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Bureau of Fish Management
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A LITERATURE REVIEW ON THE VALUE OF TESTOSTERONE TO
CONTROL THE REPRODUCTION OF FISH .

by

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This literature review on testosterone has been provided to furnish background on a compound which is known to act as a depressant on the sexual activity of fish. A literature review of the potential value of a compound of this kind was considered essential before suggesting further research or management trials.

The review has been reproduced under the numbered series of management reports with the expectation that the information would be a useful starting point for others.

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Zarrow, Yochim and McCarthy outline the physiological actions of testosterone:

A. Spermatogenesis

1. Inhibition via the pituitary and/or hypothalamus
2. Direct stimulation of spermatogenesis
3. Prolongation of epididymal sperm life

B. Accessory glands and tissues

1. Stimulation of growth and secretory activities of seminal vesicles, prostate, coagulating, bulbourethral and preputial glands
2. Stimulation of growth of penis and scrotum

C. Secondary sex characteristics

1. Distribution of body hair
2. Configuration of body
3. Comb, wattles, spurs, feathers of birds; clasping pads of amphibia; markings, dorsal spine of certain fishes
4. Pitch of voice
5. Behavior, sexual and aggressive

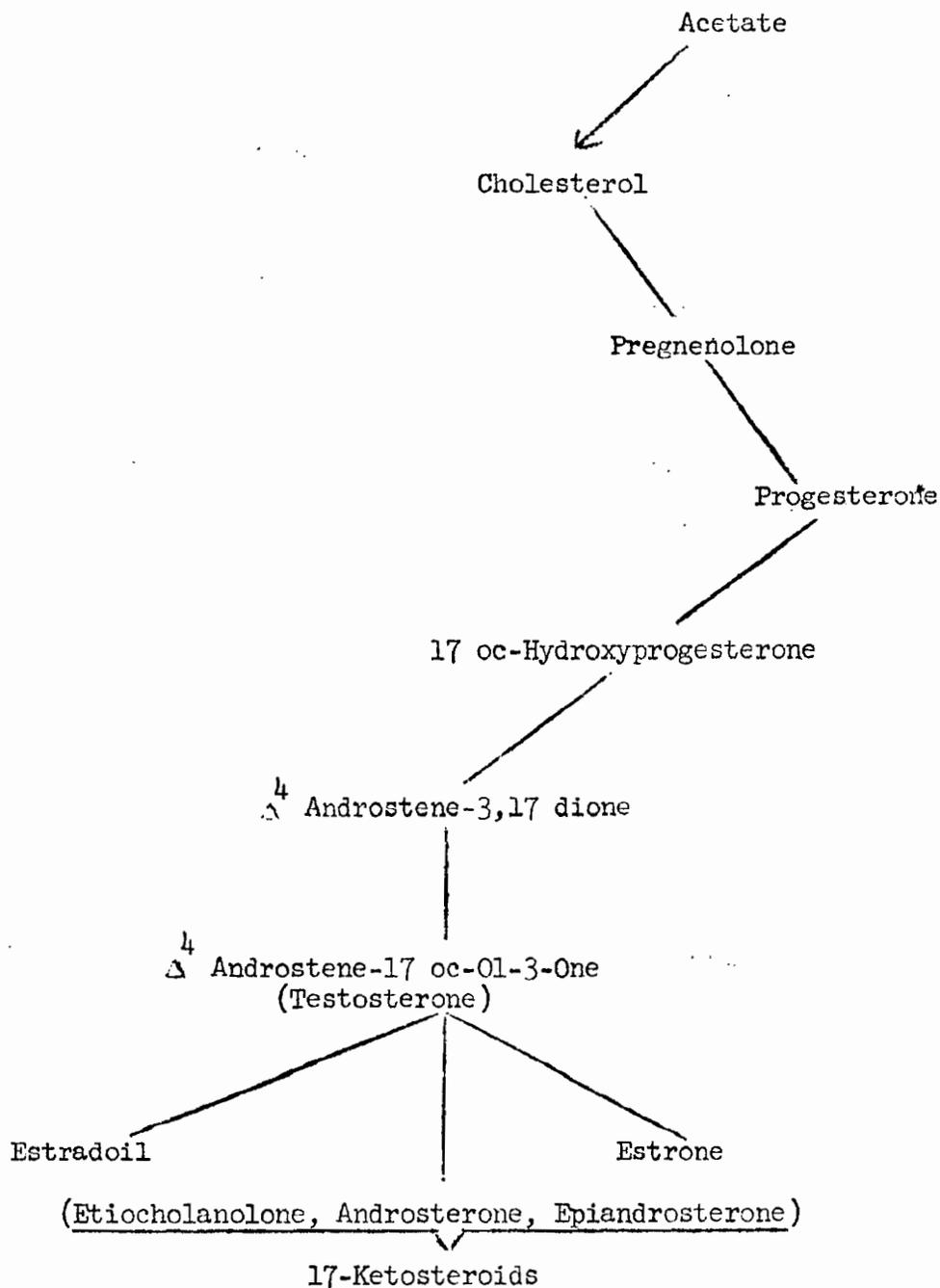
D. Metabolism

1. Promotes nitrogen retention; protein anabolic action, enzyme synthesis
2. Increased storage of creatine

Small doses of testosterone influence linear growth by sealing the epiphyseal cartilage.

Zarrow (cont'd.)

Biosynthesis and catabolism
(simplified from Dorfman, 1961)



* * * *

Young (1961) reports the following information which may be pertinent to an understanding of testosterone:

Yamamoto in 1957 showed that 50 mg/gm wt. of methyl testosterone produced neuters of full grown fish. Intermediate doses produced XX males which were fertile but yielded all female progeny.

Male hormones generally produce hypertrophy of the Wolffian ducts in larval amphibians of either sex and also in the embryos of birds and many mammals.

High concentrations of steroids invariably elicit paradoxical results but such concentrations are usually above the physiologic tolerance levels.

Prolonged treatment with sex hormones destroys the balance of the endocrine system.

Intermediate doses of testosterone propionate increase the level F.S.H. in female mammals by suppressing the elaboration and release of L.H.

Erogenous androgens produce the following in male rats:

1. In low-moderate concentrations
 - a. Severe testicular injury
 - b. Reduction in testicular weight
 - c. Inhibition of spermatogenesis
2. In large concentrations
 - a. Maintains testis size
 - b. Induces spermatogenesis
 - c. Decreases hypophysis

In mammals androgens are produced by Leydig cells in the testis. Most fish lack these cells but since interstitial cells undergo maximal development before breeding, they may be a source of androgens.

Testosterone accelerates the rate of spermatogenesis in carp. In lamprey eels it evokes development of sperm escape ducts.

In Scyliorhinus, testosterone propionate positively influences the development of female characters while in most other species it causes ovarian disintegration. If given to pregnant females, all that live are born males.

Methyl testosterone causes premature maturation of the testis and their subsequent degeneration in Lebistes sp.

There is a reduction in mating behavior after withdrawal from hormone treatment. It is gradual in males and abrupt in females indicating that the female system is controlled by several hormones.

Psychologic factors can be as important as hormones in the peck orders of chickens and mice.

Tavolga (1949) describes the differential effects of estradiol, estradiol benzoate and pregnenolone on Platyplecillus maculatus:

Although pregnenolone is generally considered a progestogen in mammals, it is decidedly an androgen in fishes.

Powdered hormone, when administered as a powder, is first taken up as food. Later the fish differentiate between food and hormone, letting the hormone settle to the bottom. A mixture of both is effective.

Pregnenolone appears to have a toxic effect, for 48 percent of the treated animals did not survive the 8-week test (95 percent of the controls survived). (Metabolism effect?)

The hormone was found to dissolve and to be excreted in metabolic wastes. It remained stable for at least 30 minutes, but was reduced 3 weeks later.

Precocious sexually aggressive behavior was observed after one week of treatment (fish are two weeks old) in males and to a slight degree in females. Gonopods developed in male and female with neither being true structural gonopods.

Under treatment, testis reached maturity far in advance of the controls. Secondary, spermatocysts, spermatics and spermatophores were present. Cysts had formed, each containing one stage of spermatogenesis as in mature adults. Many sperm were found in the ducts. Often the testis occupied the major part of the small body cavity. Interstitial tissue was very sparse compared to that of a normal testis of the same size.

Ovaries of females averaged slightly smaller than controls and were very abnormal in appearance, being much shrunken. Little interstitial tissue was found. Eggs were smaller than controls and appeared to be degenerate. Membranes have irregular depressions with one side usually concave. Nuclei are greatly misshapen. Cytoplasm is mottled. Yolk deposition was absent and the ovary ducts were poorly formed of ragged epithelium.

In two cases, bi-partite ovaries were found, probably a result of the inhibitory effect of the hormone.

The liver cells of the treated animals were much larger than controls. Cells were vacuolated. Increased vascularization was evident with a capillary for every seven to ten cells. Sudanophilic globules were very abundant whereas the controls had very few.

No sign of testis degeneration was found as in the case of Eversole (1941) using Lebistes.

No hormonal sex reversal was recorded as by Berkowitz (1937). Platyfish are presumably more stable than guppies.

In mammals, the liver inactivates steroid hormones. In this experiment the liver was affected by the toxicity of the steady doses to a point where it could no longer inactivate pregnenolone.

Baldwin and Goldin (1939) reported on the effects of testosterone propionate on the female viviparous teleost, Xiphophorus helleri (Heckel):

They state that when administering 0.5 mg. testosterone propionate in 0.02 cc. sesame oil per week, 50 percent of the treated fish experienced sex reversal. All showed progressive absorption of the ovaries and generation of testis, with or without spermatogenesis. Secondary sex character reversal preceded histological reversal, including the development of a nonfunctional gonopod.

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If testosterone propionate (Grobstein, 1940) is administered to the adult Platypoecilus maculatus female in 5-20 mg. doses within 20 days after amputation, the anal fin will regenerate into a structure very similar to the gonopod of a male. If administered after 20 days, only a modification of the typical female anal fin occurs.

The ultimate effect of testosterone propionate on gonopod development is dependent upon the maturity of the female and on the length of time since the anal fin was amputated. Immatures develop a more male-like structure, but it is still structurally incomplete.

Interestingly, testosterone propionate, when injected into juvenile males at 5-25 mg/cc of fish, does not produce a typical male gonopod but one which structurally resembles that of a testosterone propionate influenced regenerated female anal fin. During the growth phase, the hormone produces atypical gonopods.

Turner has theorized that low concentrations of androgens (e.g., ethinyl testosterone) induces gonopodal growth, whereas high concentrations induce differentiation. (Grobstein, 1942)

Concentrations less than optimal fail to produce gonopodal structures in females, whereas those above optimal produce abnormal effects.

In the normal male, there appears to be distinct areas of development of the gonopodal suspensorium, each of which is susceptible to a particular concentration of hormone. (Turner, 1942)

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Further work of Grobstein provided these effects on sexual activity:

Injection of 2 mg/cc of various androgens to the adult Platypoecilus maculatus females showed androsterone to be by far the most stimulating in regenerating a masculine gonopod. However, its effect required 45 days for regeneration and the results were variable. Testosterone propionate required 30 days and methyl testosterone 13 days. Testosterone response was similar to that of testosterone propionate but proceeded more slowly. (Grobstein, 1942).

Administration of methyl testosterone produces a marked decline in the regenerating capacity of the fish. (Grobstein, 1947).

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Hopper and others reported on the effects of testosterone on other species:

In Lebistes reticulatus, regeneration is not affected by even massive doses of testosterone.

At a concentration of 1 ppm of ethynyl testosterone, it was not until the 50th day of post-gastula development that the hormone had any effect. (Hopper, 1949)

Burger (1942), noting effects of testosterone, found that injected testosterone propionate causes the appearance and maintenance of the yellow breeding coloration. Testosterone is much less effective.

Testosterone propionate produces a marked hyperplasia of the ducts of the testis; testosterone showed no effects. Neither show more than slight stimulating effects on spermatogenesis.

Doses as low as 0.04 IU of testosterone acetate produce 100 percent nuptial coloration in males. Doses as high as 35 IU were needed to effect 66 percent of the females positively. One IU has no effect. (Owen, 1927)

Scott (1944) discovered that males fed pregnenolone developed premature gonopods and were subsequently smaller than controls. Bone deposition occurred only in the anal fin region. This ossification is opposite the effect pregnenolone has on higher vertebrates.

Testis powder produces bi-lobed and retarded ovaries in very young animals which exhibit a high mortality. Sex reversal was induced. (Regnier, 1938)

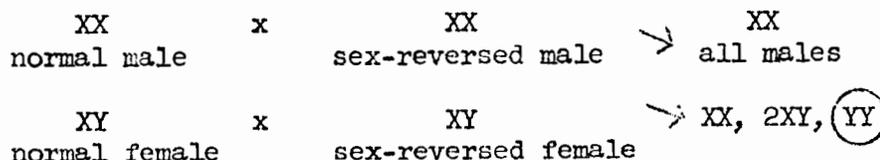
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Wolff (1936), Yamamoto (1953) and others describe sexual effects:

Wolff reported that of the 30 chicken embryos treated, 15 developed into intersexes, 10 into genetic males and 5 into genetic females.

Yamamoto found that injections of oestrogens into males produces functional females with a male genotype.

Injections of androgen (ethynyl testosterone) produce functional males from females with a female genotype.



Testosterone propionate, when injected into adult minnows, produces the following:

1. Only tubercles in males.
2. Tubercles and anal papillae in females. (Ramaswami and Hasler, 1965).

Estrone is significantly more potent in producing testis-ova than is testosterone propionate. In both cases, immersion in an aqueous solution is more effective than tissue implanting.

Okada in 1944 reported that large doses of methylidihydro-testosterone produce testis-ova. Initially, upon administration of moderate amounts to mature fish, large amounts of sperm are released. This is followed by a period of inhibited spermatogenic activity which slowly recovers to the former state. It is during the recovery period that the testis-ova develop. High concentrations of hormones cause atrophy of gonadal tissue.

In aqueous solution, estradiol has a lower threshold concentration than estrone. (Egami, 1953-1956).

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Hoar became interested in testosterone for use with salmon. Testosterone produces an increase in the activity of the goldfish. In salmon it induces feeding behavior associated with spawning migration. (Hoar, Keenleyside and Goodall, 1955).

Vanderplank (1938) discovered that oestrone inhibits an artificially produced conditioned response in these fishes.

Administration of progesterone invariably causes death, the quickness being dependent upon the concentration of the dose.

Neither prolactin nor oestrone was capable of inducing spawning.

* * * *

Methyl testosterone, by injection (0.01 mg.) or immersion (3 mg/liter) consistently produced secondary sexual characters. Implants were inconsistent for the tissue encases the pellet. Reproductive behavior, although increased, did not culminate in nest building and agonistic behavior. It is evident that sexual behavior is induced by steroids and gonadotrophins. Castrated Bathygobius soporator continue courtship response but lose combat behavior while Gasterosteus in contrast loses courtship response but retains aggressive behavior.

It appears that androgens and gonadotrophins are species specific.

Castrated males are active in courtship but are not discriminatory, chasing gravid and non-gravid females as well as fellow males. (Hoar, 1962).

Chum and coho salmon fry, when immersed in methyl testosterone (1:3,200,000), showed no behavioral change over 14 days. Chum, however, swam faster and schooled more than the controls or the coho. (Hoar et al. 1952).

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Noble and Greenberg (1941) found that testosterone propionate treated male Anolis carolinensis showed both characteristic male and female mating behavior. The mating position taken appears to be partially related to dominance, i.e. he will mate as a male with a fish lower in the order but as a female with a fish higher in the order.

After prolonged treatment, males remained in mating position for a longer time than normal males which stop immediately after mating is complete. (The hormone seems to reinforce copulatory reflexes).

Grajcer and Idler (1963) state that both sexes of sockeye salmon have similar total testosterone contents in plasma but the male has much more conjugated testosterone, the female much more free.

	male	female
free	1.7	7.8 ug/100 ml.
conjugated	13.7	7.6 "

Administration of testosterone propionate produced an increase in sexual maturity of both male and female adults but had no effect on ammocaetes. The hormonally mature males had pre-season spermatogenesis.

Calvet (1932) found that injections stimulated the follicles. (Knowles, 1939).

Administration of testosterone propionate (0.03 mg/injection bi-weekly) to female Lebisthes reticulatus suppressed body growth and ovogenesis and induced the development of some, but not all, of the male secondary sex characters. Similarly treated males showed no change.

Estrogen caused sex reversal in males, but testosterone propionate did not cause sex reversal in females. (Eversole, 1939).

11-Ketotestosterone exhibits androgenic properties in the salmon by influencing skin thickness and coloration flesh pigmentation and spermatogenesis in males. In females the hormone affects only the skin and flesh and to a much less degree. It appears that 11-ketotestosterone is the prime sex hormone in salmon being comparable to mammalian testosterone.

It was found that extradiol significantly increases the mass of female gonads. (Idler and Schmidt, 1961).

Hormone clearance in mature and spawned salmon (O.nerko) is impaired, thus producing relatively high levels of steroids. However, by comparisons with the Atlantic salmon which returns to the sea, it appears that the impaired metabolism is associated not so much with spawning but with the imminent post-spawning death. (Idler and et al., 1963).

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The following is a resume' of a discussion with Dr. Meyers of the Dept. of Zoology, University of Wisconsin, concerning the possible use of sex hormones as a control of the reproductivity of fish.

From previous experience with higher vertebrates such as man, apes and rodents, it would seem most reasonable to apply a control androgen during the breeding season. Presumably the hormone would inhibit sexual desires and responses and thus prevent ovulation and/or copulation on the part of the female. However, since very little research has been done with fish, the actual effects are unpredictable. The literature indicates that androgens do indeed have the desired controlling effects upon selected fish through one mechanism or another but, since all the work was done under restrictive laboratory conditions, few of the problems of practical application have been encountered. Foremost among these is the affect on the remaining biotic community. How will the hormone affect plants, plankton, invertebrates, other fish, and other vertebrates who live in the same water or who use it for drinking (e.g. man) or breeding (e.g. waterfowl)? A preparation must be found which is species specific.

The problem of administration appears to be a most formidable one. Since it appears that our present hormones are not species specific and since the breeding season of the bluegill extends into that of other fish, it is conceivable to put the hormone in a food eaten primarily by the bluegill. In this way it will affect only those that eat the food since most derivatives of testosterone are only very slightly soluble in water. (However, the chemical composition of a lake may alter this).

Assuming that a selective food or an alternate method is found through which the androgen can be administered, how much of it can be given? The concentration must be high enough to inhibit reproduction in the bluegill but low enough so as to deposit a minimum of androgenic compounds in the tissue of the fish. Since each fish has its own tolerance level, the level of effective androgen concentration in the body of the bluegill must be such that it will not affect any of its predators such as the bass which will receive a concentrated dose with each fish it eats. One four-inch bluegill may have the ability to concentrate enough androgen to sterilize a five-pound bass. Also, some hormone will be excreted in a probably more soluble reduced form which could be taken up by the gills or dermis of any fish.

Because the breeding season of panfish is long under the present concepts of hormonal actions, administration will have to be over an extended period. Frequency of application will depend on the stability of the hormone with relation to the physical and chemical nature of the lake.

The present state of knowledge of the physiology of fish, the biochemistry of sex hormones and the interaction of the aquatic environment indicates that a vast program of research must be undertaken before a control measure such as an androgen could meet the minimum standards of health as set forth by the Federal Food and Drug Administration. As is so often the case, research will undoubtedly show our present concepts limited or erroneous but will uncover new and more profitable avenues of discovery.

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