall to a short photoperiod (8L/16D). Experimental animals were then exposed to an initial period of six hours of light plus an additional two hours of light at various times in the ensuing dark period (Fig. 1). Sexual maturation was maximal in the groups of animals given the two hours of light between

14 and 16 hours after the onset of the six hour light period. These data imply that a long photoperiod per *se is* not required to induce gonadal matuation, rather animals are photosexually sensitive at some time after the onset of the light period (dawn). Light at dawn is apparently needed to activate the rhythm. The earlier work of van den Eeckhoudt (1946) lends support to Baggerman's (1972) theory.

McInery and Evans (1970), in an attempt to define the action spectrum of the photosexual response in *G. aculeatus,* exposed groups of fish to four discrete segments of the visible spectrum during the gonadal preparatory period. The rate of gonadal maturation in these four groups was compared with wild fish and fish maintained under normal laboratory illumination. These investigators observed that gonadal development occurred at approximately the same rate in all groups and concluded that the action spectrum of the photoperiod mechanism controlling sexual maturation in *Gasterosteus* is rather broad. Adequate controls were not, however, included in this experiment; more specifically, gonadal maturation could have occurred under any conditions. Since no animals were maintained in total darkness or on a short photoperiod to prove that sexual maturation could be retarded under some conditions, one must use caution in interpretation of these data.

Sundararaj and Sehgal (1970a) collected groups of the Asian catfish, *Heteropneustes fossilis* (family Clariidae) in the postspawning season; one group was maintained on a 12L/12D photoperiod, whereas another group was initially exposed to a 12L/12D photo-regime which was decreased to 9L/15D and then increased to 14L/10D. The temperature condition for both groups was 25° C. Sehgal and Sundararaj (1970a) previously found that 14L/10D photoperiod treatment during the postspawning season would not initiate ovarian recrudescence. The decreasing followed by an increasing daylength was, however, effective in stimulating ovarian recrudescence and in increasing the number and size of pituitary basophils (gonadotropic cells?). These authors concluded that *Heteropneustes* is refractory to long photoperiod stimulation of oogenesis unless first exposed to a decreasing or short photoperiod. Whether photoperiodism in this species is temperature dependent was not discussed. In other experiments Sehgal and Sundararaj (1970b) and Sundararaj and Sehgal (1970b) obtained catfish in the prespawning season. Groups of intact fish were maintained on a short photoperiod, on a long photoperiod and in total darkness. Groups of blinded fish were exposed to a short photoperiod or total darkness. All groups were maintained at 25° C. The rate of ovarian recrudescence was more rapid in fish with intact eyes, but neither blinding nor total darkness prevented gonadal maturation. This experiment was repeated in the postspawning season with essentially the same results. These authors concluded that although the eyes are important in the photosexual response of *H. fossilis,* they are not indispensable, and they further suggested that ovarian recrudescence is dependent on an endogenous rhythm. Before this hypothesis

can be accepted, these experiments must be repeated at temperatures equivalent to winter conditions as recrudescence in all of these experiments could be a function of warm temperatures. Furthermore, light reception could occur by some extra-retinal means. Recent data obtained in Sundararaj's laboratory (personal communication) suggest that the photosexual response in *Heteropneustes* is based on a circadian rhythm of sensitivity to light and that photoperiodism in this species is indeed temperature dependent.

Investigations have recently been conducted with the Centrarchid, *Lepomis cyanellus* (Kaya and Hasler, 1972). Fish were collected during the gonadal quiescent phase (winter). Groups of animals were exposed to a 16L/8D photoperiod at 5°, 15° or 20° C; other groups were maintained at 24° C on photo-regimes of 5L/19D, 10L/14D or 15L/9D. Gonadal recrudescence was only initiated in thet 20° C-16L/8D and 24°-15L/9D groups. Apparently a combination of warm temperatures and a long photoperiod are required to activate gonadal development in *Lepomis*. The effects of a low temperature-short photoperiod combination were not examined. In a follow up experiment Kaya (1973) exposed groups of *Lepomis* obtained during the spawning season to a low temperature (10.5° C) and either a 4L/20D or 15L/9D photoperiod. Other fish were maintained at 24° C on a 4L/20D or 15L/9D photoperiod. The rate of gonadal regression was more rapid at 24° C, but was not affected by photoperiod. Low temperatures did not prevent gonadal regression, so this researcher concluded that this species cannot be maintained in the spawning condition by manipulation of environmental factors.

Haydock (1971) exopsed immature Gulf croakers, *Bairdella icistia* (family Sciaenidae), collected in October to a 16L/8D photoperiod at 22° C or to a 8L/16D regime at 14° C. Fish in the latter groups matured rapidly, whereas those in the former did not. Whether photoperiod or temperature was the important factor cannot be determined. Mature female croakers collected in the postspawning season (November) and maintained on a 15L/9D light regime at temperatures between 14° and 16° C matured rapidly. These data imply that long photoperiods can stimulate ovarian maturation, but are not conclusive since control groups were not included. Female fish captured in the spawning season quickly reabsorbed their ovaries when held on a short photoperiod at either 14° or 22° C; ovarian regression was slower in animals on a long photoperiod. Interestingly, Haydock noted that male croakers matured under all conditions used in the laboratory suggesting sexual variation in response to environmental factors in this species. Sexual differences in responsiveness to environmental factors have been observed in other teleosts (cf., de Vlaming, 1972a, c).

Recently investigations were undertaken during the different phases of the sexual cycle of the longjaw goby, *Gillichthys mirabilis*, to examine the effects of various photoperiod and constant temperature regimes on reproductive function (de Vlaming, 1972c). In one experiment fish were obtained during the

gonadal regression phase (July); groups of animals were exposed to a 8L/16D photoperiod at 13°, 20° and 27° C. Other fish from this sample were maintained on a 15L/9D photo-regime at 13°, 20° and 27° C. The ovaries and testes of all fish in the 20° and 27° C groups remained regressed regardless of photoperiod treatment. Gonadal recrudescence occurred in both photoperiod groups at 13° C suggesting that low temperatures promote sexual maturation in this species. Recrudescence was more rapid in the short photoperiod group, but gonadal maturation also occurred in the long photoperiod group. The effect of photoperiod in *Gillichthys* thus appears to be temperature dependent. During the postspawning season (Aug.-Sept.) 20° C treatment with light periods of 8, 12 or 15 hours initiates ovarian and testicular recrudescence in *Gillichthys* (de Vlaming, 1972c). In another experiment *Gillichthys* were collected during the spawning season (May) and exposed to a 8L/16D or 15L/9D photoperiod; at each photoperiod one group of fish was maintained at 13° C and another group at 27° C. Active gametogenesis was maintained in both photoperiod groups at 13° C, whereas 27° C treatment caused gonadal regression. Several other experiments initiated at many different times of the year showed that temperatures between 24° and 32° C always cause ovarian and testicular regression in *Gillichthys*. Gobies obtained at the end of the gonadal preparatory phase (January) were placed on a 15L/9D or 8L/16D photoperiod; at each photoperiod, groups of fish were maintained at 13°, 20° or 27° C. Whether on a long or short photoperiod active spermatogenesis was maintained in all fish in the 13° and 20° C groups; testicular regression occurred at 27° C. Both 10° and 20° C treatment on a 10L/14D photo-regime prevented gonadal regression at the normal time in fish obtained at the end of the spawning season. Combined these data show that low temperatures stimulate gonadal recrudescence and maintain active gametogenesis in *Gillichthys,* whereas higher temperatures provoke gonadal regression. The effects of photoperiod on reproductive cycling in this species appear to be less pronounced.

Photoperiod effects on reproduction function in *Gillichthys* were more evident in other of de Vlaming's (1972c) experiments. During the postspawning season (September) a short, but not a long, photoperiod initiates testicular recrudescence at 20° C. Short, but not long, photoperiods maintain active spermatogenesis at 22° C during the gonadal preparatory season in *Gillichthys*. On the contrary, a long, but not a short, photoperiod maintains active spermatogenesis at 22° C during the spawning season.

The experiments (de Vlaming, 1972c) discussed above dealt only with constant temperatures and such thermal conditions are not realistic from an ecological standpoint since the population of *Gillichthys* studied occurs in very shallow saline ponds. This population of *Gillichthys* experiences diurnal fluctuations in temperature as great as 6°-7° C throughout much of the year (de Vlaming, 1972d). De Vlaming (1972d) examined the effects of diurnal thermoperiod treatments (i.e., thermocycling) on reproductive function in *Gil-*

lichthys. Figure 2 shows the thermocycle regimes used in these experiments. Fish in the spawning condition were exposed to a thermocycle with a maximum of 29° C (Fig. 2-I) and a minimum temperature of 13° C; the length of exposure to 29° C was 1.5 hours per day. This condition caused testicular regression. To determine the effects of different lengths of exposure to thermocycles with a maximum of 27° C, experiments were initiated in the gonadal preparatory period (November), during the regression phase (July), in the postspawning season (September) and at the end of the spawning period (June). Fish were exposed to 27° C for either two hours (Fig. 2-III) or nine hours per day (Fig. 2-II). The 2 hour-27° C treatment maintained active gametogenesis in the preparatory and spawning seasons, whereas the 9 hour-27° C treatment initiated gonadal regression. During the gonadal regression and postspawning periods neither thermoperiod regime initiated gonadal recrudescence. The data from the July and September experiments show that gonadal recrudescence will not occur in *Gillichthys* if they are exposed for two or more hours to 27° C.

To determine how an increase in the lower temperature of the cycle would influence the gonadal response in *Gillichthys,* de Vlaming (1972d) initiated an experiment at the end of the preparatory period (December) in which fish were exposed to a maximum of 27° C for three hours per day and the low temperature of the thermocycle was 16° C (Fig. 2-V). This thermoperiod regime maintained testicular activity, but initiated ovarian regression. Thermocycles with the same minimum temperature (16° C), but with 6 hours (Fig. 2-IV) or 7.5 hour (Gig. 2-VI) exposures to 27° or 25° C, respectively, initiate gonadal regression in *Gillichthys* during the early spawning season.

An experiment at 24° C, a temperature which, at a constant level, is the threshold for gonadal regression (de Vlaming, 1972c) was begun in the spawning season (April) to determine whether 24 hour exposures to this temperature are necessary to induce gonadal involution (de Vlaming, 1972d). Groups of *Gillichthys* were exposed to a maximum of 24° C for 5 (Fig. 2-IX), 8 (Fig. 2-VIII) or 13.5 hours per day (Fig. 2-VII); the minimum temperature of thermocycles was 15° or 15.5° C. Both the 8 and 13.5 hour treatments at 24° C caused gonadal regression whereas the 5 hour exposure maintained the gonads in an active state.

De Vlaming (1972d) concluded from these experiments that exposure to a temperature of 24° C for 8 hours a day is sufficient to induce gonadal regression in *Gillichthys;* shorter periods of heat treatment at temperatures above 24° C also cause gonadal involution. The gonadal response to thermoperiod does not depend on the average daily temperature or the total heat dose, but rather the length of time spent at or above 24° C. Furthermore, longer exposures to a given high temperature are required to cause gonadal regression than are required to inhibit recrudescence.

The studies discussed above (de Vlaming, 1972c, d) favor the view that

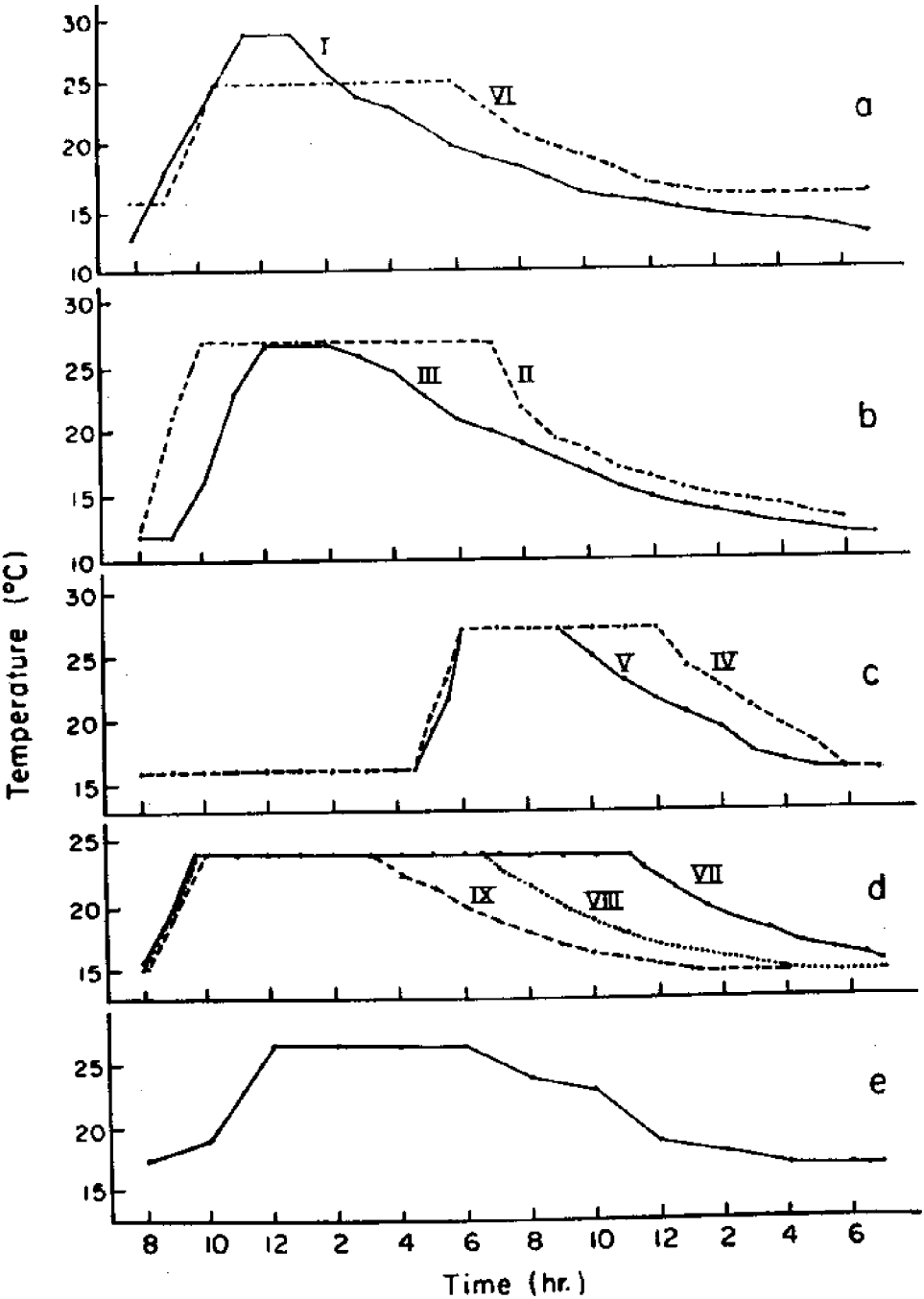

Figure 2. Diurnal thermocycle regimes (a, b, c and d) used in experiments (de Vlaming, 1972d). Diurnal temperature changes (e) in natural habitat of *Gillichthys* on 10 July 1969.

reproductive cycling in *G. mirabilis* is primarily regulated by seasonal temperature variations; in particular, gonadal regression appears to be a function of increasing summer temperatures, whereas recrudescence depends on low temperatures of fall and winter. Since gonadal regression in the population studied occurs at a time when productivity is decreasing and salinity is increasing in the estuarine habitat of this species, investigations were conducted to examine the effects of decreasing food availability and increasing salinity on gonadal involution (de Vlaming, 1971).

Inanition for 23 days at a temperature which normally maintains gametogenesis initiates gonadal regression in *Gillichthys* at the beginning (January) of the spawning season (de Vlaming, 1971). During the spawning season (May), however, starvation does not accelerate the rate of gonadal regression which occurs at 27° C. De Vlaming (1971) also noted that inanition failed to block the initiation of gonadal recrudescence of *Gillichthys* obtained in the postspawning season at either 16° or 20° C. High salinity was not effective in causing gonadal regression in *Gillichthys* and although the rate of recrudescence was reduced by high salinity, gonadal deevlopment was not inhibited. Thus, the regression phase of the reproductive cycle of *G. mirabilis* may be due to increasing summer temperatures combined with decreasing food availability in their environment. A summary of the effects of environmental factors on reproductive function in *Gillichthys* is presented in Table 1.

Ovarian maturation in *Cyprinodon rubrofluviatilis* (family Cyprinodontidae) collected in December and maintained under various photoperiod and temperature regimes was accelerated by low temperatures (Echelle *et al.*, 1973). Photoperiod apparently has little effect during this phase of ovarian development. Whether warmer temperatures or photoperiod influences the final maturation phases and spawning was not examined. In another cyprinodontid *Fundulus heteroclitus*, warm temperatures accelerate spermatoogonial proliferation and seem to be necessary for spermiation (Pickford *et al.*, 1972). A summary of the effects of temperature and photoperiod on gonadal cycling in teleosts is presented in Table 2.

The potential role of environmental factors in regulating reproductive functions in tropical fishes has received very little attention. Near the equator daylength is approximately 12 hours throughout the year and annual temperature fluctuations are very small. Many tropical areas, however, are characterized by distinct seasons of rainy and dry periods. Recently, Munro *et al.* (1973) examined on a monthly basis the gonadal condition of 35 species of Caribbean reef fishes around Jamaica (18° N) for over two years. Their data show that a majority of these reef fishes spawn throughout the year, but a peak in spawning is reached when water temperatures are minimal in February, March and April. Furthermore, the biomass of reef fish eggs in the plankton is approximately 12 times greater in the above mentioned period than in the period from June to December. Spawning in the groupers (Serranidae)

Table 1. The effects of environmental factors on gonadal function in *Gillichthys mirabilis* during different phases of the reproductive cycle.

SPAWNING PERIOD (January-June)	REGRESSION PERIOD (July)	POST-SPAWNING PERIOD (August-September)	PRE-PARATORY PERIOD October-December)
1. 13°-20° C maintains gametogenesis on L[1] & S[2] photoperiod	1. 13°-16° C initiates gonadal recrudescence on S & L	1. 10°-20° C initiates gonadal recrudescence on S & L	1. 22° C initiates testicular recrudescence on S photoperiod
2. 10°-20° C prevents gonadal regression on S & L photoperiod	2. S photoperiod accelerates gonadal recrudescence at 13° C	2. 21° S initiates gonadal recrudescence on S photoperiod	2. 22° C causes testicular regression on L photoperiod
3. 24°-32° C causes gonadal regression on S & L photoperiod	3. 20°-27° C prevents gonadal recrudescence on S & L	3. 21° C prevents gonadal recrudescence on L photoperiod	3. 25°-30° C causes gonadal regresson on S or L photoperiod
4. 22° C maintains spermatogenesis on L photoperiod	4. 2 hrs./day at 27° C prevents gonadal recrudescence	4. 2 hrs./day at 27° C inhibits gonadal recrudescence	
5. 22° C causes ovarian regression on L photoperiod	5. Inanition does not block gonadal recrudescence		
6. 22° C causes gonadal regression on S photoperiod			
7. 1.5 hrs./day at 29° C initiates testicular regression			
8. 6 hrs./day at 27° C initiates gonadal regression			
9. 2 hrs./day at 27° C maintains gametogenesis			
10. 9 hrs./day at 24° C initiates gonadal regression			
11. 5 hrs./day at 24° C maintains gametogenesis			
12. Inanition at 16° C initiates gonadal regression			

[1] L = long.
[2] S = short.

Table 2. A partial summary of the effects of environmental factors on reproductive function in teleost fishes.

Family	Effects of environmental factors
Salmonidae	(*Salvelinus fontinalis* and *Onchorhynchus nerka* Initiation of gonadal development independent of environmental factors. Short photoperiod accelerates final oocyte growth only if fish exposed to long photoperiod during early phases of secondary growth phase. Temperature independent.
Cyprinidae	(*Phoxinus laevis*) Warm temperatures in late fall accelerate completion of spermatogenesis. Warm temperatures stimulate early phases of spermatogenesis and oogenesis.
	(*Notropis bifrenatus*) Long photoperiod at warm temperature in fall accelerates gonadal maturation.
	(*Carassius auratus*) Long photoperiod at warm temperature in fall accelerates gonadal maturation.
	(*Couesius plumbeus*) Low temperatures promote meiotic phase of spermatogenesis and warm temperatures promote spermiation and spermatogonial proliferation.
Clariidae	(*Heteropneustes fossilis*) Long photoperiod and warm temperatures during preparatory period stimulate ovarian recrudescence. "Refractory" to photoperiod during the postspawning season.
Cyprinodontidae	(*Fundulus heteroclitus*) Low temperatures promote early phases and warm temperatures required for final stages of spermatogenesis. Long photoperiod-warm temperature regime prevents testicular regression at "normal" time. Warm temperatures promote spermatogonial proliferation and spermiation.
	(*Fundulus confluentus*) Low temperatures promote early phases and warm temperatures promote later phases of oogenesis.
	(*Cyprinodon rubrofluviatilis*) Low temperatures accelerate the early phases of vitellogenesis at any photoperiod in winter.
	(*Oryzias latipes*) Long photoperiod at warm temperature accelerates gametogenesis.
Poecilidae	(*Gambusia affinis*) Warm temperatures promote sexual maturation in winter.
Gasterostidae	(*Culaea inconstans*) Long photoperiod promotes gametogenesis, but is temperature dependent.
	(*Gasterosteus aculeatus*) Early phases of gonadal development occur at any temperature-photoperiod regime. The final phases of sexual maturation require a long photoyperiod. Warm temperatures accelerate gametogenesis if photoperiod correct. Animals "refractory" to long photoperiod-warm temperature stimulation in postspawning season.
Centrarchidae	(*Lepomis cyanellus*) Warm temperature-long photoperiod regimes initiate gonadal recrudescence in winter. High temperatures accelerate gonadal regression.
	(*Ennaecanthus obesus*) Long photoperiod at warm temperature causes complete testicular recrudescence in fall. Early phases of oogenesis stimulated by low temperature and short photoperiod. Later stages of oogenesis promoted by long photoperiod-warm temperature regime.
Embiotocidae	(*Cymatogaster aggregata*) Low temperatures promote testicular restitution. Long photoperiods stimulate spermatogenesis; warm temperatures accelerate spermatogenesis at long photoperiod. Ooocyte formation promoted by warm temperatures, while oocyte maturation fostered by low temperatures.

is virtually confined to the period between January to May. Spawning in the parrot fishes (Scaridae) and surgeon fishes (Acanthuridae) is mainly confined to the period between January and June with most spawning in February and March. The jacks (Carangidae) and the snappers (Lutjanidae) spawn throughout the year but more ripe females were collected in the coolest months. Some spawning also occurs in all months in the grunts (Pomadasyidae) but most spawn between February and April.

Species of *Tilapia* occurring at the equator in Lake Naivasha (Kenya) at 6230 feet have been studied by Hyder (1969a 1970). Males of *T. leucosticta* *T. nigra* and *T. zilli* in various stages of sexual maturity were encountered at all times during the year (Hyder, 1969a). Gonadal activity was lowest and no breeding was observed in female *T. leucosticta* during the coldest and driest months (Hyder, 1970). Ovarian development in this species occurs during the period of highest temperatures and maximal sustained sunlight. Spawning in *T. leucosticta* corresopnds to the period of highest temperatures and the onset of the rainy season. Although very interesting, it is difficult to segregate the regulating environmental factors in Hyder's studies.

In eight different teleost species representing six different families from the Murray-Darling River system of New South Wales warm temperatures and floods appear to act as the triggering mechanisms for final gonadal maturation and spawning (Lake, 1967). Lake also suggested that the factor(s) which stimulate these fish to spawn are produced when flood waters come into contact with dry soil. These data do not allow one to predict what environmental factors are involved in gonadal recrudescence, but Lake (1967) observed that during years when floods and high rivers are common throughout the winter and early spring gonadal development in these fish occurs earlier.

Evidence has been produced which shows a close relationship between flooding and spawning in several other tropical species (Khanna, 1958; David, 1959; Tang, 1963). The correlation of spawning with rainy seasons (or flooding) does not permit conclusions with regard to environmental control of gametogenesis. An intriguing question is how do these tropical fishes anticipate the rainy season and attain a state of gonadal readiness so that spawning can occur when the ideal environmental situation is realized. Is it possible that in tropical fishes an endogenous physical rhythm proceeds independently and maintains a prespawning condition for a long period or brings about gonadal maturation shortly before the onset of the rainy season?

The question now arises as to how changes in the environment control reproductive physiological processes in teleost fishes. Considerable evidence is available which shows that the endocrine system serves as a link between the environment and gonadal function. More specifically the hypothalamo-hypophyseal neuroendocrine axis is of ultimate importance in mediating the effects of environmental variations on sexual cycling.

THE ENDOCRINE CONTROL OF REPRODUCTION

An extensive literature has accumulated which deals with teleost reproductive endocrinology. Various aspects of this literature have been reviewed by several authors (Dodd, 1955, 1960, 1972; Hoar, 1955, 1957, 1965, 1966, 169; Pickford and Atz, 1957; Ball, 1960; Barr, 1965, 1968; Dodd and Wiebe, 1968; Lofts, 1968; Fontaine, 1969; Yamazaki, 1969; Reinboth, 1972; Donaldson, 1973).

THE PITUITARY GLAND

The central role of the adenohypophysis in regulating gonadal function in teleosts is well established (see below). The organization and morphology of the teleost pituitary has been the subject of much research (see reviews of, van Oordt, 1968; Ball and Baker, 1969; Sage and Bern, 1971; Rai, 1972). The pars distalis in teleosts is typically divided into rostral and proximal regions (pro- and meso-adenohypophysis). Gonadotropin secreting cells have usually been identified by their changes during the sexual cycle and are most frequently located in the proximal pars distalis (cf., van Oordt, 1968; Ball and Baker, 1969; Sage and Bern, 1969; Rai, 1972). Moreover, Ball and Baker (1969) have recently stated that the distribution of cell types within the teleost adenohypophysis is extremely regular so that to a large extent the topographical location of a cell type can support its identification on tinctorial grounds. Frequently then, gonadotropic cells can be distinguished on the basis of tinctorial features, position, cell size, granule size and other morphological features. In essentially all teleosts examined, gonadotropic cell secretory granules are basophilic and stain with periodic-acid-Schiff reagent (PAS), aldehyde fuchsin, alcian blue and aniline blue in the common trichrome and tetrachrome cytological techniques.

In a variety of teleost species a correlation between the proximal pars distalis basophils (gonadotropic cells) and the gonadal cycle can be observed, this cell type showing hyperplasia, hypertrophy and other signs of increased activity in association with the ripening of the gonads (Buchmann, 1940; Kerr, 1948; Rasquin, 1949; Scruggs, 1951, Stolk, 1951; Atz, 1953, Verhoeven and van Oordt, 1955; Beach, 1959; Honma, 1969; Matty and Matty, 1959; Sundararaj, 1959, 1960; Olivereau and Herlant, 1960; Stahl *et al.*, 1960; Sokol, 1961; Robertson and Wexler, 1962a, b; Stahl and Leray, 1962; Honma and Tamura, 1963, 1965; Rizkalla, 1963; Sathyanesan, 1963; Stahl, 1963; Olivereau and Ball, 1964; Schreibman, 1964; Lagios, 1965; Leray, 1965; Lehri, 1966, 1970; Rai, 1966a, b; Olivereau, 1961, 1962a, 1967; Blanc and Abraham, 1968; Honma and Suzuki, 1968; Marmorino *et al.*, 1969; Nagahama and Yamamoto, 1969; Tamura and Honma, 1969; Mattheij, 1970; Singh, 1970; Moisieva, 1971; Zambrano, 1971; Lambert *et al.*, 1972). Kallman *et al.* (1973) recently reported that a sex-linked gene controls the age at which gonadotrophs differentiate in *Xiphophorus maculatus*.

Various investigators, using both light and electron microscope techniques, have described two different gonadotropic cell types. Two gonadotrophs have been observed in the pituitaries of *Anguilla* (Olivereau and Herlant, 1960; Knowles and Vollrath, 1966a, b; Olivereau, 1961, 1967, 1968, 1969), *Mugil* (Leray and Carlon, 1963; Stahl, 1963; Leray, 1966; Olivereau, 1968), *Carassius* Olivereau, 1962b; Leray, 1965; Leatherland, 1972), *Oncorhynchus* (Olivereau and Ridgeway, 1962; Cook and van Overbeeke, 1972), *Caecobarbus* (Olivereau and Herlant, 1954), *Salmo* (Olivereau, 1954), *Tor* (Rai, 1966a, b) and *Zoarces* (Oztan, 1966a). Examination of the eel pituitary by electron microscopy indicates one gonadotroph (Type I) contains secretory granules 1,900 A in diameter whereas the second type (Type II) contains secretory granules 1,300 A in diameter (Knowles and Vollrath, 1966a, b).

Antisera to mammalian luteinizing hormone (LH), but not to mammalian follicle stimulating hormone (FSH), binds with only one basophilic cell type in the pituitary of *Oncorhynchus nerka* (McKeown and van Overbeeke, 1971). These data indicate that one of the salmon gonadotropins possesses structural similarities to mammalian LH, but does not rule out the possibility of a second gonadotropin which will not react with anti-LH. In fact, Cook and van Overbeeke (1972), in an electron microscope study of the pituitary of the same species (*O. nerka*), suggested the possibility of two gonadotropic cell types, one being active in the early phase of sexual maturation and the other in the final stages of gonadal development. In contrast, the electron microscopy investions of Nagahama and Yamamoto (1970) showed only one gonadotropic cell type in *O. keta*; however, two types of secretory granules were present in this cell-granules 500 to 3000 mμ in diameter and granules 300 to 500 mμ in diameter. The small granules are depleted at the time of ovulation, whereas the large granules disappear after spawning. Another point is worth noting here. Using light microscope techniques McBride and van Overbeeke (1969) concluded that there was only one gonadotroph in the pituitary of *O. nerka*, but electron microscopy revealed two (Cook and van Overbeeke, 1971). These findings suggest that caution should be used in interpreting light microscope studies since cellular changes may be too subtle to be observed at this level.

Only one gonadotropic cell type has been reported in *Gasterosteus* (Follenius, 1968b; Leatherland, 1970a), *Oncorchychus* (van Overbeeke and McBride, 1967; McBride and van Overbeeke, 1969), *Heteropneustes* (Sundararaj, 1959, 1960), *Clarias* (Dixit, 1970), *Bathygobius* (Stoll, 1957), *Gillichthys* (Zambrano, 1971), *Embiotoca* (Lagios, 1965), *Cymatogaster* (Leatherland, 1969), *Poecilia* (Ball and Baker, 1969; Sage and Bromage, 1970), *Anoptichthys* (Mattheij, 1970), *Serarnus* (Marmorino et al., 1969) and *Carassius* (Nagahama and Yamamoto, 1969). Only one gonadotropic cell has been identified in the proximal pars distalis of *Cyprinus carpio* using immunological techniques with antisera to a purified carp gonadotropin (Billard *et al.*, 1971a). If there are

two gonadotropins in carp, however, antisera to one would not necessarily cross react with the second.

A number of investigators have examined the seasonal variation in gonadotropin activity in the teleost pituitary (Gerbilskii and Kaschenko, 1937; Gerbilskii, 1940; Kazanskii, 1951, 1952; Fontaine and Chauvel, 1961; Clemens and Sneed, 1962; Clemens and Johnson, 1965). The data indicate that pituitary gonadotropin activity is low following spawning or during gonadal regression, but is relatively high during most other parts of the year. Swift and Pickford (1965) stated that maximal gonadotropic activity in the pituitary of *Perca fluviatilis* was highest just prior to spawning and lowest during the gonadal quiescent phase; similar observations have been reported in *Pleuronectes platessa* (Barr and Hobson, 1964) and *Mystus vittatus* (Singh, 1970). In the bream, *Abramis brama,* gonadotropin potency of the pituitary is as great in the spring as in the fall, but cytological investigations suggested that gonadotropic cells are most active in the spring (Gerbilskii, 1940). Vitellogenic potency of fish pituitaries decreases as vitellogenesis is completed, well before ovulation (Kazanskii, 1951, 1952), whereas the ovulation-inducing potency does not decline until after spawning (Gerbilskii, 1940). De Vlaming (1972e) reported that pituitaries obtained from *Gillichthys mirabilis* with regressing gonads were just as effective as pituitaries from fish undergoing testicular recrudescence in stimulating spermatogenesis in hypophysectomized fish of the same species. These assay data imply that there is little difference in gonadotropin content of pituitaries from *Gillichthys* with regressed or active gonads. In an electron microscope study Zambrano (1971) observed that the pituitary gonadotropic cells in *Gillichthys* with recrudescing gonads are large and show several characteristics of an actively secreting cell, including numerous secretory granules. In contrast, the gonadotropic cells of fish with regressing gonads were small and contained few secretory granules. Although there may be little difference in pituitary gonadotropin content in *Gillichthys* with regressing or active gonads, Zambrano's data (1971) suggest that there may be differences in the rates of hormone secretion.

A circadian pattern of pituitary gonadotropin release in teleosts has been proposed by O'Connor (1972). In *Salvelinus fontinalis* on a 10L/14D photoperiod pituitary gonadotropin activity decreased significantly shortly after the onset of the light period; pituitary gonadotropin activity remained high during the remainder of the 24 hour period. A similar rhythm was observed in the pituitary gonadotropin activity of *Salmo gairdneri.* O'Connor interpreted the decrease in pituitary gonadotropic activity as reflecting a release of hormone. Obviously the decrease in gonadotropic activity could have been due to a decrease in hormone synthesis. Interestingly, the rhythm of pituitary gonadotropin activity remained approximately the same in July for female *Salvelinus* on a 14L/10D photoperiod as for males in April on a 13L/11D photoregime. Pituitary gonadotropin activity was assayed using ^{32}Phosphorus

uptake by chick testes. The specificity of this assay for fish gonadotropin is not known. Furthermore, mammalian FSH was used as a standard in the assay and piscine gonadotropins appear to be more similar to mammalian LH in activity (see below).

HYPOTHALAMIC CONTROL

Since reproduction is typically synchronized with seasonal changes in temperature and/or day length, it follows that the gonadotropic cells are probably under control of the brain. The pituitary in teleosts has no obvious median eminence or hypophyseal portal system as is present in tetrapods. The teleost pars distalis is, however, penetrated by nerve fibers from the neurohypophysis originating in cells of the nucleus lateralis tuberis (NLT) of the hypothalamus (cf., Ball and Baker, 1969; Sage and Bern, 1971; Zambrano, 1972; Zambrano *et al.*, 1972). The fibers originating in the various regions of the NLT provide a possible means of exerting hypothalamic control over the gonadotropic cells. Although a typical median eminence is lacking in teleosts, control of gonadotrophs by substances released into the blood is still possible since the blood supply to the adenohypophysis usually passes through neurohypophysial tissue (cf., Ball and Baker, 1969; Sage and Bern, 1971). Moreover, one of the major components of the neurohypophysial core is neurosecretory nerve fibers which probably arise in the nucleus preopticus (NPO) of the hypothalamus (cf., Ball and Baker, 1969; Sage and Bern, 1971).

Two types of neurosecretory fibers penetrate into the teleost adenohypophysis. One type of fiber usually stains with aldehyde fuchsin, chrome-alum hematoxylin, aldehyde thionin or alcian blue following permanganate oxidation, and appears to correspond to the Type "A" (stainable) neurosecretory fiber distinguished at the ultrastructural level (Knowles, 1965; Knowles and Vollrath, 1966c, d). Classic "neurosecretory stains" do not react with the other type of fiber. These "nonstainable" fibers seem to correspond to the Type "B" described by Knowles using the electron microscope. Type "A" fibers contain typical elementary neurosecretory granules, 1400-1600 A in diameter and usually terminate in the distal part of the neurohypophysis, but may penetrate into the pars distalis in some species (Sathyanesan, 1965, 1966). Type "A" fibers presumably arise in the NPO (cf., Follenius, 1965a, Ball and Baker, 1969; Sage and Bern, 1971; Zambrano, 1972). Type "B" fibers usually contain large granulated vesicles, 900-1000 A in diameter. Many Type "B" fibers traverse the basement membrane separating the neurohypophysis from the different lobes of the adenohypophysis and come into more or less intimate contact with all adenohypophysial cell types, including the gonadotrophs (Follenius, 1965a, b; Knowles and Vollrath, 1966c, d; Vollrath, 1967; Leatherland, 1970a, b, 1972; Kasuga and Takahashi, 1970; Zambrano, 1970b, 1971; Bern *et al.*, 1971; Zambrano *et al.*, 1972). Presumably Type "B"

fibers originate in the NLT (cf., Ball and Baker, 1969; Sage and Bern, 1971; Bern *et al.*, 1971; Zambrano, 1972; Zambrano *et al.*, 1972).

Type "B" fibers appear to be monoaminergic in *Anguilla anguilla*, (Knowles, 1965), *Gasterosteus aculeatus* (Follenius, 1967, 1968b, 1969, 1972a), *Anguilla japonica* (Urano, 1971), *Gillichthys mirabilis* and *Tilapia mossambica* (Zambrano, 1970b; Bern *et al.*, 1971). Treatment of *Oryzias latipes* with reserpine, an adrenergic blocking agent, apparently activated the pituitary gonadotropic cells, suggesting that the hypothalamic control system may be aminergic (Egami and Ishii, 1962). Zambrano (1972) has determined the noradrenaline, dopamine and 5-hydroxytryptamine contents of portions of the pituitary of *Mugil platanus*. In whole pituitary extracts these amines were present in relatively low concentrations. In the portion of the gland containing the gonadotropic cells, dopamine was present in higher concentrations than noradrenaline. Zambrano (1972) suggested that the protein component in the large granulated vesicles of Type "B" fibers may be the releasing factor and that monoamines may have a role in the release of these factors. The amines could be a functional part of the neurosecretory neuron itself or the neurosecretory neuron could be innervated by aminergic fibers from "higher" brain centers.

Other investigators have not been able to demonstrate the presence of catecholamines in the Type "B" fibers in the pituitaries of *Carassius auratus* (Baumgarten and Braak, 1967), *Leucopsarion petersii* (Honma and Honma, 1970) and *Leuciscus rutilis* (Ekengren, 1973). These differences may be due to species variation, differences in photoperiod and temperature acclimation of experimental animals or differences in the reproductive condition of the experimental animals. Recently, Follenius (1972b) suggested that gamma aminobutyric acid may be involved in regulating cells in the proximal lobe of the pituitary of *Gasterosteus aculeatus*.

Zambrano (1971) studied with electron microscopy the hypothalamic NLT neurons and Type "B" fibers making synaptoid contacts with pituitary gonadotrophs in *Gillichthys mirabilis* following experimental treatmetns designed to alter gonadotropin secretion. He reported a direct relationship between gonadal activity and neurosecretory activities in the lateral group of NLT neurons and in the Type "B" fibers innervating the gonadotrophs. More specifically, he found that low temperatures activated the NLT neurons, Type "B" fibers and gonadotrophs and high temperatures reduced activity. These data further confirm de Vlaming's (1972a, b) suggestion that low temperatures stimulate gonadal development and high temperatures cause gonadal regression in *Gillichthys*. Furthermore, Zambrano (1971) found that after castration the lateral group of NLT neurons became extremely active; this reaction was abolished with supplementary androgen therapy. Zambrano's data (1970a, b, 1971) combined with that of Knowles and Vollrath (1966c, d) indicate that Type "B" fibers originating in the NLT and innervating the

gonadotropic cells exert a stimulatory influence on gonadotropin secretion.

Cytological signs of neurosecretory activity in the NLT have been correlated with reproductive activity in a variety of teleost species (Polenov, 1950; Stahl, 1953, 1954, 1957; Zaitsev, 1955; Brehm, 1958; Schiebler and Brehm, 1958; Billenstein, 1962; Stahl and Leray, 1962; Oztan, 1963; Honma and Tamura, 1965; Szabo and Molnar, 1965; Dixit, 1967; Honma and Suzuki, 1968; Samuelsson *et al.*, 1968). By use of stereotaxic electrolytic lesions in various parts of the hypothalamus of the goldfish, Peter (1970) demonstrated that destruction of the posterior region of the NLT causes regression of gonads and gonadotropic cells; lesions in the NPO were ineffective. These data point to the NLT as the hypophysiotropic center for controlling gonadotropin secretion.

A positive correlation between apparent activity in the NPO and sexual maturation has also been reported in other teleosts (Barannikova, 1961; Sokol, 1961; Stahl and Leray, 1962; Honma and Tamura, 1965; Sathyanesan, 1965; Oztan, 1966; Honma and Suzuki, 1968; Kasuga and Takahashi, 1970; Sundararaj and Viswanathan, 1969, 1971). Kasuga and Takahashi (1970) and Oztan (1966) noted that photoperiod activates both the gonads and NPO in *Oryzias latipes* and *Zoarces viviparus*. Ovariectomy of *Clarias* presumbly increases neurosecretory material in the NPO whereas estrogen therapy caused a degranulation in the NPO (Dixit, 1970). Tamura and Honma (1969) observed that in *Leucopsarion petersi* neurosecretory material accumulates in the neuhypophysial core prior to spawning and is depleted upon spawning. A similar situation is seen in *Poecilia reticulata* (Sage and Bromage, 1970). Reductions in stainable neurohypophysial neurosecretory material at the time of spawning have been reported in several other teleost species (Olivereau and Herlant, 1960; Sokol, 1961; Egami and Ishii, 1962; Sawyer and Pickford, 1963; Rai, 1966a, b; Olivereau, 1967). Wilson and Smith (1971) noted an increase in the oxytocic activity in the pituitary of sexually maturing *Oncorhynchus tschawytscha* and suggested that this substance could be involved in gonadal development. Neurohypophysial neurosecretory material may be associated with the spawning reflex rather than control of the gonadotropic cells (see below).

Gonadal atrophy and/or gonadotroph regression have been reported in teleosts with autotransplanted or homotransplanted pituitaries (Atz, 1953; Roy, 1964; Ball *et al.*, 1965; Ball and Olivereau, 1966; Johansen, 1967; Olivereau, 1970, 1971). These data suggest that the hypothalamus exerts a positive control on pituitary gonadotropin secretion. Other investigators, however, have suggested an inhibitory action of the teleost hypothalamus on gonadotropin secretion (Egami and Ishii, 1962; Sage and Bromage, 1970; Leatherland, 1970a, b). Possibly variations are due to differences in reproductive condition of the experimental fish or to the physical conditions (e.g., temperature) under which animals were maintained. Recently evidence has been

presented which shows that hypothalamic extracts stimulate release of gonadotropin from *Cyprinus carpio* pituitaries *in vitro* (Breton *et al.*, 1972); the increase in release into the incubation medium was measured using a radioimmunoassay developed for carp gonadotropin (Breton *et al.*, 1971a).

FEEDBACK CONTROL OF GONADOTROPIN SECRETION

Evidence is available which indicates that gonadotropin secretion in teleosts is influenced by gonadal steroids. McBride and van Overbeeke (1969) showed that castration of mature sockeye salmon (*O. nerka*) induces degranulation of the gonadotropic cells which could be interpreted as release of hormone. Other studies employing castration have yielded similar results (Sokol, 1955; Robertson, 1958; Robertson and Wexler, 1962c; Schreibman, 1964). In two different species of *Serranus* castration led to activation of gonadotrophs and estradiol monobenzoate therapy reverse this trend (Febvre and Lafaurie, 1971). Treatment wtih estrogens inhibits vitellogenesis in teleosts suggesting a negative feedback control on gonadotropin secretion (Berkowitz, 1941; Bullough, 1942; Tavolga, 1949; Egami, 1954, 1955). Several investigators have reported a negative feedback of gonadal steroids on gonadotroph activity (Geske, 1956; Sokol, 1961; Egami and Ishii, 1962; Schreibman, 1964; Goswami and Sundararaj, 1968; McBride and van Overbeeke, 1969).

Ovariectomy of *Clarias* increased neurosecretory material in the NPO and estrogen treatment of ovariectomized fish caused a degranulation of these cells implying that gonadal steroids may act at the hypothalamic level (Dixit, 1970). The electron microscope studies of Zambrano (1971) showed that castration of *Gillichthys* activated the NLT and gonadotrophs; androgen therapy reversed this influence.

THE PINEAL

Hypothalamic control may not be the only means of controlling gonadotropin secretion in teleost fishes; there is some evidence that the pineal is involved in regulating reproductive function. A pineal-gonadal relationship is well established in mammals (for review see, Reiter, 1973). Pinealectomy in mammals frequently stimulates the genital system, whereas treatment with pineal extracts or pineal indolamines inhibits reproductive function. The active pineal principle(s) may be one or more of the biogenic amines synthesized in this gland; it has been suggested that melatonin is a potent antigonadotropin (cf., Reiter, 1973). Photosensitivity of the pineal's biochemical rhythms, coupled with the photodependency of effects of pinealectomy on the mammalian reproductive system has led many investigators to infer that the pineal acts as a neuroendocrine transducer of photoperiod information which is used for endocrine and physiological timing (e.g., Ariens Kappers, 1971; Anton-Tay, 1971; Axelrod, 1971).

The teleost pineal is a well-vascularized organ lying on the dorsal surface

of the posterior diencephalic region of the brain (e.g., van de Kamer, 1955, 1956, 1958, 1965; Holmgren, 1959, 1965; Ariens Kappers, 1965; Oksche, 1965; Rüdeberg, 1966, 1968; Takahashi, 1969; Omura and Oguri, 1969; Owman and Rüdeberg, 1970; Rizkalla, 1970; Hafeez, 1971). The pineal body frequently lies beneath a partially depigmented and translucent area of the cranial roof (Holmgren, 1959; Hafeez, 1971) or is located in a depression in the roof of the skull which may allow for the passage of light (Rivas, 1953). For a more detailed discussion of the origin and structure of the teleost pineal the reader is referred to the reviews of Ariens Kappers (1965, 1971), Fenwick (1970a) and Hoffman (1970).

Its location dorsally in the brain orients the pineal directly toward the primary source of light and renders it well suited for detecting changes in light intensity or for acting as a light dosimeter. Histologically, the pineal body of fishes frequently resembles a sensory structure and is largely composed of three cell types: sensory cells, supporting cells and ganglion cells (Holmgren, 1959; Breuker and Hortsmann, 1965; Oksche and Vaupel-von Harnack, 1965; Oksche and Kirschenstein, 1966, 1967; Rüdeberg, 1970; Oksche *et al.*, 1971).

Several light and electron microscope studies have shown that the sensory cells present in the teleost pineal epithelium are similar to the ciliary type of photosensory cells present in the retina of the eyes (N. Holmgren, 1918; U. Holmgren, 1959; Breucker and Horstman, 1965; Oksche and Kirschenstein, 1966, 1967; Rüdeberg, 1966, 1968, 1969; Hafeez and Ford, 1967; Collin, 1968, 1971; Collin and Meiniel, 1968; Omura and Oguri, 1969; Bergmann, 1971; Hafeez, 1971). Furthermore, electrophysiological studies by Dodt (1963, 1966) de la Motta (1963, 1964), Morita (1966), Hanyu (1967) and Hanyu *et al.* (1969) demonstrated a photosensory potential in the pineal of various teleosts. Recently Morita and Bergmann (1971) made electrical recordings of the pineal stalk in the cichlid, *Pterophyllum scalare* and found that light inhibited spontaneous discharge of the nervous elements; these investigators reported that the pineal organ discharges maximally in dim light and in the absence of light.

Afferent nerve fibers connecting the pineal with other parts of the teleost brain have been described (Holmgren, 1959; Ariens Kappers, 1965; Rüdeberg, 1968, 1969; Hafeez and Ford, 1967; Omura and Oguri, 1969; Hafeez, 1971). Nervous connections between the pineal and posterior commissure (Ariens Kappers, 1964, 1965, 1967; Hafeez and Ford, 1967; Rüdeberg, 1968; Chèze, 1971), the superior commissure (Holt, 1891), the right habencular nucleus (Ariens Kappers, 1964), the optic tectum (Holmgren, 1918), and subcommisural organ (Holmgren, 1959; Chèze, 1971) have been reported in various teleosts. These nervous connections may link the pineal with efferent brain centers which in turn could influence hypothalmo-hypophysial function. Unfortunately there is a paucity of data in this area, and studies of pineal-brain relationships seem in order.

There is also a possibility that the pineal in teleost fishes functions as an endocrine organ. Histological examination of the pineal in various species of teleosts revealed secretory gland characteristics (Holmgren, 1958; van de Kamer, 1956, 1958, 1965; Altner, 1965; Hafeez and Ford, 1967; Takahashi, 1969; Rizkalla, 1970; Hafeez, 1971). Chèze and Lahaye (1969) presented evidence for photic influence on the secretory activity in the pineal of *Gambusia affinis*. In the glandular appearing pineal of *Symphodus melops* there is a diurnal variation in stainable granulation with degranulation occurring during the dark phase of the photoperiod (Chèze, 1969); furthermore, the activity of the glandular appearing cells varies with the stage of gonadal development, being inactive during the period of greatest gonadal activity. In addition, melatonin has been isolated from the pineal gland of teleosts (Fenwick, 1970b). Other histochemical and biochemical evidence is available which indicates an active indolamine metabolism in the teleost pineal (Quay, 1965; Hafeez and Quay, 1969, 1970; Quay *et al.*, 1969; Owman and Rüdeberg, 1970). A neuroendocrine function of the teleost pineal is therefore not improbable.

Krockert (1936a, b) noted that the appearance of secondary sexual characteristics was delayed in *Poecilia reticulata* fed bull pineal glands. In the same species pinealectomy accelerates sexual maturation (Pflugfelder, 1954). Other investigators (Schonherr, 1955; Rasquin, 1958; Pang, 1967; Peter, 1968) have suggested that pinealectomy has little influence on gonadal activity in teleost fishes. Unfortunately, most of these investigators failed to mention photoperiod conditions and the time of year when the experiments were conducted. The importance of such information is indicated in recent studies. Pinealectomy of *Carassius auratus* had no effect on gonadal size during most of the year, but when the organ was removed prior to the onset of the final maturation phase, gonadal size increased more rapidly than in fish with intact pineals (Fenwick, 1970c). Furthermore, Fenwick (1970b) demonstrated that melatonin treatment inhibited the increase in gonadal size stimulated by increasing photoperiod. In *Oryzias latipes*, pinealectomized in December and maintained under continuous illumination or on the natural photoperiod, gonadal development was retarded and no oviposition occurred in spring (Urasaki, 1972a). However, gonadal size in pinealectomized *Oryzias* increased more rapidly than in controls if the operation was completed in October and the animals were maintained in continuous darkness or under natural photoperiod conditions (Urasaki, 1972b). If *Oryzias* is pinealectomized in November and maintained on a short photoperiod the ovaries are larger than in controls, but if the fish are maintained on a long photoperiod the ovaries of pinealectomized animals are smaller than in control animals (Urasaki, 1972c). Urasaki (1972c) also reported that melatonin treatment of either pinealectomized or intact fish on a long photoperiod in January inhibits gonadal development, but is without effect in fish maintained on a short photoperiod. Melatonin treated *Fundulus similis* collected in January or May and maintained on a

long photoperiod had smaller gonads than control fish (de Vlaming *et al.*, 1974). We also found that melatonin treatment of *F. similis* maintained on a short photoperiod in May retarded testicular enlargement; melatonin treatment of animals on a short photoperiod in January was without effect. Thus, it appears that the effects of pinealectomy and melatonin treatment vary with photoperiod acclimation and season.

HYPOPHYSECTOMY

The dependence of gonadal function on the pituitary in teleosts was first demonstrated by Vivien (1938, 1939a, b, c, 1941) and Matthews (1939). Vitellogenesis is suppressed in the absence of the pituitary and all yolky oocytes undergo atresia (Matthews, 1939; Vivien, 1939a; Buser-Lahaye, 1953; Yamazaki, 1961, 1962, 1965; Barr, 1963a; Belsare, 1965; Sundararaj and Goswami, 1968a; de Vlaming, 1972e). The secondary growth phase of oocytes, during which yolk deposition occurs, is primarily affected by hypophysectomy. Barr (1963a) noted that if *Pleuronectes platessa* were hypophysectomized prior to the annual mitotic oogonial proliferation stage, the number of oogonia was considerably reduced but not totally suppressed; he also observed a reduction in the number of primary oocytes in fish maintained for six months after pituitary removal. Yamazaki (1965) concluded that hypophysectomy inhibits oogonial proliferation in *Carassius;* pituitary extracts from goldfish or salmon promoted oogonial proliferation in hypophysectomized fish. These data imply that gonadotropin may be required for oogonial divisions, but Barr (1968) points out that hypophysectomy alters the entire hormonal mileau and some other factor may be involved in stimulating proliferation of oogonia. Following the proliferation stage, oogonia transform into primary oocytes and begin the primary growth phase. This phase of growth of yolkless oocytes does not appear to require gonadotropin support (Vivien, 1939a, 1941; Barr, 1963a; Yamazaki, 1965).

Hypophysectomy in teleosts usually blocks spermatogenesis by inhibiting the transformation of spermatogonia to spermatocytes (Vivien, 1938, 1939b, c, 1941; Matthews, 1939; Burger, 1941, 1942; Buser-Lahaye, 1953; Pickford, 1953a, b, 1954; Tavolga, 1955; Yamazaki, 1962; Barr, 1963b; Belsare, 1965; Ahsan, 1966; Lofts *et al.*, 1966; Sundararaj and Nayyar, 1967; Yamazaki and Donaldson, 1968a; Pandey, 1969a; de Vlaming, 1972e). Different opinions exist as to the effects of hypophysectomy on spermatogonial mitoses. Some investigators have reported that pituitary removal blocks the proliferation of spermatogonia (Barr, 1963b; Ahsan, 1966; Yamazaki and Donaldson, 1968a; Pandey, 1969a), whereas others have suggested that mitoses continue, but at a slower rate (Burger, 1941; Pickford, 1953; Lofts *et al.*, 1966; Sundararaj and Nayyar, 1967). Belsare (1965) claimed that hypophysectomy had no effect on spermatogonial proliferation in *Ophicephalus punctatus.* If *Gillichthys mirabilis* is hypophysectomized while in phases of active spermato-

genesis, mitotic proliferation of spermatogonia does not occur (de Vlaming, 1972e). If fish are hypophysectomized during testicular regression, spermatogonial proliferation is not inhibited. Funk and Donaldson (1972) presented evidence showing that gonadotropin does promote spermatogonail mitoses in *Oncorhychus gorbuscha.*

The fate of later maturational stages in spermatogenesis following hypophysectomy also seems to vary according to species. Spermatocytes may continue maturation with sperm being formed as in *Pleuronectes* (Barr, 1963b) and *Poecilia* (Pandey, 1969a) or all stages may undergo degeneration as in *Fundulus* (Lofts *et al.*, 1966) or *Gillichthys* (de Vlaming, 1972e). Hypophysectomy has been shown to bring about regressive changes in interstitial tissue, lobule boundry cells and Sertoli cells in various species (Buser-Lahaye, 1953; Ahsan, 1966; Lofts *et al.*, 1966; Sundararaj and Nayyar, 1967). Yamazaki and Donaldson, 1968b; Pandey, 1969a; de Vlaming, unpublished data).

The effects of hypophysectomy probably vary as a function of the gonadal condition at the time of surgery (season) and with the temperature of maintenance. The discrepancies reported above may be due, in part, to variation in these parameters.

In recent years a pharmacological agent, the dithiocarbamoylhydrazine derivative, (I.C.I. 33,828) methallibure, has been used to block reproductive function in teleosts. Methallibure is a potentially useful tool because hypophysectomy leads to an interference with a whole spectrum of physiological functions which are under hypophyseal regulation. Treatment with methallibure appears to have the same effect as hypophysectomy on gametogenesis, but frequently has side effects on the thyroid as well. Mackay (1973) reported a decrease in the proportion of yolky oocytes and increases in nonyolky oocytes and atretic follicles in *Hypselotris galii* treated with methallibure at the beginning of the breeding season; he also noted a dose dependency between methallibure and gonosomatic index (GSI). Oogenesis is also blocked by methallibure in *Carassius* and *Gasterosteus aculeatus* (Hoar *et al.*, 1967); *Cymatogaster aggregata* (Wiebe, 1969b) and *Tilapia* (Hyder, 1972). Wiebe (1969b) noted that methallibure treatment failed to block oogonial proliferation in *Cymatogaster.* A number of investigators (Hoar *et al.*, 1967; Wiebe, 1968, 1969b; Leatherland, 1969; Pandey and Leatherland, 1970; Billard *et al.*, 1970a; Martin and Bromage, 1970; Pandey, 1970; Mackay, 1971, 1973; Calvo and Borriconi, 1972; Hyder, 1972; de Vlaming and Licht, unpublished data) have shown that methallibure inhibits the transformation of spermatogonia to spermatocytes in the teleost testis. Calvo and Morriconi (1972) found that methallibure retards mitotic proliferation of spermatogonia in *Jenynsia lineata,* but spermatogonial divisions are apparently not inhibited in *Carassius, Cymatogaster, Gasterosteus* (Hoar *et al.*, 1967), *Poecilia reticulata* (Martin and Bromage, 1970), *Tilapia* (Hyder, 1972) and *Gillichthys mirabilis* (de Vlaming and Licht, unpublished data). The development of sper-

matocytes into spermatozoa is not inhibited by metallibure (Martin and Bromage, 1970; Pandey 1970; Hyder, 1972; Mackay, 1973) suggesting that gonadotropin is not required for these divisions and transformations or that methallibure does not inhibit the gonadotropin involved in this process.

Methallibure inhibits Leydig cell activity in *Cymatogaster* (Wiebe, 1968) and *Tilapia* (Hyder, 1972). Secondary sex characters are not influenced by methallibure treatment in *Poecilia* (Martin and Bromage, 1970).

Pandey and Leatherland (1969), Leatherland (1969) and Mackay (1971) described degranulation of pituitary gonadotropic cells following methallibure administration indicating that this compound acts to block synthesis and/or release of gonadotropic hormone. Whether the site of action is on the pituitary or at the hypothalamic level is not presently known. Replacement therapy with fish pituitary homogenates or mammalian gonadotropins override the methallibure gametogenic blockage (Wiebe, 1968, 1969b; Billard *et al.*, 1971b; Calvo and Morriconi, 1972; Hyder, 1972). Recently, de Vlaming and Licht (unpublished results) found that pituitaries from methallibure treated fish were more effective at stimulating *in vitro* ovulation in ovarian segments of *Gillichthys* than were pituitaries from non-treated fish. Since methallibure was effective in blocking spermatogenesis, it is possible that this compound inhibits release of gonadotropin.

EFFECTS OF MAMMALIAN GONADOTROPINS

The effects of purified mammalian gonadotropins on reproductive processes in teleosts can give some insight into the nature of piscine gonadotropic hormone(s). Treatment of both intact and hypophysectomized teleosts with mammalian luteinizing hormone (LH) and follicle stimulating hormone (FSH) suggests that fish gonadotropins are more similar to mammalian LH. Evidence is also available which indicates that piscine gonadotropins may be similar to human chorionic gonadotropin (HCG) and pregnant mares serum gonadotropin (PMSG). For a thorough review of the earlier literature see Pickford and Atz (1957).

Pickford and Atz (1957), Dodd (1960) and Hoar (1966) have reviewed the literature dealing with the effects of mammalian gonadotropins on the gonads of intact fishes. It is evident from these reviews that LH and HCG frequently increase gonadal weight or cause spawning in many teleosts. Few investigators, however, report whether hormones promote spermatogenesis and vitellogenesis, or whether they simply cause gonadal hydration. In addition, one must obviously apply caution in interpretation of these data obtained with fish having intact pituitaries since exogenous hormones could be acting in concert with the endogenous hormonal mileau.

In sticklebacks maintained on a photoperiod which is normally non-stimulatory to gonadal development, LH was considerably more potent than FSH in stimulating vitellogenesis (Ahsan and Hoar, 1963); HCG and PMSG

were also effective, but not as active as LH. Ovine LH, but not FSH initiated spermatogenesis in intact *Blennius sphinx* with regressed testes (Blüm, 1972). De Vlaming (1972e) showed that ovine LH or HCG stimulated testicular hydration and accelerated vitellogenesis in *Gillichthys mirabilis* with intact pituitaries at 12° C and 20° C; LH or HCG therapy failed, however, to prevent testicular regression in fish maintained at 27° C. Partially purified salmon gonadotropin (SG-G100) maintains spermatogenesis in intact *Gillichthys* at 27° C and homoplastic pituitaries initiate spermatogenesis in intact fish with regressed gonads at 28° C (de Vlaming, 1972e). The point to be made here is that gonadal sensitivity to hormones may depend on temperature.

Pickford (Pickford and Atz, 1957) observed a log dose response between testicular weight and mammalian LH in hypophysectomized *F. heteroclitus*; FSH was not active. LH, but not FSH, reinitiates spermatogenesis in hypophysectomized *Couesius plumbeus* (Ahsan, 1966). In hypophysectomized *H. fossilis* LH restores spermatogenesis but not vitellogenesis (Sundararaj and Nayyar, 1967; Sundararaj and Anand, 1972). Wiebe (1969b) found that mammalian LH reinitiates spermatogenesis in methallibure treated *Cymatogaster aggregata*. Contrary to the above data Billard *et al.* (1970) reported that a highly purified mammalian LH would not activate spermatogenesis in hypophysectomized goldfish. HCG was not active in stimulating vitellogenesis in hypophysectomized goldfish (Yamazaki, 1965) or spermatogenesis in hypophysectomized *Gillichthys* (de Vlaming, 1972e).

Singh (1970) concluded that mammalian FSH was more potent than both LH and HCG in promoting an increase in ovarian weight in hypophysectomized *Mystus vittatus*. No histological data was presented to varify this claim so possibly hormonal treatment caused ovarian hydration. Furthermore, the doses of hormone given were extremely large and due to the highly impure nature of the hormones used, especially FSH, little can be said about the physiological meaning of these data.

Recent intriguing data (Pickford *et al.*, 1972) suggest that other pituitary hormones may act on the teleost testis. In hypophysectomized *F. heteroclitus* only mammalian LH stimulated the maturation of spermatogonia. LH, however, did not significantly affect spermatogonial multiplication, whereas mammalian growth hormone (GH) was effective in this regard. GH did not stimulate spermatogonial maturation.

Available data indicate that mammalian LH and HCG can activate the interstitial tissue in intact fishes (Berkowitz, 1941; Baldwin and Li, 1942; de Vlaming, 1972e). Hyder *et al.* (1970) reported that HCG therapy increases plasma androgens in intact *Tilapia leucosticta*. Using histological criteria Ahsan (1966) stated that LH activates the lobule-boundry cells in hypophysectomized *C. plumbeus*. In methallibure treated *Cymatogaster* LH apparently activates both the interstitial tissue and Sertoli cells (Wiebe, 1969b). LH also increases the 3β-HSDH activity in the interstitial tissue of hypophy-

sectomized *F. heteroclitus*. Combined these data favor the view that androgen secretion in the teleost testis is controlled by pituitary gonadotropins.

PISCINE GONADOTROPINS

Numerous investigators have treated intact teleosts with homoplastic pituitary material or pituitary material from other non-mammalian species; in most cases the recipient species were near sexual maturity so little can be concluded about the ability of these homogenates to stimulate the entire gametogenic process (for thorough review see, Pickford and Atz, 1957). In most of these stuides recipient fish completed gonadal development rapidly or were induced to spawn.

To date, various attempts have been made to extract gonadotropin from fish pituitary glands and some of their physiological activities have been investigated (Otsuka, 1956; Robertson and Rinfret, 1957; Fontaine and Gérard, 1963; Clemens *et al.*, 1964, 1966; Schmidt *et al.*, 1965; Yamazaki and Donaldson, 1968a; Sinha, 1969; Burzawa-Gérard, 1971; Donaldson *et al.*, 1972a; Hattingh and du Toit, 1973). Despite the number of purification attempts, gonadotropins of relatively high specific activity have been prepared and characterized from only two teleost species, *Cyprinus carpio* (Fontaine and Gérard, 1963; Burzawa-Gérard, 1971) and the chinook salmon, *Oncorhynchus tshawytscha* (Yamazaki and Donaldson, 1968a; Donaldson *et al.*, 1972a). The purification procedures used in obtaining both carp and salmon gonadotropin, the chemical and physical characteristics of each has been reviewed in detail by Donaldson (1973). The problem of whether one or two gonadotropic hormones are produced by the teleost pituitary has become a central issue which will be discussed below.

Witschi (1955) and Otsuka (1956) suggested that the pituitary of *Oncorhynchus* contains both luteinizing and follicle-stimulating activities; both investigators, however, used non-teleostean bioassays. Acetone plus acetic acid extracts from the pituitaries of *O. keta* and *O. tshawyscha* were shown to induce development of the infantile testes of rainbow trout, *Salmo gairdnerii* (Robertson and Rinfret, 1957; Schmidt *et al.*, 1965).

Salmon gonadotropin (SG-G100) was first prepared by gel filtration on Sephadex G-100 (Yamazaki and Donaldson, 1968a). Further purification of SG-G100 on DEAE-cellulose resulted in a fraction SG-DEAE-2 with a specific activity approximately five times higher than SG-G100 (Donaldson *et al.*, 1972a). Donaldson *et al.* (1972a) suggested that SG-DEAE-2 is a single protein due to results obtained in polyacrylamide disc electrophoresis studies. Carp gonadotropin (c GTH) is obtained by gel filtration on Sephadex G-50 and chromatography on DEAE-cellulose; the appropriate fraction is then applied to Sephadex G100 followed by preparative electrophoresis and finally rerun on Sephadex G100 (Burzawa-Gérard, 1971; Burzawa-Gérard and Fontaine, 1972).

C-GTH is a glycoprotein (Burzawa-Gérard, 1969) as are the mammalian gonadotropins. Both carp and salmon gonadotropins are more similar to mammalian FSH than LH in that they are acidic in nature (Fontaine and Gérard, 1963; Donaldson *et al.*, 1972a). The amino acid composition c-GTH is more similar to mammalian FSH than LH (Burzawa-Gérard, 1969; Burzawa-Gérard and Fontaine, 1972). Antiserum to c-GTH, however, does not cross react with either mammalian LH or FSH (Burzawa-Gérard and Fontaine, 1972). Papkoff (1965) has shown that removal of sialic acid from mammalian FSH with neuraminidase reduces its biological activity, whereas LH is unaffected. Neuraminidase treatment of SG-G100 did not reduce its activity in stimulating testicular growth in immature pink salmon, *O. gorbusha* (Donaldson, 1973). The molecular weight of c-GTH has been estimated using sucrose gradient ultracentrifugation at 27,000 and there is some evidence that this gonadotropin consists of two subunits (Burzawa-Gérard, 1971; Burzawa-Gérard and Fontaine, 1972) as do the mammalian gonadotropins (Papkoff, 1972). Molecular exclusion chromatography indicates that the molecular weight of SG-G100 is approximately 29,000 (Donaldson *et al.*, 1972a); recent studies imply that SG-G100 consists of two subunits (Donaldson, 1973).

Burzawa-Gérard and Fontaine (1965, 1966) reported that a single purified gonadotropic factor (c-GTH) or a group of factors characterized by the same molecular weight from the carp pituitary gland induces spermiation in the frog, uptake of ^{32}Phosphorus in the testis of the eel and swelling of tadpole testes. Although these data imply that there is a single gonadotropin in the carp, they are not totally convincing. Bioassay data using a teleost is required to show that this fraction stimulates all reproductive activities (e.g., spermatogenesis, spermiation and androgen biosynthesis; oogenesis and ovulation). In hypophysectomized goldfish c-GTH does restore spermatogenesis and induce spermiation (Billard *et al.*, 1970). Mammalian FSH and LH increase adenyl cyclase activity in the ovary of rats, but neither hormone is active in immature goldfish ovaries; c-GTH is active in the later test (Fontaine *et al.*, 1970). SG-G100 also stimulates adenyl cyclase activity in homogenates of goldfish ovaries, but is about 36 times less active than c-GTH (Fontaine *et al.*, 1972).

In hypophysectomized *Carassius* SG-G100 stimulates spermatogenesis and spermiation in males and evokes vitellogenesis and ovulation in females (Yamazaki and Donaldson, 1968a, b). Furthermore, SG-G100 increases 3β-HSDH activity in the testes of hypophysectomized goldfish (Yamazaki and Donaldson, 1969). Liley and Donaldson (1969) observed a restoration of vitellogenesis in hypophysectomized *Poecilia reticulata* treated with SG-G100. In *Gillichthys mirabilis* SG-G100 inhibits testicular regression and stimulates spermiation at a temperature which normally causes gonadal involution (de Vlaming, 1972e). SG-G100 maintains spermatogenesis and causes spermiation (Sun-

dararaj *et al.*, 1971) and restores oogenesis and evokes ovulation (Sundararaj *et al.*, 1972a) in hypophysectomized *H. fossilis*. Spermatogenesis and spermiation (Donaldson *et al.*, 1972b; Funk and Donaldson, 1972a) and vitellogenesis (Funk and Donaldson, 1972b) can be induced prematurely in immature *O. gorbusha* by SG-G100 therapy. Menon and Smith (1971) reported that SG-G100 increased the formation of cyclic AMP in slices of testis from *O. tshawytscha*. The activity of SG-G100 in stimulating both *in vivo* and *in vitro* ovulation in several other teleost species is discussed below. All of these data combined favor the view that a single gonadotropin is elaborated in the pituitary of *O. tshawytscha*. Furthermore, SG-G100 is chromatographically homogenous (see Donaldson, 1973). However, if two gonadotropins do exist in this species and they are structurally and chemically similar one might expect them to appear in the same chromatographic fraction. Even if one gonadotropin occurs in the salmonids, one must be careful not to assume that a similar situation exists throughout the diverse teleost group.

Sinha (1969) has prepared three Sephadex G-100 fractions of *Puntius gonionotus* pituitary. Of these, only fraction II is active in inducing ovulation (Sinha, 1971) in the same species. Sinha (1972) also reported that fraction II of his preparation stimulates gonadal hydration in *Puntius*. However, other fractions also stimulated gonadal hydration, no statistical analysis was made and intact fish were used. Furthermore, fraction II was not examined for gametogenic activity. Fraction II of Sinha's preparation is capable of initiating and maintaining vitellogenesis in hypophysectomized *H. fossilis*, as well as stimulating ovulation and spawning (Sundararaj *et al.*, 1972c). Since there is no proof that fraction II consists of a single protein, one cannot conclude that a single gonadotropin exists in this species.

Pituitary glands of the mudfish, *Labeo umbratus* have been fractionated by preparative polyacrylamide electrophoresis and chromatography on DEAE-cellulose and Sephadex G-50 (Hattingh and du Toit, 1973); eight fractions were obtained. Fractions 3 and 4 contained gonadotropic activity as determined by the technique of Yamazaki and Donaldson (1968a). These authors suggested that the gonadotropin has a molecular weight of 14,000 to 20,000. Unfortunately, these investigators did not present their data on spermiation and no evidence was presented as to whether fractions 3 and 4 promote spermatogenesis (i.e., the data do not negate the possibility of two gonadotropins).

Breton *et al.* (1971a) developed a radioimmunnoassay (using antisera to c-GTH) for measuring plasma gonadotropin levels in fishes. Using this assay a circadian rhythm of plasma gonadotropin was observed in pre-ovulatory goldfish; on the day of ovulation plasma gonadotropin levels increased approximately five fold compared to the pre-ovulatory maximum (Breton *et al.*, 1972). A technique has also been developed for radioimmunoassay of SG-G100 (Crim *et al.*, 1972).

ANDROGENS AND SPERMATOGENESIS

Gonadotropin activation of spermatogenesis may be due primarily to a stimulation of androgen secretion which in turn functions in testicular maturation. Evidence is presented above which indicates that interstitial cell or lobule boundry cell steroidogenic activity is tempered by gonadotropin. Eversole (1939, 1940) and Bullough (1942) were the first to suggest that androgens can accelerate germ cell maturation in teleosts; these investigators, however, used fish with intact pituitaries so case must be used in interpretation of their data. Testosterone presumably accelerates testicular maturation in *Poecilia* (Svardson, 1943), *Xiphophorus* (Laskowski, 1953) and *Gillichthys* (de Vlaming, 1972e) with intact pituitaries. Pandey (1969b), however, claimed that methyl testosterone stimulated only spermatogonial proliferation and differentiation of spermatogonia into primary spermatocytes in hypophysectomized *Poecilia reticulata*. In contrast to Pandey's observations, androgen administration stimulated maturation of regressed testes in hypophysectomized *F. heteroclitus*, but did not induce spermatogonial proliferation (Lofts *et al.*, 1966). Furthermore, in *F. heteroclitus* (Lofts, 1968) and *Tilapia nigra* (Hyder, 1970) the interstitial tissue is most regressed when proliferation of spermatogonia is greatest.

Complete restoration of spermatogenesis in hypophysectomized *H. fossilis* (Sundararaj and Nayyar, 1967; Sundararaj *et al.*, 1971) and *Carassius* (Yamazaki and Donaldson, 1969) has been observed following androgen treatment. Contrary to these observations, plasma androgen levels are low and interstitial tissue is poorly developed during the most active phase of spermatogenesis in *G. aculeatus*, suggesting that sex steroids may not be involved in testicular maturation in this species (Gottfried and van Mullem, 1967). Testosterone failed to activate spermatogenesis in methallibure treated *Cymatogaster* (Wiebe, 1969b) and *Tilapia* (Hyder, 1972). Yamazaki (1972) reported that methyl testosterone suppressed spermatogenesis and caused degeneration of spermatogonia in the salmonids, *Oncorhynchus gorbuscha* and *O. nerka*.

The contrasting data presented above may be explained in part by species differences and/or variation in experimental conditions. This area is ripe for further investigation, but at the present time it appears that certain stages of spermatogenesis are controlled directly by gonadotropin(s), but androgens may be the ultimate controlling agents for other phases of testicular maturation.

ESTROGENS AND OOGENESIS

Estrogens possibly mediate some actions of gonadotropin(s) on the teleost ovary. In *Poecilia* (Svardson, 1943) and *Xiphophorus* (Laskowski, 1953) with intact pituitaries estrogen therapy presumably promotes vitellogenesis. Estradiol benzoate treatment of hypophysectomized gravid *H. fossilis* maintained yolky oocytes and prevented follicular atresia (Sundararaj and Goswami, 1968b). Oogonial mitotic proliferation was stimulated by esterone adminis-

tration in intact *Phoxinus laevis*, but vitellogenesis was inhibited (Bullough, 1942). Inhibitory effects of estrogens on vitellogenesis have been reported in other species (Berkowitz, 1941; Tavolga, 1949; Egami, 1954, 1955).

ENDOCRINE CONTROL OF OVULATION

Evidence is accumulating which suggests that ovulation (ejection of the ripe oocyte from the follicle) is hormone induced. Hypophysectomy blocks ovulation in *Carassius* (Yamazaki, 1961, 1962, 1965), *Pleuronectes* (Barr, 1963a) and *Ophicephalus* (Belsare, 1965). Although the ovulatory effects of mammalian and piscine gonadotropins were briefly mentioned above, a general discussion of this process bears further discussion.

High doses of HCG, PMSG or salmon (*O. tshawytscha*) pituitary powder induce ovulation in intact gulf croakers, *Bairdiella icistia* (Haydock, 1971). Ovulation and spawning can also be induced with HCG in blind cave fish, *Anoptichys* jordani, with intact pituitaries (Zeitlin, 1973). Mammalian LH and HCG promote ovulation in hypophysectomized *H. fossilis* (Sundararaj and Goswami, 1966a). Recently, Hirose (1972) reported that HCG stimulates ovulation in incubated ovarian fragments of *Oryzias latipes*. Ovulation can be induced in intact or hypophysectomized gravid goldfish with HCG or PMSG (Yamamoto and Yamazaki, 1967).

Purified salmon gonadotropin (SG-G100) induces ovulation in hypophysectomized goldfish (Yamazaki and Donaldson, 1968b). Both SG-G100 and partially purified carp gonadotropin promote ovulation in both intact and hypophysectomized gravid *H. fossilis* (Sundararaj *et al.*, 1972a, c). Hirose and Donaldson (1972) demonstrated that SG-G100 was more effective in stimulating *in vitro* ovulation in ovarian fragments of *O. latipes* than ovine LH. SG-G100 and carp pituitary extracts induced final oocyte maturation *in vitro* in *Salmo gairdnerii* (Jalabert *et al.*, 1972). In the ayu, *Plecoglossus altivelis*, with intact pituitaries SG-G100 was much more effective in causing ovulation than HCG (Ishida *et al.*, 1972). Ovulation can also be induced in mature *Mugil cephalus* with intact pituitaries by SG-G100 therapy (Shehadeh and Kuo, 1972; Shehadeh *et al.*, 1972).

Kirshenblat (1959) showed that cortisone induced *in vitro* ovulation of *Misgurnus fossilis* oocytes whereas LH was not effective. Beoxycorticosterone acetate (DOCA) also promotes ovulation in *H. fossilis* with intact pituitaries (Ramaswami, 1962), but is not effective in *Bairdiella icistica* (Haydock, 1971). Based on work with hypophysectomized *H. fossilis* Sundararaj and Goswami (1966a, b, c, 1969) have proposed that gonadotropins may not act directly on the ovary to stimulate ovulation, but via the interrenal and corticosteroids. DOCA and cortisol also stimulate *in vitro* ovulation of oocytes in *H. fossilis* (Goswami and Sundararaj, 1971a, b), but SG-G100 and LH are ineffective (Sundararaj *et al.*, 1972b). The ability of gonadotropins to promote corticosteroidogenesis in *H. fossilis* interrenal slices *in vitro* has been demonstrated.

Using three different teleost species Colombo *et al.* (1972a) demonstrated that ovarian tissue *in vitro* is capable of corticosteroid biosynthesis. This interesting discovery suggests that if corticoids are involved in ovulation they perhaps originate within the ovary.

Oocyte final maturation can be induced in *in vitro* ovarian fragments of *Salmo gairdnerii* by purified carp gonadotropin or progresterone, but not by estrogens or corticosteroids (Jalabert *et al.*, 1972). No hormone treatment was effective in stimulating ovulation. These investigators (Jalabert *et al.*, 1972) suggest that gonadotropin stimulates the ovary to produce progestogens which in turn stimulate the final maturation of oocytes; ovulation presumably occurs spontaneously after this final maturation. Yamazaki (1965) also noted progesterone promoted ovulation in hypophysectomized goldfish. *In vitro* ovulation is stimulated in ovarian fragments of *O. latipes* with both progesterone and hydrocortisone, but hydrocortisone alone and in combination with HCG was most effective (Hirose, 1972); interestingly, the effects of all hormones depended on the time of day ovarian incubations were initiated.

ENDOCRINE CONTROL OF SPERMIATION

Spermiation consists of testicular hydration with concomitant ejection of spermatozoa from the lobules into the sperm duct. The process of spermiation appears to be mediated in part by the columnar cells of the sperm duct or the Sertoli cells. Pituitary regulation of spermiation in teleosts has been discussed by Clemens and Grant (1964, 1965), Clemens *et al.* (1964), Yamamato and Yamazaki, 1967) and Grant *et al.* (1969). These investigators demonstrated that mammalian LH elicits testicular hydration in carp, goldfish and rainbow trout.

Yamazaki (1962) and Yamazaki and Donaldson (1968a) showed that hypophysectomy blocks spermiation in *Carassius*, but SG-G100 was effective in stimulating spermiation in these fish. In a later report Yamazaki and Donaldson (1969) presented data which indicates that in goldfish gonadotropins act on interstitial tissue evoking androgen secretion which in turn activates the Sertoli cells in the spermiation response. In *H. fossilis* spermiation is induced by SG-G100, but not by LH or testosterone (Sundararaj *et al.*, 1971). Spermiation can be induced in immature *Oncorhynchus gorguscha* by SG-G100 therapy (Donaldson *et al.*, 1972b; Funk Donaldson, 1972a). SG-G100 also accelerates spermiation in intact *Mugil cephalus* (Donaldson and Shehadeh, 1972; Shehadeh *et al.*, 1972). In hypophysectomized goldfish purified carp gonadotropin (c-GTH) is capable of evoking spermiation (Donaldson and Shehadeh, 1972). The pituitary is apparently not required for spermiation in *Pleuronectes platessa* (Barr, 1963b) or *Couesius plumbeus* (Ahsan, 1966). Apparently then, species differences exist with regard to control of spermiation.

ENDOCRINE CONTROL OF THE SPAWNING REFLEX

Arginine vasopressin and synthetic oxytocin have been reported to stimulate reflex movements similar to the spawning act in *F. heteroclitus* (Wilhelmi *et al.*, 1955). Neurohypophyseal extracts from *Perca fluviatilis* evoked a similar response in *Fundulus* (Swift and Pickford, 1965). These investigators concluded that the effects of neurohypophyseal compounds are probably mediated by the nervous system since it does not require the presence of the gonads. Injection of neurohypophyseal extracts into gravid *O. latipes* caused egg laying; other species, however, did not respond in a similar fashion (Egami and Ishii, 1962). Recently, Heller (1972) reported that arginine vasotocin (which occurs in the teleost neurohypophysis)stimulates contraction in isolated oviducts and oviposition in eight species of oviparous and four species of ovoviviparous teleosts.

SUMMARY

Among the teleosts there is evidence that temperature, photoperiod, food availability, salinity changes and environmental flooding can activate neuroendocrine centers which regulate reproductive cycling. The adenohypophysis plays a central role in controlling gonadal funtcion. Certain basophilic cells in the ventral proximal pars distalis elaborate a glycoprotein gonadotropic hormone which stimulates various gonadal activities. In some teleost species two gonadotrophs have been described while in others only one gonadotropic cell has been identified. The pars distalis basophils show cyclic activity which can be correlated with seasonal gonadal changes. The secretary activity of the gonadotrophs appears to be controlled by the hypothalamus. Evidence is accumulating which indicates that neurosecretory neurons originating in the nucleus lateralis tuberis penetrate into the pars distalis directly innervating and exerting a positive influence over gonadotropic cell secretion. Secretion of gonadotropin can apparently be modified by a negative feed back of gonadal steroids on the pituitary and/or hypothalamus. Recent research implies that the teleost pineal may also be involved in regulating reproductive function. Possibly the pineal acts as a neuroendocrine transducer of photoperiod information and exerts a negative influence on gonadal activity. Hypophysectomy blocks ovarian development by inhibiting vitellogenesis; pituitary removal also retards ovulation in some teleosts. Spermatogenesis is inhibited by hypophysectomy due to a failure of spermatogonia to transform into spermatocytes. Frequently removal of the pituitary blocks androgen production by the testes and also spermiation.

Carp and salmon gonadotropins have been isolated in a relatively pure state. Salmon gonadotropin has been shown to restore all reproductive functions in hypophysectomized fish of two species.

Androgens may be the actual mediators of spermatogenesis in some species. Ovulation in some teleosts seems to be controlled directly by gonadotropin,

whereas in other species interrenal steroids may be the ovulation inducing agents. Figure 3 presents a summary of the environmental and endocrine factors which influence teleost reproduction.

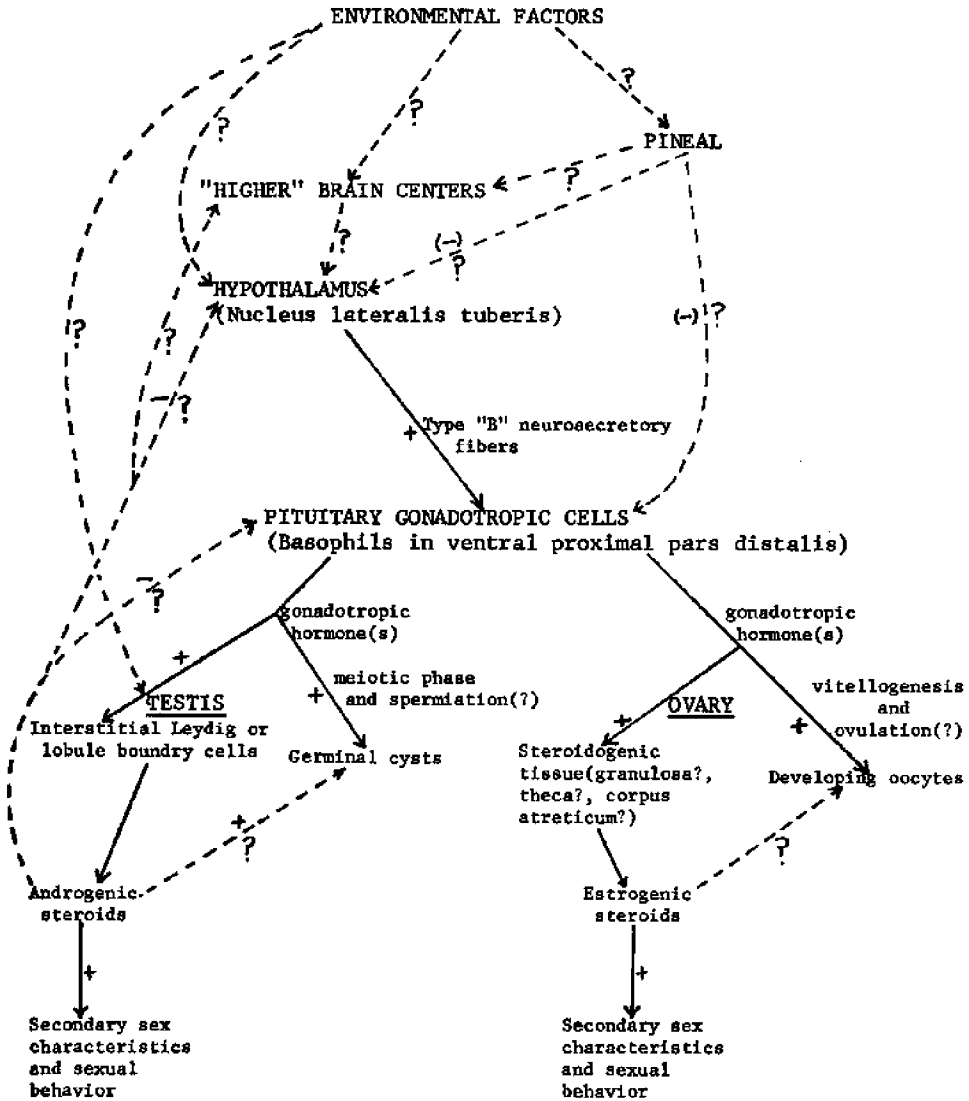

Figure 3. Schematic diagram of possible control mechanisms involved in teleost reproduction. Solid lines represent pathways which are relatively well established; dashed lines indicate possible control pathways which lack definitive experimental proof.

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HORMONAL TREATMENT AND SEX MANIPULATION IN FISHES

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Abstract

Sex hormones have been used extensively in experiments dealing with sex reversal in fishes. Androgens and estrogens may cause genetic females and genetic males, respectively, to differentiate into functional, fertile adults of the heterologous sex. Gestagens and corticoids are ineffective as sex reversal agents. Unisexual populations of fish have broad management potential, including such areas as population control and weed eradication.

Introduction

The preceding papers by R. W. Harrington and V. L. de Vlaming point out the various sequences of events believed to be involved in sex determination and differentiation and environmental and hormonal control of reproduction in fishes. The sex and reproduction of fishes can, potentially, be artificially manipulated by affecting any link in the sequences of events controlling the reproductive processes outlined in the following figures. Details on pathways and specifics on interactions can be obtained from the preceding papers.

Sex determination is depicted by Figure 1. Natural selection "programs" the genetic material to prescribe a certain sex or sexes. However, the environment may also influence the sex of an organism by supplying the raw materials (such as essential amino acids) required for successful genomic expression; the term "genetotropism" was applied by Williams (1963) to this process. The genome of the organism is ultimately responsible for determining sex and apparently works via an additive rather than an all or none process (Yamamoto, 1969). Once sex has been established, a series of finely controlled events lead up to the initiation and culmination of the spawning process.

Environmental stimuli are translated by organisms into responses controlling reproduction (Fig. 2). Nutrition affects reproduction as an ultimate factor. Proximate factors are involved in more direct control of reproductive cycling. Timing mechanisms, of which photoperiod and temperature are important, function in establishing seasonal periodicities in reproduction. Triggering mechanisms initiate the actual spawning process. Stimuli for both mechanisms are received centrally where they are translated into neural and/or hormonal responses. The pituitary is linked via neural and/or circulatory messengers (releasing factors) to the hypothalamus of the brain. The pineal very likely affects reproduction by inhibiting stimulatory activity at either the hypothalmic or the hypophyseal level.

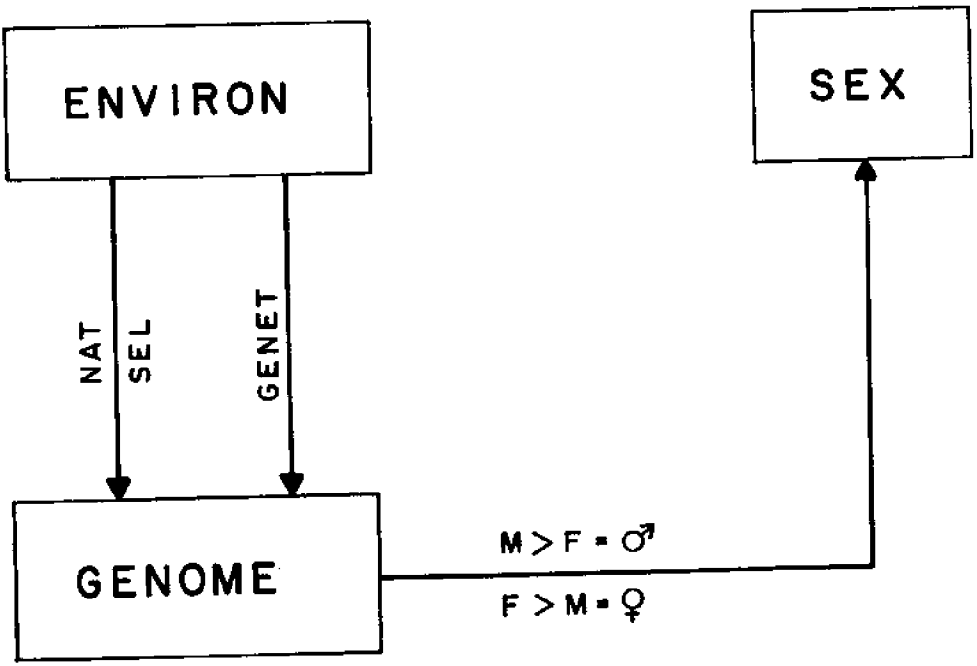

Figure 1. Simplistic representation of the determination of sex. Environ = environment; Nat Sel = natural selection; Genet = genetotrophism; M = male producing "quality"; F = female producing "quality."

The endogenous control of reproduction is represented in Figure 3, a simplification of de Vlaming's Figure 3. In brief, hormones elaborated by the pituitary, gonads and possibly interrenal gland are responsible for controlling gametogenesis, spermiation, ovulation and secondary and accessory sex structures. Feedback mechanisms control the production and release of these hormones.

Although sex and reproduction can be influenced at any step along the sequences presented above, two areas of concern to the fisheries scientist have received extensive study: the administration of hormonal substances to induce spawning and the use of exogenous hormonal substances to direct sex in fish. I review the latter, the ability to produce unisexual populations of fish by sex reversal induced via sex hormones.

The production of unisexual populations of fishes is of interest to both academic and management scientists. Sex hormones administered to juvenile fish can direct the sex of an individual. Genetic males can be induced to become functional females by treatment with estrogenic compounds, and genetic females can be turned into functional males by administering androgenic compounds. Although several other means of producing unisexual populations of fishes are being explored, the role of hormonal substances remains the subject of intensive study for two main reasons: 1) Synthetic hormones are inexpensive and can easily be administered to young fish, thus having potential as a tool in

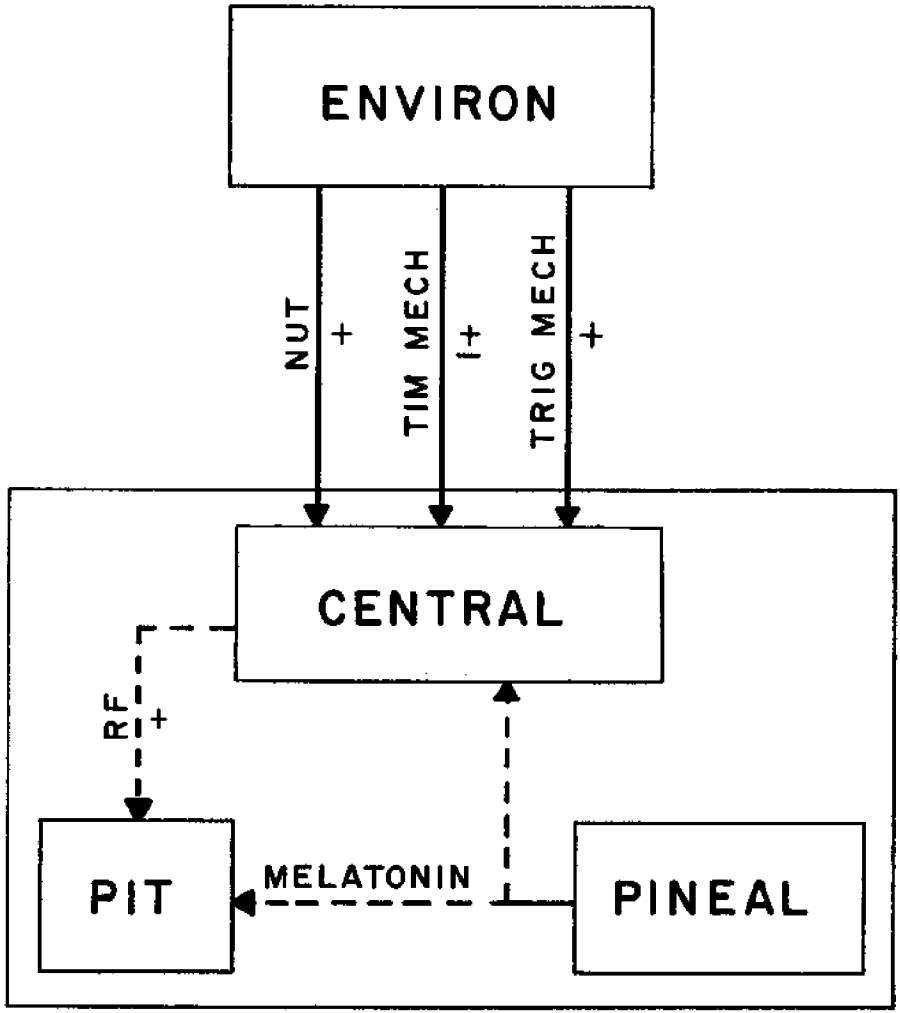

Figure 2. Environmental control of reproduction. Environ = environment; Tim Mech = timing mechanism; Trig Mech = triggering mechanism; Central = central nervous system; RF = releasing factor; Pit = pituitary; Solid line = established response; Broken line = likely response; + = positive response; − = negative response.

fisheries management; and 2) the possible role of steroid hormones as sex inducers, the substances responsible for turning an individual into one sex or the other, is of interest from a scientific and medical standpoint. This latter factor was discussed more fully in the chapter by R. W. Harrington and will not be considered here.

The intention of this chapter is to review the work concerning administration of hormonal substances to juvenile fish. The tabulated format of this review should allow easy reference to specific compounds, species involved and general effects.

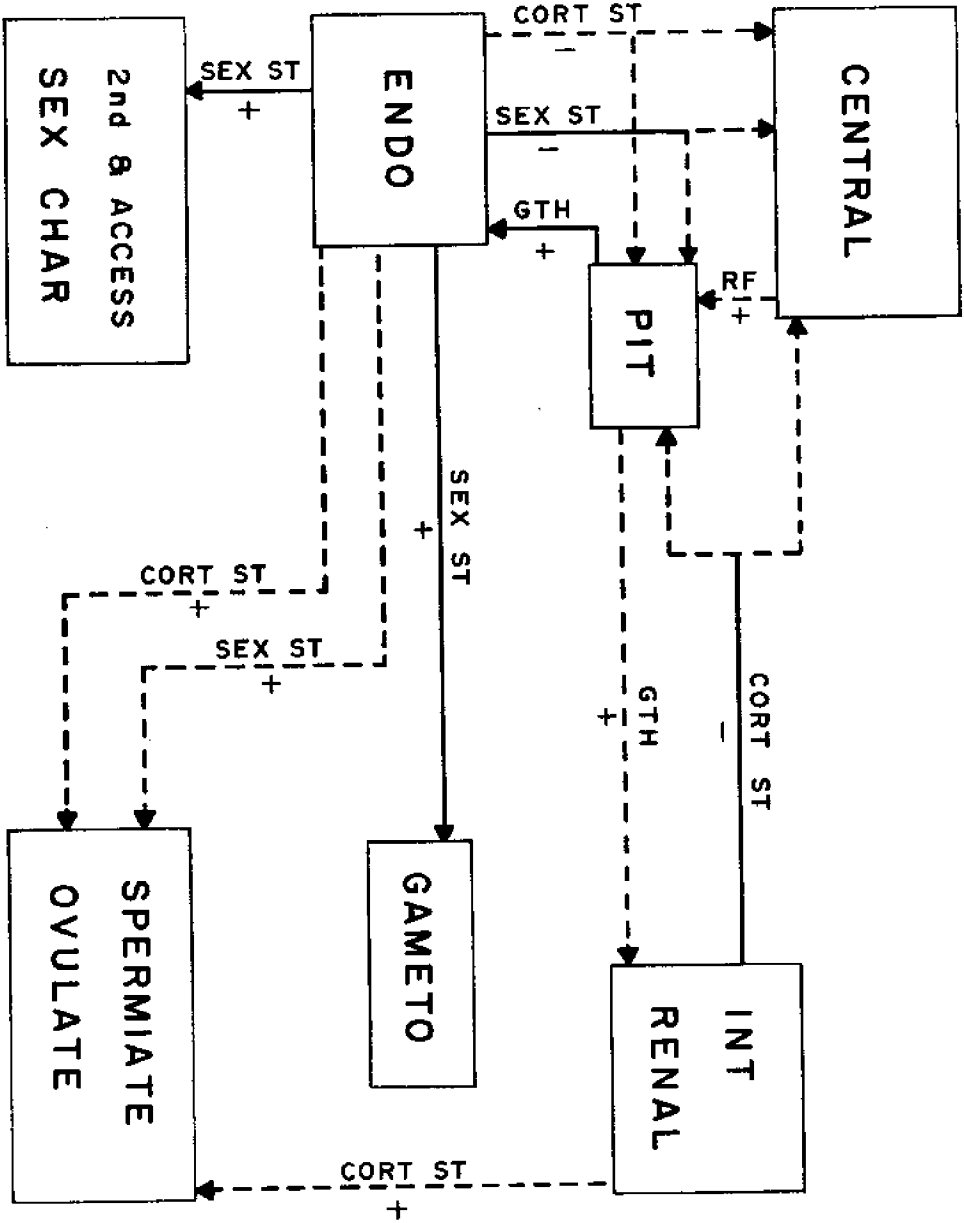

Figure 3. Endogenous control of reproduction. Control = central nervous system; Pit = pituitary; Int Renal = interrenal; Endo = endocrine portion of the gonad; Gameto = gametogenic portion of the gonad; 2nd and Access Sex Char = secondary and accessory sex characters; RF = releasing factor; GTH = gonadotropic hormone; Sex St = sex steroid; Cort St = cortiosteroid; Solid line = established response; Broken line = likely response; + = positive response; − = negative response.

Discussion

Sex reversal studies suggest the possibility of producing larger individuals than would occur normally. Theoretically, the treatment of young fish with anabolic substances should produce increased growth rates. However, Bulkley (1972) found that catfish (*Ictalurus punctatus*) fed Diethylstilbestrol (DES) at 0, 0.1, 1.0 and 10 mg/454g feed had an inverse relationship between growth and dosage. Bulkley and Swihart (1973) also found no improved weight gains in catfish and goldfish (*Carassius auratus*) fed the anabolic steroid Stanozolol. Increased growth was obtained by Poston (1970) who water-hardened brown trout (*Salmo trutta*) eggs in 10 ppm Δ^1-Testolactone for 30 minutes. Most recently, McBride and Fagerlund (1973) did find an increase in weight and length but not condition factor in juvenile coho (*Oncorhynchus kisutch*) and chinook (*O. tshawytscha*) salmon given 17α-methyltestosterone *per os* at 10 mg/kg body weight for 42 days and 1 mg/kg body weight for 48 days, respectively. Testes of fish receiving 10-50 mg/kg were degenerative, and the fish had thickened skin; ovaries were apparently not affected.

Attention is now also being given to use of hybridization or radiation plus hormone treatment to insure permanence of reversal in fish. Hickling (1962), an early proponent of the use of hybridization for cultural purposes, discussed the use of this method for production of all-male populations of *Tilapia*. The genetics involved was discussed by Hickling (1960). Radiation of juvenile fishes has been shown to produce altered sex ratios. However, Bonaham and Donaldson (1972) and Donaldson *et al.* (1972) found no alteration in sex ratios of premigratory smolt chinook salmon irradiated with 0.5-50 R/day for the first 80 days of life. Gonadal development was retarded in fry and spermatogonia or oogonia were lacking in fingerlings having received more than 10 R/day. The number of these fish returning from the ocean as adults was not different from controls. Anders *et al.* (1969) were able to reverse genetic male *Platypoecilus maculatus* into females by irradiation of their pregnant viviparous mothers at 1,000-2,500 R. Stanley and Sneed (1973) reviewed the effects of the production of gynogenesis in fishes via irradiation of sperm (Hertwig effect), and the reader is referred to that work for details. Suffice it to say, that sperm irradiated at 100-200 KR can inseminate an ovum but the genetic material of the sperm does not enter into the karyotype of the egg. Unviable haploids and some gynogenetic diploid eggs result. Warm or cold temperature shock increases the incidence of the viable diploid eggs. All offspring are generally always female, not possessing paternal genetic material. The direction of Stanley and Sneed's (1973) research concerns the grass carp (*Ctenopharynogodon idella*) in which non-reproducing populations would be highly desirable. To insure against mishap and possible reproduction in the field, the females are fed androgens as juveniles to hopefully reverse them into males. Similarly, sex reversed fish can be used to sire unisexal broods.

That is, androgens can be administered to change homogametic females into functional, homogametic males. These males can be bred with normal females to produce all female offspring. Estrogens can be employed in species where the male is homogametic.

Fishes react somewhat differently than higher vertebrates to the administration of sex hormones early in life. In the higher vertebrates only the secondary and accessory sex characters can be changed to those of the heterologous sex; the primary sex structures are not changed. Yamamoto (1969) reviewed mechanisms responsible for sex hormone action on sex differentiation in fishes. Many of the studies concerning administration of hormonal compounds to juvenile fish to induce sex reversal are by Yamamoto (1953, 1955, 1958, 1959a, b; 1961, 1962, 1964a, b; 1965, 1968; Yamamoto and Kajishima, 1968; Yamamoto and Matsuda, 1963). These studies concern not only data on reproductive responses of the treated fish but also genetic analysis of their progeny and viability of genetic rarities (e.g., YY zygotes).

Review of Yamamoto's contributions and the others presented in the following tables allow generalities to be drawn. Androgens can cause genetic females to become functional, viable males, thus acting as andro-inducers (androtermones). Estrogens have the capability of forming genetic males into functional, viable females, thus acting as gyno-inducers (gynotermones). Steroids with side chains at C_{17} ($COCH_3$ or $COCH_2OH$) such as the gestagens and corticoids have no effect on directing sex. To effect sex reversal, the androgenic or estrogenic compounds must be administered during the "indifferent" stage of gonadal development, and treatment must continue through the stage of gonadal differentiation. For example, in the medaka (*Oryzias latipes*) this period is treating for 7-10 weeks post hatching when treated *per os*. Dosage is extremely important, with undetreatment generally affecting only secondary and accessory sex characters and overtreatment, particularly with androgens, causing atrophy of the gonads.

Various modes of administration of the compounds have been successful in affecting sex reversal. These include injection, immersion of eggs and/or fry in water containing the substances and feeding. Attempts at sex reversal have been under highly controlled conditions. To be applicable under culture conditions, however, large-scale more loosely controlled treatment would be desirable. Schreck and Flickinger (1972, unpublished) attempted to cause sex reversal in fathead minnows (*Pimephales promelas*) under such conditions. Eight hundred and fifty fry in a 5 x 5 m earthen outdoor pond were fed Diethylstilbestrol at 1 mg/g food for 70 days after hatching. At 9 months, when the fish were large enough to be sexed, there were 139 females and 106 males, determined by the method of Flickinger (1969). This sex ratio did not differ from that found in controls. Possible reasons for failure to induce reversal of males into females was that under these field conditions other nutrients in the pond may have provided a substantial portion of the

Table 1. Androgens administered to juvenile fishes.

Compound	Species	Treatment	Remarks	Reference
Androstenediolacetate	*Lebistes reticulatus* *Platypoecilus variatus* *Xiphophorus helleri*	Immersion—0.2 ppm for 4 weeks after birth	Ovary not affected except in *X.* it became an ovotestis.	Querner (1956)
Androstenedione	*Oryzias latipes*	Per os—125-16,000 ug/g food to 12mm stage	Female intersexes formed, retarded testes at high concentrations. ED_{50} = 400-500 ug/g food to reverse females.	Yamamoto (1968)
Androsterone	*Oryzias latipes*	Per os	ED_{50} = 580 ug/g food to reverse females	Yamamoto *et al.* (1968)
Dehydroepiandrosterone (Dehydrotestosterone)	*Hemihaplochromis*	Immersion—afterbirth	Inhibited testicular development	Hackmann (1971)
	Oryzias latipes	Per os	$ED_{50} > 3200$ ug/g food to reverse females.	Yamamoto (1969)
	Tilapia aurea	Per os—15-60 ppm for weeks.	No reversal	Guerrero (1973)
Dehydroisoandrosterone acetate	*Lebistes reticulatus* *Platypoecilus variatus* *Xiphophorus helleri*	Immersion—0.2 ppm for 4 weeks after birth	Ovary not affected except in *X.* became an ovotestis.	Querner (1956)
Ethynyltestosterone (Pregneninolone)	*Lebistes reticulatus*	Per os—5-10mg/week for 14-56 days	Precocious male characters; testicular maturation, ovarian inhibition.	Eversole (1941)
	Oryzias latipes	Per os	ED_{50} = 3.4 ug/g food to reverse females.	Yamamoto (1969)
	Tilapia aurea	Per os—15-60 ppm for 3 weeks	Sex reversal in females at highest dose.	Guerrero (1973)
Fluoxymesterone (Halotestin)	*Oryzias latipes*	Per os	ED_{50} = 1.2 to reverse females.	Yamamoto (1969)
11-Ketotestosterone	*Hemihaplochromis*	Immersion after birth	No reversal. Induced male secondary sex characters. Spermatogenesis not affected, oogenesis was.	Hackmann (1971)

Table 1. Androgens administered to juvenile fishes.—(Continued)

Compound	Species	Treatment	Remarks	Reference
11-ketotestosterone (Continued)	*Oryzias latipes*	Per os	10 x more potent than testosterone in producing male secondary sex characters in the female.	Arai (1967)
	Oryzias latipes	Per os—25-500 ug/g food	ED_{50} = 110 ug/g food to reverse females 98.5% reversed at 200 ug/g food.	Hishida and Kawamoto (1970)
Male Hormone	*Tilapia*	Per os—Immersion after birth	Obtained sex reversal; positive effect on growth.	Yashouv and Eckstein (1965)
Methylandrostenediol	*Oryzias latipes*	Per os	ED_{50} = 7.8 ug/g food to reverse females.	Yamamoto (1969)
Methyltestosterone	*Carassius auratus*	Per os—25 ug/g food for 2 months	All females reversed into males except 1.	Yamamoto and Kajishima (1968)
	Halichoeres poecilopterus	Implanted	Masculinized females, did not induce sperm.	Okada (1962)
	Hemihaplochromis Tilapia mossambica	Immersion	Caused predominance of female H.; Feminized gonads in *T.*	Hackmann (1971)
	Hemihaplochromis multicolor	Immersion of embryos	Male secondary sex characters induced, gonads not reversed; oocytes stimulated, spermatogenesis hindered.	Muller (1969)
	Ictalurus melas Lepomis macrochirus Salmo gairdneri	Immersion (?)	LC_{50} at 96 hours was 12.2 ppm for *I.*, 12.2 ppm for *L.* and 8.9 ppm for *S.*	Lennon (1968)
	Lepomis macrochirus	Per os—3 weeks Immersion—eggs for 7 days	Possible reversal in *per os* treatment.	Lennon (1969)

Table 1. Androgens administered to juvenile fishes.—(Continued)

Compound	Species	Treatment	Remarks	Reference
Methyltestosterone (Continued)	*Lebistes reticulatus*	Per os—20-30 ug/g food for 60 days	Reduced growth rates. Up to 90% males produced. Possible behavioral deficiencies and sterilization induced	Clemens *et al.* (1966)
	Lebistes reticulatus	Immerison—3 ppm for 24 hours for pregnant female	Reversal in females with respect to fin development.	Dzwillo (1962a, b)
	Lebistes reticulatus	Immersion—3-4 ppm for 14 days to pregnant female	Most females reversed to males but were infertile.	Dzwillo (1965)
	Lebistes reticulatus	Per os—1 mg/g food	Sex reversal in females	Haskins *et al.* (1970)
	Lebistes reticulatus *Platypoecilus variatus* *Xiphophorus helleri*	Immersion—4 weeks after birth	No effect on germ cells of ovary in *L.* Ovaries in many *X.* became ovotestis.	Querner (1956)
	Oncorhynchus keta *O. kisutch*	Immersion—1-2 weeks at 1:30,200,000 for fry	No change in behavior.	Hoar *et al.* (1952)
	Oncorhynchus gorbuscha *O. keta*	Per os—50-100 ug/g food for 2 weeks	Thickening of skin; ovarian eggs degenerated; spermatogenesis suspended; spermatogonia and interstitial cells hypertrophied.	Yamazaki (1972)
	Oryzias latipes	Per os—1.25-2,500 ug/g food	Mortality increased with dose; female mortality higher. 100% reversal of females at 25 ug/g food. Females at lower dosages not reversed had barren, reduced ovaries. Above 250 ug/g food produced androgenic castration in both sexes.	Yamamoto (1958)

Table 1. Androgens administered to juvenile fishes.—(Continued)

Compound	Species	Treatment	Remarks	Reference
Methyltestosterone (Continued)	*Oryzias latipes*	Per os—12.5-25 ug/g food	Reversal of females; fertile but impotent in some.	Yamamoto (1961)
	Platypoecilus maculatus *P. xiphidium* *P. variatus* X *P. xiphidium* *Xiphophorus helleri*	Immersion—2 ppm of young for 24-36 hours	Masculinity produced.	Dzwillo (1964)
	Tilapia aurea	Immersion—0.05-0.8 ppm of fry for 5-6 weeks	Variable results; involuted gonads in some	Eckstein and Spira (1965)
	Tilapia aurea	Per os—15-60 ppm for 3 weeks	Sex reversal of females 97.6% at 30 ppm. Survival not affected.	Guerrero (1973)
	Tilapia mossambica	Per os—10-59 ug/gm food	10-30 ug/g food produced reversal. Many abnormalities.	Clemens and Inslee (1968)
19-Nor-ethynyltestosterone	*Oryzias latipes*	Per os	$ED_{50} = 1.0$ ug/g food to reverse females.	Yamamoto (1969)
Testosterone	*Cyprinus carpio*	Injected	Sex reversal of male characters; spermatogenesis affected.	Castelnuovo (1937)
	Gambusia holbrookii	Per os	Development increased. Spermatogenesis in both sexes; oogenesis in females	Lepori (1945)
	Salmo irutta	Immersion0.05-0.60 ppm for fry	No sex reversal; germinal tissue inhibited.	Ashby (1954)
	Tilapia aurea	Immersion—1 ppm for 5-6 weeks	Variable results; involuted gonads in some.	Eckstein and Spira (1965)

Table 1. Androgens administered to juvenile fishes.—(Continued)

Compound	Species	Treatment	Remarks	Reference
Testosterone propionate	*Cichlasoma biocellatum* *Hemihaplochromis* *Tilapia hendeloti* *T. mossambica*	Immersion	Produced hermaphroditism in *C.*, predominance of females in *H.*; feminized gonads in *T.*	Hackmann (1971)
	Halichoeres poecilopterus	Injection—0.1 mg	Ovary became testis.	Okada (1964)
	Hemihaplochromis multicolor	Immersion—embryos	Male secondary sex characters induced in all fish; gonads not reversed; growth of oocytes stimulated, spermatogenesis hindered.	Muller (1969)
	Lebistes reticulatus	Injected —0.3 mg/week for 4 months. Per os—for 2-3.5 months	Injection had greater effect; growth in female suppressed and developed male secondary sex charatcers and suppressed ovogenesis. Testis in male degenerated, inhibited gametogenesis.	Eversole (1939)
	Lebistes reticulatus	Injected—0.025 mg/week for 8 weeks; increased dosage to 0.25 mg	Inhibited male color; precocious male secondary sex characters and testicular maturation.	Eversole (1941)
	Lebistes reticulatus *Platypoecilus variatus* *Xiphophorus helleri*	Immersion—0.2 ppm for 4 weeks after birth	Ovary not effected except in *X.* became ovotestis.	Querner (1956)
	Lebistes reticulatus *Xiphophorus helleri*	Per os Injected	Inhibited ovary in *L.*; induced maleness in *X.*	Regnier (1938)
	Lebistes reticulatus *Xiphophorus helleri*	Immersion—15 ppm	Maleness produced in both; ovaries degenerated, testis little effected in *L.*; testicular development in *X.*	Regnier (1939)
	Oryzias latipes	Per os	ED_{50} = 560 ug/g food to reverse females.	Yamamoto *et al.* (1968)

Table 2. Estrogens administered to juvenile fishes.

Compound	Species	Treatment	Remarks	Reference
Diaethyldioxystilben-diphosphate (Honvan, ST-52-Asta)	*Tilapia aurea*	Immersion—1 ppm for 5-6 weeks	Gonadal destruction.	Eckstein and Spira (1965)
p,p^1-Dicarboeth-oxyoxy-trans-α, β-diethylstilbene (Euvestin)	*Oryzias latipes*	Per os	$ED_{50} = 0.8$ ug/g food to reverse males.	Yamamoto (1969)
Diethystilbestrol	*Oryzias latipes*	Per os—3-25 ug/g food for 7-10 weeks	No mortality due to treatment; 100% reversal at 25 ug/g food.	Yamamoto and Matsuda (1963)
	Pimephales promelas	Per os—1 mg/g food for 70 days	No change in sex ratios.	Schreck and Flickinger (1972, unpublished)
Estradiol	*Carassius auratus*	Per os—100 ug/g for 2 months	Reversal of males; no differential mortality.	Yamamoto and Kajishima (1968)
	Lebistes reticulatus	Per os—20 mg/g food	Sex reversal of males.	Haskins *et al.* (1970)
	Lebistes reticulatus *Platypoecilus variatus* *Xiphophorus helleri*	Immersion—0.2 ppm for 4 weeks	Testis became hermaphroditic in *L.*	Querner (1956)
	Oryzias latipes	Per os—5-50 ug/g food for 7-10 weeks	Mortality directly related to dose; 100% reversal at 12.5 or more ug/g food.	Yamamoto and Matsuda (1963)
	Salmo trutta	Immersed—0.05-0.3 ppm for fry	Growth reduced mortality high; no reversal.	Ashby (1957)
Estradiol benzoate	*Halichoeres poecilopterus*	Injected—0.1 mg	No reversal; necrosis and disintegration of spermatogonia.	Okada (1964)

Table 2. Estrogens administered to juvenile fishes.—(Continued)

Compound	Species	Treatment	Remarks	Reference
Etradiol benzoate (Continued)	*Lebistes reticulatus*	Injected—0.002-0.03 mg	Inhibited male secondary sex characters, stimulated female sex characters; formed ovotestes in male.	Berkowitz (1941)
Estradiolbutyryl acetate	*Hemihaplochromis*	Immersion—fry	100% feminization	Hackmann (1971)
	Hemihaplochromis multicolor	Immersed-embryos	Sex reversal.	Muller (1969)
Estradiol propionate	*Lebistes reticulatus*	Injected—2 ug	Inhibited male secondary sex characters; stimulated female sex characters and formed ovotestis in male.	Berkowitz (1941)
Estriol	*Oryzias latipes*	Per os—25-1600 ug/g food for 7-8 weeks	100% reversal of females above 400 ug/g food; at 25 ug/g food female urogenital papilla enlarged	Yamamoto (1965)
Estrogenic compound	*Salmo trutta*	Immersion—40 ppm for eggs	No sex reversal; large size; males had much semen.	Poston (1968)
Estrone	*Carassius auratus*	Per os—100 ug/g food for 2 months	Sex reversal in males.	Yamamoto and Kajishima (1968)
	Cyprinus carpio	Injected	Spermatogenesis in males.	Castelnuovo (1937)
	Gambusia holbrokii	Per os	Accelerated development in both sexes.	Lepori (1945)
	Hepatus hepatus	Immersion	Produced spermatocytes.	Padoa (1939a)
	Oryzias latipes	Per os—125 g/g food for 8 months	Sex reversal in males; mortality.	Yamamoot (1953)
	Oryzias latipes	Per os—125 ug for 4 weeks—7 months	Sex reversal in males.	Yamamoto (1959a)
	Oryzias latipes	Per os—0.5-150 ug/g food for 6-10 weeks	100% reversal in males above 25 ug/g food. Intersexes at lower doses.	Yamamoto (1959b)
	Oryzias latipes	Per os—50-125 ug/g food for 8-10 weeks	125 ug/g food had higher mortality; sex reversal in males.	Yamamoto (1961)

Table 2. Estrogens administered to juvenile fishes.—(Continued)

Compound	Species	Treatment	Remarks	Reference
Estrone (Continued)	*Oryzias latipes*	Per os—50 ug/g food	Sex reversal in males.	Yamamoto (1963)
	Salmo gairdneri	Immersion	Reminization, males inhibited.	Padoa (1937, 1939b)
Ethynyl estradiol	*Oryzias latipes*	Per os	$ED_{50} = 1.7$ ug/g food to reverse males.	Yamamoto (1969)
Female hormone	*Tilapia*	Per os—Immersion of fry	Sex reversal in males when *per os;* Immersion retarded growth, caused high mortality, retarded gonad development, no mortality.	Yashouv and Eckstein (1965)
Hexesterol	*Oryzias latipes*	Per os	$ED_{50} = 0.4$ ug/g food to reverse males.	Yamamoto (1969)
Progynon tablets	*Lebistes reticulatus*	Per os—45 R. U. of estrone and estradiol for 5 months	Size and secondary sex characters female; suppression of spermatogenesis.	Berkowitz (1937)
	Lebistes reticulatus	Per os—45 R. U. of estrone and estradiol for 1-5 months	Size and secondary sex characters female; longer feeding caused ovotestes in males; females not affected.	Berkowitz (1938)
	Lebistes reticulatus	Per os—30-600 R. U. of estrone and estradiol for 2-3 weeks	Secondary sex characters female; inhibited testes and formed ovotestes.	Berkowitz (1941)
Stilbestrol	*Lebistes reticulatus*	Injected—0.05-0.1 mg	Secondary sex characters female; inhibited testes and formed ovotestes.	Berkowitz (1941)
	Oryzias latipes	Per os—250 ug/g food for 8 months	Sex reversal in males.	Yamamoto (1953)
	Oryzias latipes	Per os—62.5 ug/g food for 4 weeks—7 months	Sex reversal in males.	Yamamoto (1959a)

Table 2. Estrogens administered to juvenile fishes.—(Continued)

Compound	Species	Treatment	Remarks	Reference
Stilbestrol diphosphate	*Tilapia aurea*	Immersion—0.05-1 ppm for 5-6 weeks	Concentrations less than 0.05 ppm for 3 weeks did not affect gonads. At 0.2 ppm or higher high mortality. High concentrations had inhibitory effect on gonads.	Eckstein and Spira (1965)

Table 3. Other sex-related compounds administered to juvenile fishes.

Compound	Species	Treatment	Remarks	Reference
Cortisone acetate	*Lebistes reticulatus* *Platypoecilus variatus* *Xiphophorus helleri*	Immersion—1 ppm for 4 weeks for fry	No effect; reduction in growth.	Querner (1956)
	Oryzias latipes	Per os—125-1000 ug/g food for 7-1 weeks	No sex reversal.	Yamamoto and Matsuda (1963)
Cyproterone acetate	*Oryzias latipes*	Per os 50-500 ug/g for food for 12 weeks	Possible sex reversal in males.	Irons and Schreck (1974)
Desoxycorticosterone acetate	*Lebistes reticulatus*	Injected—0.1 mg/week for 7 weeks to fry	No effect on sexual development.	Eversole (1941)
	Lebistes reticulatus *Platypoecilus variatus* *Xiphophorus helleri*	Immersion 1 ppm for 4 weeks for fry	No effect; reduction in growth.	Querner (1956)
	Oryzias latipes	Per os—125-500 ug/g food for 7-10 weeks	No reversal; may possibly inhibit development of ovary and oviduct; increased sterility.	Yamamoto and Matsuda (1963)
	Salmo trutta	Immersion—0.1-0.4 ppm for 52 days for fry	No sex reversal, but more females; germinal tissue inhibited.	Ashby (1957)
17α-Hydroxyprogesterone	*Oryzias latipes*	Per os—125-2,000 ug/g food	No sex reversal.	Yamamoto (1968)
Pregnandiol	*Lebistes reticulatus*	Injected—0.1 mg/week for 7 weeks to fry	No effect on sexual development.	Eversole (1941)
Pregnenolone	*Oryzias latipes*	Per os—400-3,200 ug/g food for 7-10 weeks	No sex reversal.	Yamamoto and Matsuda (1963)
Progesterone	*Lebistes reticulatus*	Injected—0.1 mg/week for 7 weeks into fry	No effect on sexual development.	Eversole (1941)

Compound	Species	Treatment	Remarks	Reference
Progesterone (Continued)	*Salmo trutta*	Immersed—0.05-0.1 ppm for 52 days for fry	No sex reversal, but more females; germinal tisue.	Ashby (1957)

diet and even though the concentration of Diethylstilbestrol was high, not enough of it was consumed to be effective.

The above experiments concern the administration of hormonal substances to fishes. What, however, would be the effect on sex differentiation of fish if one could remove the respective endogenous sex hormone during the period of gonadal differentiation? Recent developments with respect to anti-hormones opens a new realm of research potential. One such substance is the antiandrogen cyproterone acetate (1,2α-methylene-6-chloro-$\Delta^{4,6}$-pregnadiene-17α-ol-3, 20-dione-17α-acetate). This synthetic steroid inhibits development of male secondary and accessory sex characters in mammals apparently by inhibiting the action of testosterone by competitively blocking the androgen at the "receptor" level. In a preliminary experiment with the medaka Irons and Schreck (1974) administered 0, 50, 250, and 500 ug cyproterone acetate/g diet for 12 weeks following hatching. External and histological examination of the fish after 6 months suggested that the highest concentration altered the typical sex ratio of 50:50 to 15 females: 5 males. Growth was not affected. Work now in progress will hopefully establish the basis for these results. Tables 1, 2, and 3 provide a reference to androgens, estrogens and other steroids, respectively, tested for their effects on sex of juvenile fishes. This listing is not intended to be complete but hopefully is comprehensive enough to reflect the scope of work done in this area. A similar review of effects of male and female sex steroids was compiled by Vanyakina (1969).

There are many benefits that could accrue to fisheries management from unisexual or asexual fish. These include the formation of non-reproducing populations of game fish to prevent stunting due to overpopulation. Self-eliminating predator popualtions would be desirable in situations where the etsablishment of the predator is undesirable. A current issue concerns the production of non-breeding herbivorous fish such as the grass carp where environmental considerations potentially make the fish undesirable to stock into public waters if it could breed and decimate desirable species or habitat. Other potential benefits from the ability to direct sex in fish include production of desirable sex ratios for culture practices. This would be desirable where an unequal ratio of male to female brood fish could yield an optimum number of progeny yet require maintenance of a minimum number of adults. Often one sex is more desirable than another because its phenotype is more valued, as in the fathead minnow where the silvery color of the female is more desired as bait, thus demanding a higher price. Stocking of non-reproducing populations of trout or kakanee salmon (*O. nerka*) in situations where there is little or no possibility of reproduction could possibly result in an improved fishery through increased production. Similarly, it would be beneficial if one could channel energy wasted on gonadal maturation into protein formation in food fishes. For example, energetics of plaice (*Hippoglossoides platessoides*) indicates that the female uses one-fifth or more of the energy intake during the growing

season for later gonadal maturation (Bagenal, 1967). Both the food industry and the consumer would benefit if such reproductive wastes in cultured forms could be channeled into usable product.

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