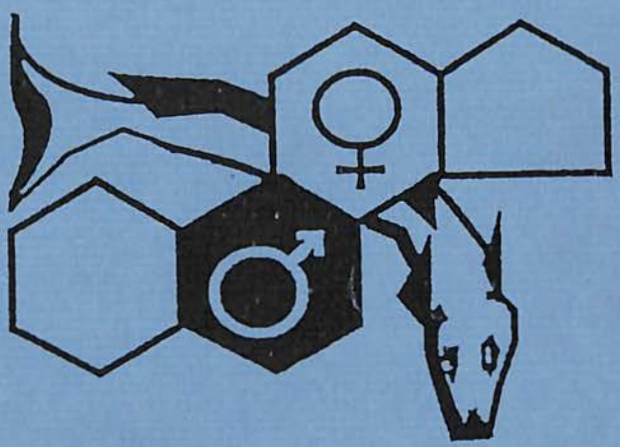
4th International Symposium

on

Reproductive Physiology of Fish



University of East Anglia, Norwich, U. K.
7-12 July 1991

Abstracts

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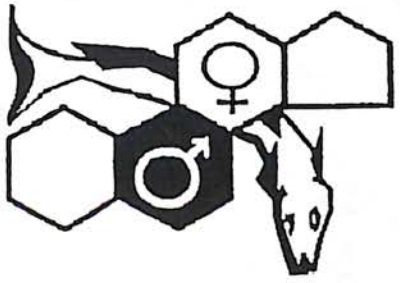

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This book contains:

Abstracts (numbered and in alphabetical order)

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1

PHOTOPERIODISM IN REPRODUCTION IN BITTERLINGS

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More than 10 species of bitterlings inhabit Japan. These bitterlings are separated into two groups by their characteristics in photoperiodism. The first group, which repeats spawning during spring or spring-summer, comprises long-day spawners. Rose-bitterling and Akahiretabira-bitterling are included in this group. Their gonadal maturation is induced in spring only by the increase in water temperature. During their breeding season, photoperiodism gradually develops. Therefore, gonadal development in autumn is inhibited, although the water temperature is at an adequate level for their maturation. Long-daylength is necessary for the induction of gonadal maturation in autumn. The second group, which repeats spawning during autumn, comprises short-day spawners. Kanehira-bitterling and Zenitanago-bitterling are included in this group. They commence spawning under decreasing daylength and repeat regular spawnings in autumn. The decrease in water temperature in late autumn inhibits spawning. Spawning in yearling Akahiretabira-bitterling is induced in June irrespective of photoperiod. The second group is considered to have diverged from the first group during the process of evolution. Photoperiodism probably reversed during their separation. Changes in photoperiodism enabled them to make use of the gill cavity of freshwater mussels in maintaining their larvae in winter. Understanding the genetic background of photoperiodism is one of the more interresting phenomena regarding fish reproduction.

2

MOLECULAR APPROACH FOR CONTROLLING SEXUAL MATURATION IN FISH

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Our attempt to prevent early sexual maturation in farmed fish is based on the key hormone GnRH. We have isolated and characterized the complete GnRH gene (2600 base pairs) from Atlantic salmon (S. salar). The gene encodes a polypeptide having a signal peptide, followed by salmon GnRH, a proteolytic cleavage site, and finally salmon GnRH asssociated peptide (GAP). This information is used to study the patterns of GnRH gene expression and is the base for novel gene constructs with potential GnRH antagonistic activity. Zebrafish (B. rerio) and medaka (O. latipes) are used as models in the transgenic fish strategy.
An immunological approach to postpone sexual maturation is also under investigation. Rainbow trout (O. mykiss) has been injected with GnRH conjugates. Preliminary results show that the fish develop anti-GnRH antibodies, but that the immune response is not sufficient to immunoneutralize the endogenous GnRH.

3

GnRH BINDING IN THE PITUITARY OF THE THREE-SPINED STICKLEBACK; SEASONAL CHANGES AND EFFECT OF PHOTOPERIOD.

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Gonadotropin-releasing hormone (GnRH) binding sites in pituitaries of sticklebacks in different physiological conditions were studied by means of displacement experiments using D-Arg^{6}-Pro^{9}-salmonGnRH-NEt as labelled ligand. Both males and females displayed marked seasonal changes in the capacity of high-affinity GnRH binding sites, with a high content in the breeding season in summer and no detectable high affinity binding in late winter-early spring. The binding capacity was lower in postbreeding fish than in breeding fish. Long photoperiod in combination with high temperature stimulated sexual maturation in winter, whereas short photoperiod in combination with high temperature did not. However, the capacity of the GnRH binding sites was the same in both photoperiods, both groups displaying levels similar to breeding fish in summer.

4

THE ROLE OF THE RIA FORMOSA IN THE MATURATION CYCLE OF THE FEMALE GONAD OF SOLEA SENEGALENSIS (PISCES, SOLEIDAE)

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The oogenesis and the maturation of Solea senegalensis in the Ria Formosa lagoon and off the south coast of Portugal were studied between May 1984 and May 1988. In this paper, the stages of oogenesis, the maturation of the ovaries and their relation with the migration of the individuals from the lagoon to the sea are described. The results obtained show that the maturation of the ovaries begins only when the individuals leave the lagoon, and continues as they move to deeper waters.

5

NEUROANATOMICAL SUBSTRATE FOR DOPAMINE-sGNRH (GONADOTROPHIN-RELEASING HORMONE) INTERACTIONS IN THE FOREBRAIN OF THE GOLDFISH

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It is now well established that dopamine and GnRH are the major factors involved in the neuroendocrine regulation of gonadotrophin release in the goldfish. In view of recent data, interactions between these two factors take place not only at the pituitary level, but also within the central nervous system (Yu and Peter, 1990; Neuroendocrinology 52: 276-283). Since the organization of the dopaminergic and GnRH neuronal systems have been studied separately, there is so far no precise indication about the possible central sites of interactions between these two factors. In the present study, the distribution of sGnRH and tyrosine hydroxylase has been studied within the forebrain of the goldfish on adjacent sections, and on the same sections using two different chromogens following an antibody elution procedure. The results confirmed the presence of a continuum of sGnRH immunoreactive (ir) structures extending from the olfactory nerves to the pituitary and that of a preotico-hypophyseal TH ir pathway (Kah et al. 1986; Cell Tissue Res. 244: 327-337; Kah et al., 1987; Neuroendocrinology 45: 451-458). In addition, TH positive neurons were detected in the olfactory bulbs and tracts, the anterior ventral and posterior dorsal telencephalon, the thalamic area and the posterior hypothalamus. Double staining studies indicate that interactions between sGnRH ir neurons and TH ir neurons most likely take place within the preoptic region and the posterior hypothalamus, although other sites cannot be excluded at this stage.

6

SYNTHESIS OF 17α,21-DIHYDROXY-4-PREGNENE-3,20-DIONE, 17α,20β–DIHYDROXY-4-PREGNEN-3-ONE, AND 17α,20β,21-TRIHYDROXY-4-PREGNEN-3-ONE IN THE OVARIES OF TOBINUMERI-DRAGONET, *REPOMUCENUS BENITEGURI*, CALLIONYMIDAE (TELEOSTEI)

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Tobinumeri-dragonet is a multiple-spawning marine fish. These fish spawn every day in the breeding season (spring and autumn).

Naturally spawning females (three individuals each) were sampled at 21:00, 1:00, 5:00, 9:00, and 13:00 hours. Ovarian fragments (about 200 mg of tissue) were incubated individually with [4-^{14}C]-17α–hydroxyprogesterone (about 3 x 10^5 dpm, 2.4 - 2.6 nmol) and NADPH (1 mg) dissolved in 5 ml of incubation medium (marine fish Ringer buffered with Hepes, pH 7.5) for 60 min.

At each sampling time, the main metabolites from 17α–hydroxyprogesterone were 17α,21-dihydroxy-4-pregnene-3,20-dione (11-deoxycortisol) and 17α,20β,21-trihydroxy-4-pregnen-3-one (17α,20β,21–triOHprog). 17α,20β–Dihydroxy-4-pregnen-3-one (17α,20β–diOHprog) was also synthesized but the extent of production of this metabolite was about 20 % of that of 17α,20β,21–triOHprog.

7

HORMONAL CONTROL OF STURGEONS' (ACIPENSERIDAE) REPRODUCTION

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During natural spawning of female Russian sturgeon the functional activity of pituitary prolactin and ACTH-cells as well as of thyroid and interrenal glands markedly increases. In males the activity of endocrine glands is usually higher than in females, these differences being connected with spawning behaviour. Some hours before spawning in Russian and stellate sturgeons considerable amounts of gonadotropin (GTH) are released in blood, its serum concentrations reaching 100 ng/ml or more (basal level - 1-2 ng/ml). After spawning GTH serum and pituitary levels decrease to basal values, the exhausted gonadotropocytes predominate in the pituitary. Thyroid and interrenal activity decreases, sex differences are absent. Sex steroids' levels are low in both sexes (Bukovskaya, 1981). Artificial spawning may be obtained in sturgeons with the aid of hypophyseal and hypothalamic hormones. Pituitary preparations induce sharp serum GTH increase to 40-80 ng/ml, which still does not reach levels as high as during natural spawning, the pituitary activity of the recepient fish is inhibited. To the time of ovulation GTH concentrations decrease but not to the initial values. LH-RH administration also causes blood GTH concentrations increase, in most cases it is lower in comparison to pituitary preparations' injections. In Russian sturgeon interrenal and thyroid glands are activated only in case of pituitary preparations administration.

8

STARVATION, ESTROGEN, AND CORTISOL EFFECTS ON HEPATIC ORNITHINE DECARBOXYLASE ACTIVITY IN BROOK TROUT.

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Ornithine decarboxylase (ODC) is a key enzyme in the regulation of protein synthesis in higher vertebrates. The longterm goal of this research is to determine how hormones act upon the liver to regulate vitellogenesis in fish, using ODC activity as tool. Some preliminary results from this research are presented here. Hepatic ODC activity declined exponentially during six days of starvation in underyearling brook trout, falling from 94.3 (±4.2) to 0.12 (±0.03) pmoles carbon dioxide/hour/mg protein. The injection of 17ß-estradiol at 1 mg/kg in yearling brook trout starved for seven days caused an over 200-fold increase in hepatic ODC activity at 2 and 3 days after injection. Cortisol injection at 20 mg/kg, both simultaneous to and at 48 hours prior to estrogen treatment, had no effect on this estrogen-induced elevation in ODC activity.

9

SEX INVERSION IN A PROTANDRIC HERMAPHRODITE, *LITHOGNATHUS MORMYRUS* (L., 1758)(TELEOSTEI, SPARIDAE) : HISTO-CYTOLOGICAL PECULIARITIES.

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The modalities of sex inversion in Lithognathus mormyrus, caught in Mediterranean Sea (Golfe du Lion), were carried out using light and electron microscopic criteria. As in most of the Sparid species, gonadogenesis in L. mormyrus leads to an heterosexual gonad (ovotestis) in which testicular and ovarian territories coexist, separated by a connective tissue.

During the protandric evolution of the gonad (o o), two major stages were identified : ovarian edification and testicular regression. When fishes are in functional male phase, ovarian edification is identified by the presence of numerous oogonia and meiotic oocytes only during the post-spawning period. This phenomenon recurring each year (over 2 or 3 years), ovarian edification occurs as a discontinuous, cyclic and small-scale process. During the functional female phase (post inversion), testicular regression proceeds first both by the degeneration of male germ cells and by the invasion of the testicular area by eosinophilic granulocytes (exibiting phagocytic activity). Afterwards, brown-bodies appear as numerous and voluminous clusters of cell remnants (chromolipoids). At the end, rare spermatogonia are scattered among numerous brown-bodies in a small testicular crest, which finally disappears. The active participation of the granulocytes in this testicular regression should be emphasized.

10

MATURATIONAL GONADOTROPHIN HORMONE (GtH) and GODANOTROPHIN RELEASING HORMONE (GnRH) CHANGES DURING GROWTH AND SEXUAL MATURATION ON FEMALE CARP (Cyprinus carpio L.)

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Fish originating from one couple of spawners were growing in a conventional carp pond. At the beginning of investigation every three months and next every month blood samples, pituitaries and hypothalami from 10 to 20 females were collected for RIA. Gonadosomatic indices (GSI) were defined also GSI was showing great variability during growth and sexual maturation.

It was found that blood GtH levels were not changing much during all investigated period, except at the beginning of vacuolisation of the oocytes when peaks of GtH were observed. GtH in the pituitary started to accumulate from 13 months and GnRH from 33 months of age, GnRH levels in the pituitaries being very low before this time, GnRH contents in the hypothalami were also low increasing during females lives and showing great variations. No significant relations between all the studied parameters were found, except significant correlation between age and GSI as well as between age and GnRH content in the pituitary.

11

OLFACTORY SENSITIVITY TO SEXUAL HORMONES IN CRUCIAN CARP (CARASSIUS CARASSIUS)

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The sensitivity of olfactory receptors to sexual hormones was studied with electro-olfactogram (EOG). Crucian carp detected 17α,20ß-dihydroxy-4-pregnen-3-one and prostaglandin $F_{2\alpha}$ which indicates that the hormones may function as sexual pheromones in the same way as previously has been shown in the closely related goldfish (Carassius auratus) (e.g. Sorensen, P.W., Stacey, N.E. & Chamberlain, K.J., 1989. Hormones and Behavior, 23, 317-332).

12

PHOTOPERIODIC CONTROL OF PLASMA GROWTH HORMONE LEVELS AND SEXUAL MATURATION OF ADULT ATLANTIC SALMON

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[1]Department of Zoophysiology, University of Göteborg, Sweden. [2]Department of Aquaculture, Matre Aquaculture Station, Norway. [3]Department of Fisheries and Marine Biology, University of Bergen, Norway. [4]Department of Biochemistry, University of Bergen, Norway.

Growth hormone (GH) has both somatic and non-somatic functions in salmonids. An important non-somatic function is an GH-induced increase in hypoosmoregulatory ability during parr-smolt transformation. The sexual maturation of Atlantic salmon may be under photoperiodic control, and during this period changes occur in both somatic and gonadal growth as well as hypoosmoregulatory ability. The aim of the study was thus, for the first time, to establish if GH may have a regulatory role during sexual maturation, and if changes in GH during this period are affected by photoperiod. Monthly plasma samples were obtained, covering the second season in SW and the subsequent spawning period. From January - June, GH levels were relatively low (0.5-1 ng/ml). A long-day (24L) treatment from January or March did not affect GH, while it caused a large decrease in the proportion of fish that matured. From July, sub-groups were exposed to SNP, 24L, or a short day (8L:16D). GH levels increased in both males and females during a 2-4 month period in the fall. Compared with females on SNP (GH increase in October; ovulation in November), short-day and long-day treatments advanced and delayed the GH increase in females by 1 month, respectively. Similar shift in the timing of ovulation also occurred. An endogenous rhythm, directly or indirectly resulting in increased GH levels a month prior to ovulation, is indicated. The timing of this endogenous rhythm is apparently fine-tuned by photoperiodic control. The correlation between the timing of the GH increase and ovulation suggests a specific role of GH in the maturation process. In contrast to smoltification, where increased day-length during spring is stimulatory for GH levels (Björnsson *et al.* 1989; Aquaculture 82:77-91), a decreased day-length during fall appears to be a similar stimulus during sexual maturation.

13

EFFECT OF 17ß-ESTRADIOL ON SEX DIFFERENTIATION IN INBRED (XX;MAS-1/MAS-1) MALES OF COMMON CARP, CYPRINUS CARPIO L.

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Sex determination in common carp is thought to be of the XX/XY sex determining system. In our laboratory however, gynogenetic reproduction of common carp occasionally resulted in a 50:50 sex ratio instead of an expected 100 % female offspring. Backcrossing experiments of these gynogenetic males indicated the presence of a mutant, recessive minor sex determining gene ("mas-1").
Oral administration of 17α-methyltestosterone results in 93 % males in an outbred population of common carp. Administration of 17ß-estradiol (17ß-E2) however failed to induce feminization, possibly due to a dominant role of endogenous androgens in presumptive male fish during the period of early sex differentiation. In a new experiment, 17ß-E2 was administered to an inbred line of common carp. Broodstock used was a XX;mas-1/mas-1 male and a XX;mas-1/+ female. An outbred male named WT (XY) was used to produce control offspring. The objective of this experiment was to determine the effect of 17ß-estradiol on sex differentiation in XX:mas-1/mas-1 males.

14

KIDNEY ANDROGEN METABOLISM IN THE THREE-SPINED STICKLEBACK

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The kidney of the male stickleback hypertrophies in the breeding season and produces a glue that is used in nest-building. This hypertrophy is androgen-dependent, 11-ketoandrogens being particularily effective.
Kidneys were incubated with tritiated androstenedione (A4) or 11-ketotestosterone (OT). After the A4-incubations the metabolites found in the largest amounts were testosterone (T), 5β-androstenedione (5βAd), etiocholanolone (Et), 5β-androstane-3α,17β-diol (5βA3α17βdiol) as well as glucuronides of T, Et, and 5βA3α17βdiol. The main pathway appeared to be A4 - 5βAd - Et - 5βA3α17βdiol - 5βA3α17βdiol-glucuronid. 5α-reduced compunds were formed to a lesser extent. After OT-incubations 11-ketoandrostenedione (OA) and 5β-androstan-17β-ol-3,11-dione were found, but not 5α-androstan-17β-ol-3, 11-dione (5αOT).
As OT was not 5α-reduced the results suggest that 5α-reduction is not important for the effectiveness of OT on kidney hypetrophy. This is also suggested by preliminary results that OT and OA are more effective than 5αOT and 5αOA in stimulating kidneys in castrated males.

15

SECONDARY SEX CHARACTERS IN A VOCALIZING FISH: INTRA- AND INTER- SEXUAL DIMORPHISM AND ANDROGENIC CONTROL. R.K. Brantley and A.H. Bass. Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853, U.S.A.

The plainfin midshipman fish Porichthys notatus (Batrachoididae) has inter- and intra-sexual dimorphisms in the sound-generating "drumming" muscles. Enlarged sonic muscle is a secondary sexual character. Territorial "Type I" males possess sonic muscles larger in both absolute (25-fold) and relative (6-fold) size than either females, or non-territorial, sneak spawning "Type II" males. This dimorphism parallels a difference in reproductive behavior: only Type I males produce a lengthy "humming" sound to attract gravid females to their nests; reproduction by Type II males is not dependent on acoustic courtship.

Paradoxically, Type II males do not possess the enlarged sonic muscle like Type I males, even though they are also reproductively mature as evidenced by enlarged testes (avg. 8% of body weight), production of viable sperm, and androgen levels comparable to Type I males. To address the androgen sensitivity of this muscle during development, juvenile males and females, and Type II males received subcutaneous pellet implants of testosterone (T), 11-ketotestosterone (KT), estradiol (E2) and cholesterol (C). Changes in sonic muscle weight, and muscle fiber number and diameter, were assessed. T and KT dramatically increased sonic muscle size in all treatment groups, whereas E2 and C had no effect. The androgen effect is partially reversible after implant removal. The natural sex dimorphisms in sonic muscle mass are determined by differences in both muscle fiber diameter *and* number. Type I fibers are characterized by an expansive peripheral zone of sarcoplasm. Androgens induced the Type I fiber morph in all treatment groups. By contrast, although Type I males have 5-fold more fibers, androgens did not induce a noticeable change in fiber number. We conclude that: (1) sexual differentiation of muscle size in the Type I males includes both androgen-dependent and -independent events, and (2) the results do not support an androgen-insensitivity hypothesis for the lack of muscle enlargement in Type II males. Alternative hypotheses will be discussed.

16

THE EFFECTS OF SEASONAL ALTERATIONS IN RATION ON FECUNDITY AND MATURATION IN RAINBOW TROUT (ONCORHYNCHUS MYKISS)

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Previously, we have shown that broodstock trout fed higher rations throughout the year-long reproductive cycle produce significantly more eggs than fish on lower rations even after allowance is made statistically for differences in fish size. Increased percentages of fish also undergo maturation on higher rations. These experiments, however, take no account of the possible effects on reproduction of any seasonal alterations in diet, changes which almost certainly occur in both wild and farmed populations. In the present work groups of 50+ one year old trout were fed on 1% (high ration) of body weight per day for 0, 4, 8 or all 12 months of the reproductive cycle and at 0.4% (low ration) for the remaining periods of time in the year respectively and the number, size and quality of eggs produced and percentages of fish which matured recorded at the first spawning. Results show that fish fed high rations for the first 4 or 8 months of the annual cycle had higher fecundities than those fed on high ration during the last 4 months of the cycle. Regarding maturation, those groups fed high ration for the first 4 months had the highest percentages of spawning fish. These effects are discussed in relation to the timing and control of the different stages of oocyte development and to the optimisation of egg production on commercial farms

This work was supported in part by a MAFF (CSG) award to N. Bromage

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ADOLESCENCE IN FEMALE POWAN, COREGONUS LAVARETUS (L.) IN LOCH ECK AND LOCH LOMOND, SCOTLAND.

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Two distinct categories of juvenile powan, Coregonus lavaretus (L.) (Salmonidae, Coregoninae) are distinguishable. Besides immature juveniles, in which no significant gametogenesis occurs, there are adolescent juveniles, in which gametogenesis begins, but it is not completed and does not culminate in spawning. Length and weight, rather than age, determine when immature powan enter the adolescent phase. Unlike the rigidly timed reproductive cycle of adults, adolescence may begin at any time of year. Adolescents are characterised by very high levels of lipid reserves regardless of season and feeding. We postulate that these high lipid levels are the consequence, not the cause, of the failure of adolescents to complete their reproductive cycle; and that it is insufficiency of protein reserves which result in the abortion (or perhaps delay) of the full reproductive cycle.

18

SEXUAL STATE CHANGES IN A PROTANDRIC HERMAPHRODITE, *AMPHIPRION FRENATUS* BREVOORT (TELEOSTEI, POMACENTRIDAE) : ULTRASTRUCTURAL ASPECTS

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Gonadal modifications related to the influence of social relations were investigated using light and electron microscopic data. Gonads of males are ovotestes (testicular and ovarian areas being contiguous), those of females are pure ovaries.
Sex inversion (♂--> ♀) is characterized by a degeneration both of male germ cells and their associated Sertoli cells and by an increase of oogenetic activity (mitotic oogonia, meiotic oocytes, beginning of auxocytosis of oocytes). Among female germ cells, primordial germ cells (PGCs) exhibiting features of undifferentiated cells (high nucleus to cell ratio, abundance in ribosomes, scarcity of membrane organelles) were identified. Their participation in building up the ovary is suggested. It is possible experimentally to early induce juveniles (γ) to a male (ß) or a female (α) orientation. In these two types of induced gonadogeneses, besides mitotic gonia and meiotic spermatocytes or oocytes, very numerous PGCs were observed and their bipotentiality revealed. It is suggested that any change of sexual state (male-ß--> female-α ; juvenile-γ--> male-ß; juvenile-γ--> female-α) makes a heavy demand on the gonad's germ potentialities since not only gonia but also PGCs participate in the transformation.

19

SERUM SEX STEROIDS' LEVELS IN STELLATE STURGEON (Acipenser stellatus Pall.) DURING INDUCED MATURATION AND OVULATION.

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Stellate sturgeon spawners were captured in nature and kept at a fish farm. In control females (non-injected or saline-injected) during 24 hours of the experiment serum sex steroids' levels (testosterone, T; progesterone, P; estradiol, E) do not change or gradually decrease. In the couse of maturation induced by purified sturgeon GTH, as well as liquid sturgeon pituitary preparation or acetonized hypophyses suspension, P and T levels increased in most fishes in 8-16 hours postinjection and E - on the contrary has a tendency to decrease. Serum steroids' dynamics in control and stimulated by pituitary preparations male sturgeons is the same as in females. During maturation under the influence of LHRH or its analogues T,P and E levels increase only in some females; in males these levels considerably increase. After combined injection of PIM+LHRH-A increase of T serum levels is observed both in males and females and P - in females.

20

TESTICULAR ADAPTATIONS FOR INTERNAL FERTILIZATION IN THE GLANDULOCAUDINE FISHES (TELEOSTEI: CHARACIDAE).

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We carried out a histological study of the gonads of representative species of the characid subfamily, Glandulocaudinae. Ovaries from eleven genera were available and internal fertilization was confirmed in each genus by the presence of spermatozoa within the ovarian ducts. Testis analysis of 13 genera (21 species) revealed that, unlike related characid genera (outgroups), all glandulocaudine testes had the posterior region specialized for sperm storage. Sperm head morphology varied from round (1.8 microns in diameter) to extremely elongated (25.3 microns in length). Based on the presence of shared characters and previous cladistic analysis, there appear to be two main groups represented. Within each group there is an initial trend toward sperm elongation followed by the packaging of spermatozoa into spermatozeugmata ("naked" sperm bundles). In addition, some species had abundant stainable material (periodic acid-Schiff reagant) within the testicular ducts which appeared to be involved in the formation of well-formed spermatozeugmata or less-organized sperm clumps. All specializations mentioned appear to relate to internal fertilization with trends toward more efficient mechanisms of sperm transfer.

21

A PHYSIOLOGICAL BASIS FOR NON-ANNUAL SPAWNING IN WINTER FLOUNDER

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Individual winter flounder (Pseudopleuronectes americanus) may omit a spawning season, forming a non-reproductive post-mature subset in the population, varying as a proportion of the population from year to year. The non-reproductive post-mature state is induced and reversed by controlled manipulation of feeding levels at specific times in the annual reproductive and feeding cycles. Omission of a spawning cycle by winter flounder is interpreted as an adaptive response to variable length, variably successful feeding seasons alternated with prolonged winter fasts. The intermittent iteroparity of a non-regular pattern is predictable, on the short term, based on condition of fish at specific times in the annual cycle.

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ELISA (ENZYME-LINKED-IMMUNOSORBENT-ASSAY) FOR VITELLOGENIN AND VITELLUS IN THE EEL (ANGUILLA anguilla) AND IN THE INDIAN MAJOR CARP (LABEO rohita).

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ELISAs were developed to measure eel and major indian carp vitellogenin (VTG) and vitellus (VT). The two assays were based upon the competition between soluble VTG and VTG adsorbed on microtiter plates for the rabbit anti- VTG serum or anti-VT serum. The adsorbed complexes were then revealed using the peroxidase linked on the second anti-serum (anti-rabbit IgG). The peroxidase activity was estimated by a chromogen transformation of the subtrate (o-phenylene diamine).

A homologous system (eel VTG and anti-eel VTG serum) was used in the eel with a sensitivity of 1.7 + 0.2 ng/ml. Intra and inter-assay variation coefficients were respectively of 6.2% and 9.1%. Parallelism of standard VTG curves and estradiol (E_2) treated eel plasma was assessed by covariance analysis (F_{obs}=0.04 (df1-21). Specificity of the assay was attested using in vivo experimental vitellogenesis in female eels after E_2 or gonadotropic treatments and in vitro eel hepatocytes primary culture with estradiol induction. The assay was used to quantify VT in ovarian extracts.

A hererologous system (indian carp VTG and anti-indian carp VT serum) was developed in the same conditions. The sensitivity was 200ng/ml with intra and inter variation coefficients respectively of 9.1% and 3.1%.

When other species VTG or VT were tested, no cross-reaction was observed for the two systems.

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AROMATASE IN GOLDFISH BRAIN: CELLULAR/SUBCELLULAR LOCATION AND ROLE IN EXPRESSION OF ANDROGEN RECEPTORS

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Circulating testosterone (T) exerts actions on the CNS directly via androgen receptors (AR) and indirectly via aromatization and binding of formed estradiol (E) to estrogen receptors. We previously observed that aromatase in teleost fish brain is elevated 100-1000-fold when compared to mammalian brain. Brain AR are overexpressed to the same extent. Also, aromatase and AR show the same tissue-specific distribution and co-vary seasonally. These correlative data suggest a common regulatory mechanism or other means of functional interdependence. To test this hypothesis, brain aromatase and AR were measured biochemically after experimental manipulation of fish in vivo (control vs. gonadx, +/-E, T, DHT, aromatase inhibitors). Data indicate that in situ aromatization not only increases aromatase per se, but also increases AR. By contrast, AR are downregulated by their own ligands. To determine whether effects of locally formed E are part of an autocrine or paracrine mechanism, the neuroanatomic distribution of aromatase cell bodies, fiber projections and terminals was mapped immunocytochemically using a human placental aromatase antibody. Studies colocalizing AR - positive cells have been initiated using rat prostate anti-AR. (Supported by NSF DCB89-16809)

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EFFECTS OF ACUTE AND CHRONIC STRESS ON TIME OF OVULATION, FECUNDITY, EGG SIZE AND EGG SURVIVAL IN BROWN AND RAINBOW TROUT.

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Groups of mature male and female brown trout (Salmo trutta) and rainbow trout (Oncorhynchus mykiss) were subjected to repeated acute stress (5 min emersion) or a single episode of chronic stress (2 weeks confinement) during the 8 months prior to spawning. Time of ovulation, fecundity and egg size were recorded in mature females from both stressed and control groups. Eggs from ovulated females were fertilized with milt from males subjected to the same treatment regime. Sperm counts were carried out on the male fish and subsequent development of the fertilzed eggs was monitored.

Exposure of female fish to stress during reproductive development resulted in a significant delay in ovulation compared to control fish, and eggs from stressed females, fertilized with milt from stressed males, displayed a significantly higher mortality rate than eggs from unstressed control fish.

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STEROIDS OF MARINE FISH.

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The gonads of teleost fishes have the potential to secrete a variety of steroids during the final stages of oocyte final maturation and ovulation. We have identified several novel C21 steroids in Pleuronectiform species in *in vitro* incubations of gonadal fragments with tritiated 17α-hydroxyprogesterone as precursor. The type of steroids produced was determined by the presence or absence and relative activities of the enzymes 3(α,ß)- and 20(α,ß)-hydroxy steroid dehydrogenases (HSD), 5ß-reductase, 21-hydroxylase, sulphate transferase and glucuronyl transferase. Among the enzymes that act on the steroid nucleus, 3α-, 3ß-HSD and 5ß-reductase were present in dab (*Limanda limanda*) and winter flounder (*Pseudopleuronectes americanus*), 3α-HSD and 5ß-reductase were present in flounder (*Platichthys flesus*) sole (*Solea solea*) and plaice (*Pleuronectes platessa*). None of these enzymes was detected in halibut, (*Hipoglossoides hipoglossoides*). Of the side-chain enzymes, 20ß-HSD was present in winter flounder, 20α-HSD was present in dab and winter flounder and 21-hydroxylase was present in halibut, flounder, sole and plaice. Sulphates were the main conjugates present. The pattern of C21 steroidogenesis in these six species was highly specific and consistent with a proposed pheromonal role. Radioimmunoassay measurements in plasma and urine samples showed very high levels and a relationship between levels and maturity stage.

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SPECIES-SPECIFIC OLFACTORY SENSITIVITIES TO POTENTIAL SEX PHEROMONES IN FISH

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Although chemical communication is apparently widespread among teleosts, the chemical identity of the pheromones involved has been established in only a few species. However, in all cases reported so far, these compounds are reproductive hormones or their metabolites that are released into water where they function as pheromones. Given the low diversity of compounds that serve as reproductive hormones in fish, together with the possibility that released hormones also function as sex pheromones, it is not clear how species-isolation is maintained in sympatrically-spawning species. Electro-olfactography (EOG) is a simple and rapid quantitative technique for determining the degree of responsiveness of the olfactory epithelium to water-borne compounds. We used EOG to investigate the olfactory sensitivity of a variety of fish species to water-borne steroids, steroid-glucuronides and prostaglandins that might function as sex pheromones. In goldfish (*Carassius*), F_2-prostaglandins (PGF's) and free and glucuronated 17α,20ß-dihydroxy-4-pregnen-3-one (17,20ß-P and 17,20ß-P-G) are potent olfactory stimulants (but free 17,20ß-P >> 17,20ß-P-G). Conversely, in minnows (*Pimephales, Phoxinus, Notropis*), only PGF's and the glucuronated form of 17,20ß-P are stimulatory. More distantly related cypriniforms of the Catostomidae (*Catostomus, Moxostoma*) are anosmic to all steroids and glucuronides tested, but are highly sensitive to prostaglandins. Representatives from a number of non-cypriniform families (*Cottus, Gasterosteus, Perca, Prosopium, Percopsis* and *Hiodon*) have so far exhibited no significant olfactory response to prostaglandins, steroids or steroid-glucuronides. These results will be compared with predicted relationships between teleost mating systems and the use of hormonal sex pheromones.

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CHANGES IN THE ELECTROPHORETIC PATTERN OF THE YOLK PROTEINS LIPOVITELLIN AND POHOSVITIN DURING VITELLOGENESIS IN THE GILTHEAD SEA BREAM *SPARUS AURATA* L.

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In non mammalian vertebrates, vitellogenin, a large lipoglycophosphoprotein produced in the liver and released into the bloodstream, is sequestered by developing oocytes, proteolytically cleaved into smaller proteins which are stored in the egg yolk as lipovitellin (LV) and phosvitin (PV). The purpose of the present investigation was to resolve LV and PV components by SDS -PAGE and to monitor changes in yolk proteins (YP) during vitellogenesis in sea bream *Sparus aurata*. Ovaries were removed from fish and placed in F.O. medium (Wallace and Selman , 1978). LV and PV were isolated according to the procedure by Wallace and Begovak (1985).Ovaries were homogenized in 0.1 M phosphate buffer (pH 7) containing 2 mM PMSF, centrifuged 1 h at 15.000 g to remove a small pellet, and the supernatant adjusted to 3 M (NH4)SO4. The resulting precipitate contains LV Coomassie blue-staining bands. Five different types of LVs were resolved in SDS-PAGE with MW of 85, 72, 39, 33 and 12.5 kDa. The (NH4)SO4 supernatant, remaining after the LV precipitation step, was desalted with Bio-Gel P6-DG column and lyophilized. The resulting PV fraction could be resolved in SDS-PAGE as a complex of PV-like components revealed by Stains-all with MW of 35, 28, 24, 19 and 13 kDa. Differences in YP components were found during oocyte growth. Oocytes at different stages (0.1-0.5 mm ø) were isolated from ovaries of individual fish and pooled oocytes of the same size were analyzed by SDS-PAGE.The Coomassie blue-stainig bands of 85 and 72 kDa almost disappeared when the oocytes reached the terminal stage of vitellogenesis (0.5 mm ø). A specific changes also occurred among PV components when oocytes progressed from 0.1 to 0.5 mm in diameter: PVs were gradually reduced , while new smaller peptides (phosvettes) appeared.
In conclusion and in accordance with previous results, our data show that the emergence of lower MW bands among both LVs and PVs most likely reflects a pronounced proteolysis during the vitellogenic process.

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STRESS AND REPRODUCTION IN A COMMERCIALLY IMPORTANT MARINE FISH, *PAGRUS AURATUS* (SPARIDAE).

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Pagrus auratus is known as snapper in Australia and New Zealand, and as red sea bream in Indonesia and Japan. The species is very important for both commercial and recreational fishermen. Attempts are currently being made to farm the species and some success has been reported. However, there are still difficulties, especially concerning reproduction, with high levels of ovarian atresia being noted. This study investigates some of the effects that stress events have on reproductive function, particularly on reproductive endocrinology, in female fish.
Plasma cortisol levels of sexually mature female snapper caught by longlining were variable (range: 0.2-62 ng/ml; mean $\pm$ sem 12.6 $\pm$ 0.6; n=84). They increased to 64 ng/ml after 1 hour, and were 43 ng/ml after 6 hours of confinement. Plasma levels of estradiol (E_2) and testosterone (T) were both approx. 70% of their initial levels 1 hour after capture, and approx. 40% by 6 hours. Plasma 17α,20β dihydroxy-4-pregnen-3-one (17,20βP) levels were approx. 300% of initial levels at both 1 and 6 hours post-capture. Ninety percent of fish kept for several days in the laboratory ovulated on the day following capture, 63% on the 2nd day, 20% on the 3rd, 3% on the 4th, and none on the 5th. Plasma cortisol levels remained at about 20 ng/ml from the day of capture until the 3rd day, thereafter increasing to about 45 ng/ml on the 4th and 5th days. Plasma E_2 levels were 1.2 ng/ml on the day of capture, subsequently most individual levels were undetectable (<0.05 ng/ml). Plasma T levels also decreased to about 40% of the initial level. Plasma 17,20βP levels tended to increase over the experimental period.

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EGG QUALITY AND FECUNDITY IN THE SEA BASS (DICENTRARCHUS LABRAX) AND THE EFFECTS OF PHOTOPERIODICALLY-INDUCED ADVANCES AND DELAYS OF SPAWNING TIME.

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Recently-developed methods of producing all-year round supplies of eggs of sea bass, by photoperiodically advancing or delaying spawning, will only receive wider commercial acceptance if the quality of the eggs and fry produced and the fecundity of these broodfish are shown to be similar to those of naturally-spawning stocks. This question is considered further in the present work which examines the effects of advancements and delays of spawning of up to 5 and 3 months respectively on the quality of the egg (%age of floating or viable), the %ages of floating eggs which hatch and in turn first-feed and the relative fecundity (number of eggs/kg post-strip fish weight) of each broodfish. Under simulated natural conditions control fish have relative fecundities averaging 285,000 eggs/kg usually produced over 1 or more spawnings. Mean egg quality of control fish is 77% of which 80% and 55% survive to hatch and first-feeding respectively. In general broodfish in which spawning is advanced have higher fecundities but produce eggs and larvae of similar quality to controls, whereas fish with delayed spawnings have lower fecundities and poorer egg and larval survivals. The presence of a significant correlation of quality with temperature suggests that the higher sea temperatures of 15°C and above extant in the Mediterranean from May to September may be a significant determinant of egg and larval quality in the sea bass.

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EFFECTS ON FECUNDITY AND EGG QUALITY OF THREE DIFFERENT DIETS SUPPLIED DURING TWO REPRODUCTIVE CYCLES TO BROODSTOCK SEA BASS (DICENTRARCHUS LABRAX)

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Broodstock sea bass was fed over a consecutive two-year period with one natural diet (ND = Boops bopps muscle) and two comercial diets (D1 = 55% (P)roteins, 11% (L)ipids, 19% (C)arbohydrates and D2 = 47% P., 7% L., 30% C.), and its effects tested on natural spawning (SP) and egg quality. In order to asses the possible effects of alternating diets, in the second year, group D2 was changed to D1 (D1' group). During the first reproductive cycle, a low relative fecundity was observed in all groups. ND and D1 did not result in differences in %ages of floating eggs, hatching rates and survival to first feeding although fish fed with D1 and D2 delayed mean SP period. D2 also had a reduced SP spread with low %age of floating eggs and null hatching rates. Moreover, the egg mean diameter was significant smaller than ND and D1, in accordance with a slightly higher relative fecundity observed. The biochemical composition of eggs from D2 did not show differences, except for glycogen. In the second year, ND resulted in a strong increase in fecundity, while in D1 and D1' decreased. These groups maintained the delay in mean SP period. The quality of eggs in D1 was poorer with null hatching rates, and D1' exhibited egg diameter egg protein content and hatching rates lower than ND. These results suggest a long time deleterious effects on fecundity and egg quality of inadequately formulated diets.

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TWO NATIVE GNRH PEPTIDES STIMULATE GONADOTROPIN RELEASE IN THE GOLDFISH BY DIFFERENT SECOND MESSENGER COMPONENTS.

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In the goldfish, two gonadotropin (GTH)-releasing hormone forms, salmon (sGnRH) and chicken-II (cGnRH-II), have been identified. Both GnRHs are released, compete for the same class of receptors on dispersed pituitary cells, and stimulate GTH release. The GTH responses to sGnRH and cGnRH-II are reduced and abolished by the dopamine agonist apomorphine (APO), respectively. This suggests the presence of different second messengers for the two GnRHs. In this study, the extracellular (e)-Ca^{2+} and arachidonic acid (AA) dependence of the signal transduction pathways mediating sGnRH and cGnRH-II stimulation of GTH release were compared using dispersed goldfish pituitary cells in static culture. Replacement of normal testing media with Ca^{2+}-deficient media (without $CaCl_2$ and with 0.1 mM EGTA) partially decreased sGnRH-induced GTH release but abolished the response to cGnRH-II. Compared to sGnRH, cGnRH-II actions were also more sensitive to inhibition by the blockade of Ca^{2+} entry with $CoCl_2$ and with the voltage-sensitive Ca^{2+} channel antagonists, verapamil and nifedipine. AA-induced GTH release was e-Ca^{2+} independent and unaffected by APO. Blockade of AA metabolism by the lipoxygenase enzyme inhibitors NDGA and ETYA abolished the AA-elicited and reduced sGnRH-stimulated responses in previous experiments, but had no effects on cGnRH-II action in this study. The NDGA- and APO- induced inhibitions of sGnRH action were additive. These results indicate that 1) the different sensitivity of sGnRH and cGnRH-II actions to e-Ca^{2+} may in part be mediated by voltage-sensitive Ca^{2+} channels; 2) in contrast to sGnRH, AA metabolism, an e-Ca^{2+} independent component, is not involved in cGnRH-II mechanisms of action; 3) AA and dopamine affect GTH release via nonoverlapping pathways; 4) the difference in AA involvement is also consistent with the differential sensitivity of these GnRH peptides to dopamine inhibition. These data support the novel hypothesis that two native GnRH peptides competing for the same receptors activate different signal transduction components to stimulate GTH release in the goldfish. (Supported by NSERC grant U-0552 to JPC.)

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CRYOPRESERVATION OF MALABAR GROUPER, *EPINEPHELUS MALABARICUS*, SPERM.

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The volume of collectible milt of protogynic hermaphroditic malabar grouper, *Epinephelus malabaricus*, was extremely limited and thus, cryopreserved sperm is of high significance for artificial propagation. Suspension solution of sperm from sliced testes or suction of sperm from genital papilla with micro-hematocrit capillary tubes were found to be efficient means to obtain raw milt. DMSO and glycerol with an addition of 15% INRA Menezo B_2 medium, a kind of tissue culture medium, gave much better dilution and cryoprotective function than DMSO and glycerol alone in freezing sperm at stepwise freezing protocol. Microwave thawing appeared to be potentially beneficial for simultaneous thawing of a batch of 0.5 ml straws containing milt mixture. Motility of prefreezing and post-thawing sperm analyzed by Hamilton-Thorn Motility analyzer has shown that one- and three-month cryopreserved grouper sperm had slightly less but still satisfactory motility as compared with fresh ones. Artificial propagation using 90 min-, 1 day-, and 17 day-cryopreserved sperm with fresh eggs resulted in fertilities of 95.7% (vs. 93.8% in control), 93.2% and 85.0% (vs. 65.6% in control), respectively.

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TESTOSTERONE TISSUE BINDING IN DWARF MALES OF ATLANTIC SALMON.

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Testosterone (T) cytosol specific binding (SB) in brain and testis significantly changes during the reproductive cycle in dwarf males and to a certain extent depends on T level dynamics in blood.In course of sexual maturation SB-T in brain increases,while in testis it decreases.By the end of functional maturity period SB-T in brain begins to decrease,but it grows in testis.During post-spawning season,in the end of winter and in spring,SB--T lowers both in brain and testis down to juvenile fish level. With the start of repeated ripenning,SB-T in both tissues increases again.T treatment of spent dwarf males in winter first results in SB-T supression in brain and testis,but then in spring follows its compensatory increase.The results are discussed in connection with differentiation mechanism of spent dwarf males into two types:1-undergoing parr-smolt transformation and failing to mature,2-repeatedly ripenning as parr.The following earlier obtained data are taken into account:a)T level dynamics during the quiescent phase of reproduction reveals individual variations;b)positive feedback regulation of gonadotropic function by T is preserved after attaintment of sexual maturity;c) T supresses gonad recrudence in spent dwarf males and prevents smolting.

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SEASONAL REPRODUCTIVE HORMONE PROFILES IN OREOCHROMIS MOSSAMBICUS

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Oreochromis mossambicus is a freshwater fish currently being considered for culture purposes in Southern Africa. This study describes the seasonal hormonal profiles for both sexes. Specimens of both sexes were collected, on a monthly basis, for a year. Gonads were homogenized, centrifuged and the supernatants used to determine estrogen, progesterone, testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels.
In the male gonadal testosterone levels reached a maximum of 12.30±4.70ng/ml during early summer, which coincided with peak gonadal development. LH and FSH radioimmunoassays suggested the possibility of a single gonadotrophic hormone, having a maximal value of 37.88±3.11mIU/ml during midwinter. Scanning and transmission electron microscopy showed a large number of developing spermatids in the testis at this time.
In the female the LH/FSH levels peaked at 46.98±4.73mIU/ml during spring. The progesterone levels reached a maximum of 49.75±2.38ng/ml toward the end of summer in February whereas estrogen levels peaked at 213.86±11.74ng/ml one month later.
These results are discussed in relation to the breeding activities of this species.

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cAMP dependence of movement Iniation *In vivo* and In demembranated trout spermatozoa

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The initiation of movement of demembraneted trout sperm was investigated in various incubation conditions relative to previous phases of *in vivo* movement and to ATP-Mg concentrations. When reactivated in the absence of cAMP and in the presence of ATP-Mg<25 uM ,all spz (100%) were active with beat frequencies(B.F.) up to 15-20 Hz whathever be their previous physiological conditions of preincubation *in vivo* (allowed or not to swim previously to reactivation) . In contrast, the increase of the ATP-Mg concentration between 25 to 100 uM (or above) decreased proportionally the fraction of active spermatozoa but increased the frequency of the active ones so that at 1mM ATP-Mg only 5% were active with a (B.F.=65 Hz) : the addition of cAMP (20 µM) restored activity to 100% spz (similar B.F.) .

At ATP-Mg > 25 µM, the presence of cAMP was true in a concentration manner only in the reactivation medium but not during the demembranation step. In addition it was found independant of a previous *in vivo* phase of movement . The antagonist effect of ATP-Mg relative to that of cAMP were tested at various concentrations of both : the apparent affinity of cAMP (measured as the concentration restoring 50% movement) was decreased from 15 nM at 0.1 mM ATP to 0.5 µM at 1 mM ATP ; conversly the ATP-Mg affinity (measured as the half maximal beat frequency) was not affected by increasing concentrations of cAMP (up to 0.5 mM).

In absence of added cAMP, the possible presence of residual low concentration of cAMP carried by the sperm through the dilution steps previous to reactivation was tested by preincubation in the presence of Phosphodiesterase or of Protein-kinase inhibitors : in both cases 100% of spz were still reactivated when 25 uM ATP-Mg was added to the demembranated spermatozoa.

Preliminary measurements of cAMP content of spz at low temperature show that a cAMP rise occurs slowly but tend to a maximum much later after 100% spermatozoa are activated (HOUDEAU et al. unpublished). It is concluded from this whole set of results that cAMP is certainly involved in regulation of trout sperm axonemal movement as already stated (MORISAWA M. & OKUNO M. 1982 Nature 295 703-705)but is not of absolute requirement and in this respect not a key event in triggering in vivo trout sperm movement.

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HORMONAL MANIPULATION OF FISH SEASONAL REPRODUCTIVE CYCLES

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Precise seasonal timing is a common feature of the annual reproductive cycles of fish in the wild. Indeed, particular reproductive events including gonadal development, spawning activities and a period of reproductive quiescence occur at specific times of the year. For fish brought into captive conditions, some species appear to function normally displaying seasonal reproductive cycles and even spawning spontaneously in laboratory tanks. In contrast, other species of fish undergoing domestication fail to accomplish the expected reproductive events in captivity or they may reproduce yielding reproductive products of questionable quality. Gonadotropic hormone releasing hormone is known to induce spawning in mature fish; in addition, recent evidence suggests this hormone accelerates seasonal development of the gonads. Testosterone, which promotes gonadal development in some fish, can effectively increase the rate of rematuration of repeat spawning broodstock fish. Hormonal therapy, especially the use of gonadotropic hormone releasing hormone and sex steroid hormone treatment, appears to offer a promising practical approach for control of reproductive cycles of fish held in captivity.

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USE OF PLASMA 11KT LEVELS FOR SEX DETERMINATION IN IMMATURE SIBERIAN STURGEON ACIPENSER BAERI B.

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Sex determination can be applied in several fields. In fishfarming it allows early selection of the breeding stock for artificial reproduction. In protected wild species it is of great interest in the study of population dynamics.

As there is no morphological difference between males and females in Siberian sturgeon, Acipenser baeri B., sex determination is only possible by means of biopsies carried out from the start of the first reproductive cycle. The problem is that this species reaches marketable size, that is 3-year-old fish weighing 1.5 to 2 kg, before its first gametogenesis. Therefore a rapid and simple test based on the detection of a sex specific plasma parameter would be of great interest to allow the selection of breeding stock from this immature population bred in fishfarm.

In previous studies in trout 11KT plasma levels were found to be significantly higher in males. This study confirms that 11KT plasma levels are also significantly higher in 18-month-old and 3-year-old immature Siberian sturgeon : 72% of 18-month-old fish were correctly sexed and this increased to 83% after carp pituitary extract stimulation ; 100% of 3-year-old fish were correctly sexed without carp pituitary treatment.

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THE EFFECTS OF FLUCTUATING SEASONAL AND CONSTANT TEMPERATURES ON THE PHOTOPERIODIC ADVANCEMENT OF REPRODUCTION IN FEMALE RAINBOW TROUT.

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Recent studies have indicated that the reproductive cycle of rainbow trout is controlled by an endogenous circannual clock which entrains to the yearly photoperiodic cycle. Most of this work has been conducted at constant temperatures of around 10°C. The effects of temperature on reproduction have not been extensively studied in salmonids although it has been suggested that temperature influences reproduction by acting directly on gonadal development rather than by altering the endogenous clock. This study was conducted in order to determine the effect of temperature on the timing and viability of reproduction. Four groups of 2 year old, post-spawned, female rainbow trout were exposed to the following photoperiod and temperature regimes, commencing in Feb; Group A-ambient photoperiod, river water (0-21°C); Group B-18L:6D to May 10, then 6L:18D, river water; Group C-ambient photoperiod, borehole water (8.5±1°C); Group D-18L:6D to May 10, then 6L:18D, borehole water. The two groups exposed to the stimulatory photoperiod (B&D) showed significant 4 month advances in spawning (Aug/Sept) compared to groups (A&C) on ambient photoperiod (Dec/Feb). Egg viability was comparable in all four groups. Groups C&D, in borehole water, both showed significant 3-4 week advances in spawning compared to groups on similar photoperiods but ambient water temperatures. Notwithstanding a modifying influence for temperature, it is clear, however, that the primary cue for the entrainment of reproduction in the rainbow trout is photoperiod.

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ABSOLUTE DAYLENGTH AND THE ENTRAINMENT OF AN ENDOGENOUS CLOCK CONTROLLING REPRODUCTION IN THE FEMALE RAINBOW TROUT.

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The seasonal reproductive cycle of rainbow trout is controlled by an endogenous circannual clock which is entrained to the yearly photoperiodic cycle. It has been suggested that it is the direction of change of daylength rather than the absolute duration of the light period which is important. This is further investigated in the present study. Five groups of virgin female rainbow trout were maintained in light-proof tanks and exposed to the following photoperiod regimes beginning in January: Group A-22L:2D to May 8, then 13.5L:10.5D; Group B-18L:6D to May 8, then 9.5L:14.5D; Group C-14L:10D to May 8, then 5.5L:18.5D; Group D-12L:12D to May 8, then 3.5L:20.5D; Group E-8.5L:15.5D to May 8, then 1.5L:22.5D. Groups A-D all commenced spawning in August, 4 months in advance of the natural spawning period. Thus the magnitude of the increase in daylength is not important for advancement spawning provided there is a rise relative to the ambient photoperiod. Group E commenced spawning during October, 2 months in advance of natural spawning but significantly later than groups A-D, showing that spawning can be advanced in fish who have not experienced a long day increase. The two month advance seen in Group E is therefore due to a single phase advance of the endogenous rhythm produced by the reduction in May. Thus, it is the actual direction of change of daylength rather than daylength per se which entrains the endogenous clock.

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GENETIC AND HORMONAL CONTROL OF SEX DETERMINATION IN CHANNEL CATFISH

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Channel catfish (Ictalurus punctatus) normally produce progeny with a 1:1 sex ratio; however, exogenous estrogens or androgens given in the diet during the first 21 days of feeding result in all-female populations. When these fish were raised to maturity and spawned with normal male fish, one half of the fish had spawns with normal sex ratios, but the other half produced spawns with a 1:3 female:male ratio. These data indicate that the genetic sex determination model is homogametic for females (XX). Fifteen of 55 spawns produced between males from the 1:3 spawns and normal female fish produced all male progeny, suggesting these 15 male fish have a YY sex genotype. When these males were crossed with a confirmed XY female, all-male populations resulted which should have XY and YY sex genotypes in equal proportions. These progeny were feminized with ethynyltestosterone, demonstrating that the YY genotype can be feminized. By these genetic and hormonal manipulations, we have produced phenotypic females which have XX, XY and YY sex genotypes and male fish which have XY and YY sex genotypes. Although a variety of aromatizable and nonaromatizable androgens have been tried, no hormonal treatment has been found to produce XX males.

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Induction of maturation by carp gonadotropin, 17α-hydroxyprogesterone and 17α,20β -dihydroxy-4-pregnen-3-one in the oocytes of Blue gourami Trichogaster trichopterus, (Anabantidae, Pallas, 1770)

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ABSTRACT

The effectiveness of 17α-hydroxyprogesterone (17-P), 17α,20β - dihydroxy-4-pregnen-3-one (17,20-P) and carp gonadotropin (cGtH) in inducing sensitivity to final maturation of oocytes was examined in vitro in female Trichogaster trichopterus (Pallas, 1770). Induced sensitivity was measured at two stages of ovary development: low and high vitellogenesis (<25% and >45%).

No influence was detected, on ovaries at the stage of low vittelogenesis, by treatment with 17-P, 17,20-P, cGtH or any combination of these hormones, in either the experimental or control groups.

In ovaries with a high percentage of vitellogensis (>45%), in vitro treatment with 100 ng/ml and 300 ng/ml cGtH significantly increased vitellogenesis. In vitro treatment with 600 ng/ml cGtH, with 300 ng/ml cGtH and with various concentrations of 17-P caused a certain percentage of maturation. Various combinations of cGtH, 17-P and 17,20-P also significantly increased the percentage of oocytes in maturation, and even induced ovulation.

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ISOLATION OF SALMONID Y-CHROMOSOMAL DNA PROBES AND THEIR APPLICATION TO MONOSEX CULTURE

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Current sea-water aquaculture facilities in British Columbia grow primarily chinook salmon, Oncorhynchus tshawytscha (about 80%), and of these, over 95% are derived from the all-female strain developed at the West Vancouver Laboratory. The synthesis of monosex strains was accomplished by crossing masculinized genetic females (XX) with regular females to produce all-female lines. This procedure utilized a test cross to distinguish genetic males and females within the masculinized broodstock, and thus required families to be raised individually, using a large number of separate tanks, and requiring two generations to complete. To circumvent these difficulties, we have used a subtractive hybridization procedure (PERT) to isolate a DNA fragment from the Y chromosome from chinook salmon. Using Southern blots, this DNA clone has accurately diagnosed genetic sex in over 100 individuals, and is now being used to generate new monosex strains in a single generation and to verify the genetic constitution of existing monosex strains. We have now sequenced this chinook salmon clone and developed a rapid PCR-based assay that allows us to determine genetic sex in a single day from a small drop of blood or fin clip.

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ESTROGEN DIRECT FEMINIZATION OF CHINOOK SALMON

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Since monosex female spermatozoa have yet to be developed for several salmonid species, it is of interest to compare the estrogenic potency of several estrogens and to establish optimal treatment regimes for direct feminization. Estradiol-17β (E2) and ethynylestradiol-17α (EE2), at a concentration of 400 µg/l, were administered to chinook salmon (Oncorhynchus tshawytscha) in a single immersion treatment (IT). Treatment durations were 1,2,4 or 8 hours. EE2 proved to be extremely potent, since 97.6% females were obtained with a single 1h IT and complete feminization was achieved with a 2h IT. However, higher doses progressively reduced the number of females to 83%. On the other hand, E2 showed a clear duration of treatment-response relationship. Thus a single 2h IT resulted in 72.2% females, while 100% females were obtained with a 8h IT. In the all-female groups produced, between 11.1 and 40 % of the females exhibited ovaries with oocytes much smaller than that those of the controls. Regarding growth, these single ITs resulted in a slight increase in both weight and length, contrary to what had been reported for estrogens in some other studies. The analysis of data showed that this increase was related to the hormone administered but not to the duration of the treatment itself.

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STIMULATION OF PITUITARY GONADOTROPIC FUNCTION IN FEMALE SILVER EEL TREATED BY A GONADOLIBERIN AGONIST (GnRH-A) AND A DOPAMINE ANTAGONIST (PIMOZIDE).

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In the European silver eel, *Anguilla anguilla*, a deficiency in pituitary gonadotropin (GTH) production is responsible for the blockage of gonadal development at a juvenile stage. External factors, encountered during the reproductive migration, are likely to trigger pubertal stimulation of gonadotropic function through the mediation of hypophysiotropic neurohormones. In eels pretreated by sexual steroids (which are potent stimulators of GTH synthesis) we previously demonstrated the GTH-releasing effect of GnRH combined with antidopaminergic drugs; a positive effect on GTH synthesis was also suggested. We investigated here the effect of a chronic treatment (2 weekly injections over 1 or 2 months) by GnRH-A (0.1 µg/g bw) and pimozide (5 µg/g bw) in female eels which had not received steroids. GnRH-A combined with pimozide induced a significant increase in pituitary GTH content as determined by radioimmunoassay, indicating a stimulation of GTH synthesis; plasma GTH levels remained undetectable but a rise in GTH release was suggested by the slight but significant increase in gonadosomatic index after 2 months. These changes in pituitary-gonadal axis were similar to those previously observed in eels submitted to high hydrostatic pressure (immersed for 3 months in deep-sea). GnRH-A or pimozide given alone were without any effect. Similar results were obtained in eels maintained in freshwater or transferred to seawater. These results obtained in absence of any steroid treatment clearly demonstrate the stimulatory effect on GTH synthesis of GnRH combined with pimozide. A stimulation of GnRH neurons and an inhibition of dopaminergic neurones are likely to mediate some of the triggering effects of external factors on eel puberty.

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A REDUCTION IN DOPAMINE TURNOVER IN THE PITUITARY IS ASSOCIATED WITH SEX PHEROMONE-INDUCED GONADOTROPIN SECRETION IN MALE GOLDFISH.

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In goldfish, the gonadal steroid, 17α,20β-dihydroxy-4-pregnen-3-one (17,20B-P), functions as a potent preovulatory female sex pheromone which stimulates rapid elevations in serum gonadotropin (GtH) levels and subsequent increases in milt production in males. GtH secretion in goldfish is known to be regulated by the stimulatory actions of gonadotropin releasing hormone (GnRH) and the inhibitory actions of dopamine (DA). This study specifically examined whether the 17,20β-P-induced elevation in male GtH is caused by pheromone mediated changes in DA inhibition at the level of the pituitary (PIT). Temporal changes in circulating levels of GtH and changes in PIT content of DA and its metabolite, dihydroxyphenylacetic acid (DOPAC), as well as possible alterations in DA turnover rate (DOPAC/DA ratio) were measured following short-term exposure of male goldfish to water-borne 17,20β-P. Water-borne 17,20B-P consistently increased serum GtH levels in males within 15 min of exposure and maintained elevated levels for up to 2 h. Although changes in pituitary DA content were not observed during periods of high GtH release, coincident reductions in PIT levels of DOPAC were measured within 45 min of exposure to the pheromone. Moreover, the pheromone caused a decrease in the rate of DA turnover in the PIT, as assessed by comparing the ratio of DOPAC to DA present, within 20 min of exposure. Since the reduction of DA turnover in the PIT is inversely correlated with periods of increased GtH release, the present results suggest that water-borne 17,20β-P causes an abatement of DA release to the PIT which reduces dopaminergic inhibitory tone on GtH secretion. Based on the latency of the GtH response to water-borne 17,20B-P, a rapid reduction of DA inhibition to the PIT may serve as a neuroendocrine trigger for pheromone-induced GtH release.

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INDUCTION OF VITELLOGENESIS IN EUROPEAN EEL (ANGUILLA ANGUILLA) WITH CARP PITUITARY SUSPENSION.

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The traditional dose of hCG, cPS and sPS for the induction of vitellogenesis, maturation and ovulation in eel results hardly in the production of viable larvae. A common recipee consists of a dosis of 10-20 mg cPS/kg fish (injected with time intervals of 2 to 7 days) resulting in estimated maximum hormone levels of 1400-2800 ng GtH/ml plasma.
In several other fish species, GtH plasma levels ranging from 3-15 ng/ml (as measured as GtH-II) correlates with succesful vitellogenesis. In order to induce comparable GtH levels in European eel, frequent injections of 0.08 mg or 0.15 mg cPS/kg fish were administered which resulted in constant plasma levels ranging between 3-13 ng and 7-25 ng ir-cGtH/ml respectively.
In a subsequent experiment (75 female silver eels) two plasma ir-cGtH levels of 6-20 ng/ml and 18-60 ng/ml (fluctuations per 48 hours per fish) were induced during a 12 week experiment. The progress of vitellogenesis was monitored.

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DIFFERENT LIGHT REGIMES AFFECT GROWTH AND SEXUAL MATURATION IN ATLANTIC SALMON POSTSMOLTS.

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Growth rate and incidence of sexual matturaration is of great importance in fish farming. We have tested the hypothesis of light serving as an inducing factor for higher growth rate and if it will affect sexual maturation. Immature postsmolts (1+) of Atlantic salmon were reared in sea cages under 6 different light regimes from 26 September 1989 to 27 July 1990. The light regimes were obtained by use of natural photoperiod (NL), and natural photoperiod with 24 hour additional illumination (AL). Additional light was supplied from 1000W halogen lamps mounted 1.2 m above sea level in each sea cage. The different light regimes were as follows: NL from September to July, NL from September to December thereafter AL, NL from September to Januar thereafter AL, AL from September to December thereafter NL, AL from September to Januar thereafter NL, and AL from September to July. All groups had the same feeding regime during the experiment. All groups showed an initial decrease in growth rate when additional light was put on, followed by an increase after approximately six week. Consequently the mean weight was 30% higher in the group which recieved additional light throughout the study compared to natural light. The incidence of sexual maturation as grilse ranged from 3 to 17% among the groups, earlier and longer periods of additional light giving higher incidence of maturation. The results demonstrate that Atlantic salmon exposed to additional light during winter will have a higher growth rate compared to natural photoperiod. The higher growth rate may however cause higher incidence of maturation.

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SEX-SPECIFIC GONADAL PROTEINS IN SALMONIDS

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The capacity of steroid hormones to direct sex differentiation in many fish species raises questions about the role that endogenously produced steroids may play during normal sex differentiation. If steroids influence differentiation of the gonads, it is likely that it is through modification of protein synthesis in the gonads. Sex-specific gonadal proteins from salmonid tissue homogenates have been visualized as early as 5 months from fertilization using electrophoretic techniques, such as SDS-PAGE. In preliminary experiments, rainbow trout (*Oncorhynchus mykiss*) were fed either estradiol or methyltestosterone to control sexual development. The production of an ovarian protein of about 50 kD molecular weight diminished after dietary treatment with either steroid. It is unclear what effect steroids have on a testicular protein of about 80 kD molecular weight. Antisera have been raised to these proteins which allow detection under normal development or experimental regimens for controlling sexual development.

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REPRODUCTIVE PROBLEMS IN LAKE ERIE COHO SALMON

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Over the past decade, coho salmon eggs from the American side of Lake Erie have consistently had a survival to hatch of 25 to 40%, relative to > 80% for other Great Lakes stocks. In a study to investigate this phenomenon, coho salmon were collected from the Fairview Fish Culture Station, Fairview, Pennsylvania, from 1987-1990. Although all females appeared healthy and eggs appeared viable, the mean fertilization rate was 41.8%. Cross-fertilization trials with a self-sustaining population from the Canadian side of Lake Erie (which averaged 84% fertilization and survival to hatch) showed that the Fairview eggs were the source of the low fertility. All Fairview females contained over-ripe eggs, usually attached to the ovarian stromal tissue in the body cavity. As the fertilization rate decreased, the incidence of "mooneyes" and fry deformities increased. The poor fertility appears to be related to a breakdown in the timing of egg final maturation, rupture from the follicle, and/or vent maturation, which cannot be accounted for by steroid differences, altered thyroid status, or contaminant residues at this time.

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ASPECTS OF SEX-STEROID BINDING PROTEIN (SBP) REGULATION IN RAINBOW TROUT

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The respective role of sex steroids and hormones involved in growth and metabolism, on SBP regulation have been studied *in vivo* an *in vitro*.
In vivo: estradiol (E_2) injection (0.5 mg/kg) or implantation (5mg/kg) respectively in mature males or juveniles, induced a 50 to 60 % increase of plasma SBP levels, that started several days after treatment.
In vitro: hepatocytes in primary culture produced an SBP. E_2 addition (1 to 100 nM) stimulated this production up to 400 %, while testosterone or 17α20ßDHP had no significant effect in these experimental conditions.

Recombinant trout growth hormone (rtGH, 0,01 to 1 µg/ml) increased SBP accumulation in hepatocyte culture medium (maximum : + 100 %) and IGF1 might be involved in this GH action. Furthermore, insulin at micromolar concentration tended to be inhibitory while thyroid hormones had no effect.

We demonstrate that the liver is a site of SBP production in trout and that this production is potentially regulated by estrogens. These results also support our previous finding that androgens have little or no inhibitory influence on SBP and that the somatotrophic axis is involved in this protein regulation.

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INHIBIN/ACTIVIN-LIKE PROTEINS MEDIATED FEEDBACK SYSTEM BETWEEN THE PITUITARY AND OVARY IN GOLDFISH

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The primary purpose of this research was to determine whether a nonsteroidal feedback system mediated by such gonadal regulators as inhibin and activin exists in goldfish. Mature goldfish ovaries were homogenized and extracted in phosphate buffered saline (PBS). The extract was then treated with charcoal-Dextran to remove the endogenous steroids. By use of a goldfish pituitary fragment perifusion system, it was found that the steroid-free goldfish ovarian extract acutely stimulates gonadotropin (GTH) release. After precipitation, ion exchange chromatography and gel filtration, two partially purified fractions with acute stimulatory effects on GTH secretion were obtained. By use of an immunoblotting technique, cross-reactivities with anti-porcine inhibin/activin subunits were demonstrated in the active chromatographic fractions from goldfish ovary. Further experiments showed that steroid-free porcine follicular fluid (pFF) containing high concentrations of inhibin and activin stimulated , rather than inhibited, GTH secretion in goldfish. Consistent with these results, porcine inhibin A and activin A both have acute stimulatory effects on goldfish GTH secretion in a dose-dependent manner. The effects of porcine inhibin A and activin A cannot be blocked by a specific GnRH antagonist, indicating their actions are not mediated by endogenous GnRH released from the nerve terminals nor by acting through GnRH receptors. These data suggest that a feedback system mediated by inhibin/activin-like proteins exists in goldfish with the actions of these proteins being stimulatory on GTH release.

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IMMUNOCYTOCHEMICAL/BIOCHEMICAL LOCALIZATION OF AROMATASE IN GOLDFISH RETINA

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To fully elucidate the autocrine/paracrine role of estrogen (E) formed in neural tissues from circulating testosterone (T), it is essential to localize aromatase cell-by-cell. We report here the presence of aromatase-positive cells in goldfish (Carassius auratus) retina. Frozen fixed sections were incubated with a rabbit anti-human placental aromatase (donated by C. Mendelson) using the avidin-biotin technique. This antibody interacts with goldfish brain aromatase, as determined by inhibition of catalytic activity in our standard assay and the presence of immunoreaction product in cells of the hypothalamus, preoptic area, and telencephalon. In retina, some labeled cells were located in the ganglion cell layer and among amacrine, bipolar and horizontal cells of the inner nuclear layer. Labeled fibers projected laterally within the outer synaptic layer and centrally toward the optic nerve. Control sections (no primary antibody) were negative. Retinal aromatase was verified by formation of [3H]E from [3H]T by retinal homogenates and cultured retinal cells. Other studies have shown GnRH immunoreactivity in terminals of teleost retina. Also, the retina recieves projections of the terminal nerve, a major pathway for pheromonal triggering of sexual arousal in fish and the locus of GnRH fibers. Being structurally well-defined and easily accessed, the retina may serve as an adventageous model in which to study estrogen modulation of sensory input. (NSF DCB899-16809).

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SOME REPRODUCTIVE CHARACTERISTICS OF COMMON DENTEX, Dentex dentex - NEW FISH IN MEDITERRANEAN AQUACULTURE

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Because of fast growth and high market value common dentex, Dentex dentex has become a very interesting fish for scientific researches and commercial breeding in Mediterranean area. In order to start production of this fish, investigations on reproduction and larval rearing were carried out from 1986-1990.

Common dentex spawn in Adriatic sea from March to the middle of June. In the captivity, males mature spontaneously which is not always the case with the females. Positive response to different doses of human gonadotropin (500 and 1500 I.U./kg) were recorded. Each spawner gave an average of about 70000 eggs per kg of wet weight. Maturation of oocytes was not synchronized and eggs were produced by several spawnings over 10-15 days. Two peaks in egg release were observed, on the first and seventh-eigth day of spawning. It was established that fish is very sensitive to handling, so it is better to leave the fish to spawn spontaneouslly than stripping. Period between hormonal treatment and first egg release lasted from 50 to 100 hours, at ambient temperature (16-18°C). Percentage fertilization varied from 70-80 during the first few days, while later it dropped to 30-40 %.

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THE ROLE OF SIGNAL TRANSDUCTION IN THE CONTROL OF FISH REPRODUCTION

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Hormones and paracrine mediators that interact with specific membrane receptors, stimulate cellular processes through the activation of *signal transduction pathways* within target cells. Research has shown that these pathways are quite varied and include at least; 1) cyclase catalyzed cyclic nucleotide production, 2) phospholipase C catalyzed phosphatidylinositol (PI) cycling, 3) phospholipase D catalyzed phosphatidylcholine (PC) breakdown, 4) lipase catalyzed eicosanoid precursor release, and 5) direct mediator stimulated protein kinase activity. In the first pathway, mediators can either increase or decrease the levels of cAMP or cGMP by modulation of adenylate or guanylate cyclase and/or phosphodiesterases. In the second, PI cycling results in the formation of inositol phosphates and diacylglycerol. Certain inositol phosphates (e.g. IP_3) promote the release of Ca^{++} from internal stores and/or possibly promote Ca^{++} entry through the plasma membrane. The Ca^{++} and diacylglycerol may then stimulate protein kinase C (PKC). The breakdown of PC results in the formation of phosphatidic acid (intrinsically active) that can also be converted to diacylglycerol (PKC activator). In the fourth pathway, eicosanoids (e.g. prostaglandins, leukotrienes) can be produced from unsaturated fatty acid precursors such as arachidonic acid. These precursors may be released by lipases acting on the diacylglycerol produced by PI or PC breakdown, or by direct receptor-mediated phospholipase A_2 stimulation. In the area of fish reproduction there have been several studies that have focused on the mechanism and physiological role of signal transduction. Investigations on the stimulation of follicular steroidogenesis by gonadotropins and the stimulation of oocyte meiotic maturation by steroids have studied the role of cyclic nucleotides, while investigations on the regulation of ovulation, steroidogenesis, prostaglandin synthesis and gonadotropin release have studied the PI/PKC and/or eicosanoid systems.

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GNRH'S IN THE AFRICAN CATFISH, *CLARIAS GARIEPINUS*: CHARACTERISATION, LOCALISATION AND RECEPTORS

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Two GnRH-like peptides were detected in the African catfish brain, catfish GnRH-I and -II. Catfish GnRH-II being identical to chicken GnRH-II.
By means of very specific antibodies both peptides could be localised in nerve endings close to the gonadotropic cells in the proximal pars distalis of the pituitary. The two peptides are not only co-localised in the nerve endings but even within the secretory vescicles.
The binding of catfish GnRH-II to GnRH receptors in the pituitary was characterised and compared to the binding of other GnRH's, catfish-II showing a relatively low binding affinity.
D-Arg6- and D-Trp6-analogue have a respectively lower and higher affinity to the catfish-II binding sites if compared to catfish-II GnRH.
Catfish-II GnRH was found to stimulate the release of maturational gonadotropin (GTH-II). This stimulation was not only counteracted by dopamine, but also by GABA.

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CLEARANCE RATES OF DIFFERENT FORMS OF GnRH FROM THE CIRCULATION OF THE GILTHEAD SEABREAM, *SPARUS AURATA*.

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Our previous research demonstrated a correlation between *in-vivo* bioactivity of native GnRH and its analogs and their *in-vitro* resistance to enzymatic degradation in *Sparus aurata*. The aim of the present study was to determine the relationships between the resistance of different forms of GnRH to degradation, their bioactivities and their relative *in-vivo* clearance rates from the circulation. Seabreams were given an intravenous injection of native salmon (s) GnRH and of three GnRH analogs. Fish were bled at frequent intervals following the injections, and circulating levels of the peptides were measured by specific radioimmunoassays. The native hormone, which is highly susceptible to enzymatic degradation, disappeared from the circulation relatively fast, its $t_{1/2}$ being 5.5 min. The studied analogs- [D-Arg^6-Pro^{9}Net]-sGnRH, [D-Ala^6-Pro^{9}Net]-LHRH and D-Trp^6-LHRH, which are resistant to enzymatic degradation, were cleared from the circulation much slower, their $t_{1/2}$ ranging from 19 to 25 min. These results indicate a good correlation between *in-vivo* bioactivity of GnRH and analogs and their both *in-vivo* and *in-vitro* disappearance rates.

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PRODUCTION OF GYNOGENETIC CHANNEL CATFISH BY MEIOTIC AND MITOTIC INHIBITION

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Meiotic gynogenesis can reduce heterozygosity in the first generation equivalent to 2-6 times that obtained by sib-mating, while mitotic gynogenesis can produce completely homozygous individuals. These techniques can be utilized to accelerate selection of catfish strains with characteristics suitable for commercial production. Eggs from channel catfish females were fertilized with irradiated sperm from blue catfish males. Meiotic gynogenesis was induced by early pressure shock (10 min after fertilization; 8000 PSI; 3 min duration), and mitotic gynogenesis was induced by late pressure (90 min). Untreated eggs fertilized with irradiated sperm exhibited slow and abnormal development, and did not survive. Survival to hatch was 2% for meiotic and 0.05% for mitotic gynogens compared to 27% for stripped controls (untreated eggs and sperm). No paternal contribution was detected in gynogenetic offspring from analysis of 13 loci polymorphic between blue and channel catfish. Estimates of gene-centromere recombination frequency for six loci polymorphic in channel catfish ranged from 0.36 to 1.00 (mean, 0.62). Genetic linkage was not detected by joint segregation analyses of five locus pairs. Supported by USDI#14-16-009-89-92.

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EGG QUALITY IN HALIBUT (Hippoglossus hippoglossus L.)

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Egg quality has been investigated in unfertilised eggs from four captive halibut (Hippoglossus hippoglossus L.) during a spawning season. Egg quality was characterised by the content of free amino acids, fatty acids and percent of fertilisation. The percent of survival to day 8 were used to determine viability. Egg diameter and dryweight were measured. The ovulatory rythmes were monitored to avoid overripening of the eggs. Free amino acids were anlysed by pre-column derivatisation with o-Phthalaldehyd/2 Mercaptoethanol on HPLC (High Performance Liquid Chromatograph). The fatty acids in total egg lipid were analysed on a Gas Chromatograph (GC).

Free amino acids are considered to be an important energy source in the development of marine teleost eggs and larvae (Fyhn 1989), and can be important quality criteria. Fatty acids, specially the n-3 PUFA, are considered to be important for survival in marine teleosts (Tocher & Sargent 1984; Ulvund & Grahl-Nielsen 1988).

The ovulatory rythmes varied between 73 to 80 hours. There were not found any significant correlations between each single parameter and viability although the content of free amino acids and percent of fertilisation as well as viability varied both between female halibuts and during the spawing season.

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GONADOTROPIN RELEASING HORMONE (GnRH) RECEPTORS IN TELEOSTS

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In teleosts, as in higher vertebrates, the secretion of pituitary gonadotropin (GTH) is stimulated by the hypothalamic decapeptide, gonadotropin-releasing hormone (GnRH). In the goldfish, and perhaps other teleosts, GnRH also stimulates growth hormone (GH) release. Recent studies have provided characterization of GnRH receptors in the pituitary of a number of teleost species, including goldfish, catfish, flounder and stickleback. Goldfish pituitary contains two classes of GnRH binding sites, a high affinity/low capacity and a low affinity/high capacity sites, whereas catfish, flounder and stickleback pituitary contain only a single class of GnRH binding site. GnRH receptors in goldfish undergo seasonal variations with higher pituitary content of both high and low affinity binding sites during the late stages of gonadal recrudescence, correlating closely with the pituitary response to GnRH agonists in terms of GTH release. Structure-activity studies in goldfish indicate that high affinity GnRH receptors with molecular weight of approximately 51 M_r are likely involved in the control of GTH release; little is known about the biological activity of the low affinity pituitary GnRH receptors in goldfish. In terms of regulation, there is evidence that dopamine, gonadal steroids and native GnRH peptides influence pituitary GnRH receptor number and responsiveness in terms of GTH release in goldfish and catfish. It is likely that these factors may play a physiological role in regulating seasonal patern of GTH release and gonadal activity in teleosts.

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FOLLICULAR STEROIDOGENESIS, STEROID PROFILES AND OOGENESIS IN THE EUROPEAN SEA BASS, *DICENTRARCHUS LABRAX*.

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The aim of this study was to partially characterize steroidogenesis throughout ovarian cycle in captivity-reared seabass and to develop a bioassay for its gonadotropin (GtH). Fish were bled at monthly intervals and their ovaries were incubated with increasing concentrations of homologous pituitary extract. Steroid levels in the incubation media and in the plasma were measured using specific radioimmunoassays. Follicular potential to produce $E_2 17\beta$ was low during endogenous vitellogenesis (July-October), increased at the onset of exogenous vitellogenesis (November) and was maximal at final stages of vitellogenesis (December-January). Testosterone (T) production was first detected at the onset of exogenous vitellogenesis and was maximal at postvitellogenic stages (January-February). Circulating levels of $E_2 17\beta$ and of T were found to be correlated with the follicular potential to produce them: maximal levels of $E_2 17\beta$ were observed at final stages of vitellogenesis while T levels peaked at postvitellogenic stages. The *in vitro* system was validated as a bioassay for the measurement of gonadotropin, and was used to monitor seasonal changes in seabass pituitary GtH content.

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EFFECTS OF PHOTOPERIOD ON PLASMA GTH I AND GTH II LEVELS DURING SEXUAL MATURATION IN ATLANTIC SALMON

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Recently, two biochemically distinct gonadotropins, GTH I and GTH II, have been isolated and characterized from several teleost species. The biological functions of these hormones have been tested, but only slight differences in biological activity have so far been observed. The development of RIAs for salmon GTHs provides an opportunity to study the presence and the changes of GTH I and GTH II during sexual maturation. Data obtained from coho salmon suggest an involvement of GTH I during true vitellogenesis, while GTH II appears as a massive peak at the time around ovulation. The aim of the present study was to characterize the plasma profiles of GTH I and II in Atlantic salmon during maturation and to investigate the effects on GTHs of an altered photoperiod. Starting in January, female Atlantic salmon were sampled monthly for a year, including ovulation. GTH I and II were analysed by a slightly modified version of the method developed for chum salmon (Suzuki *et al.* 1988; Gen Comp Endocrinol 71:459-467). In the maturing control (natural photoperiod), plasma GTH I showed small mean variations (between 1-3 ng/ml) until ovulation (around 7 ng/ml), while GTH II was generally below the detection level until ovulation (7 ng/ml). Continuous light (24L) from January reduced plasma GTH I (>0.5 ng/ml) from February to December and also reduced maturation from 90 to 11%. This clearly indicates an inhibitory effect by 24L-treatment on the synthesis and/or release of GTH in Atlantic salmon. Groups exposed to 24L or short day (8L:16D) from July delayed or advanced ovulation by approximately one month, respectively. Corresponding changes occurred also in the peaks of plasma GTH I and GTH II. In general, the present study supports previous findings on plasma levels of GTH I and II in coho salmon and rainbow trout, whereas the effects of photoperiod on GTHs have not been reported previously.

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INDUCTION OF VITELLOGENIN SYNTHESIS IN JUVENILE STRIPED WOLFFISH (*Anarhichas lupus* L.)

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The effects of estradiol-17β were studied in juvenile striped wolffish, *Anarhichas lupus* L. of approximately 60 g. Estradiol-17β, mixed in corn oil, was administered in a peritoneal injection, at a dose of 5mg/kg body weight. Control fish received corn oil only. The treatment was repeated on day 7 and samples of blood and liver were taken on day 14. The RNA/DNA ratio in liver was investigated as an indication of protein synthesis. Blood plasma samples were analyzed for protein-bound and free calcium and by SDS gel electrophoresis.
The results indicate that the injection of estradiol-17β induces *de novo* protein synthesis in juvenile striped wolffish.
The newly synthesized protein is tentatively identified as vitellogenin, by plasma calcium content and by the migratory properties on electrophoresis gels.

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CRYOPRESERVATION OF RAINBOW TROUT (Oncorhynchus mykiss) SEMEN IN A SUCROSE-GLYCEROL-EXTENDER

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Insemination results obtained with frozen-thawed rainbow trout sperm are erratic and inconsistent. In Exp. 1 of this investigation diluents were investigated that consisted of different concentrations (0.15, 0.30, 0.60 mol) of glucose or sucrose. In Exp. 2 six thawing rates (518 to 1985°C/min) were compared. Best results were arrived at when pellet-freezing semen in a 0.6 mol sucrose solution with 10% glycerol and thawing pellets at a rate of 800 to 1600°C/min. Fertilization rates were between 89 and 92% of unfrozen controls. In two subsequent experiments application of this procedure yielded average eyed egg rates of 67% (SE = 0.9) and 70% (SE = 3.5), respectively (not corrected for unfrozen controls). The procedure presented is simple and supplies consistent and satisfactory results.

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INDUCED SPAWNING OF RICE-FIELD EEL BY A SYNTHETIC NONAPEPTIDE

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A nonapeptide Thr-Cys-Ser-Val-Ser-Glu-Trp-Gly-Ile representing sequence 89-94 of human seminal plasma inhibin was purified. This peptide was found to have direct action on the rat pituitary by increasing basal release of FSH as well as GnRH stimulated FSH and LH release. The effects of this nonapeptide (NP, 1, 10 and 20 μg/kg) on ovulation and spawning of rice-field eel (*Monopterus albus*) were compared with those of des-Gly^{10} - [D-Ala^{6}]-luteinizing hormone-releasing hormone (1-9) -ethylamide (LHRHa, 20 μg/kg), (hCG, 100 IU/kg) and homologous pituitary extract (HPE, 1 mg/kg). In another series of experiments, fish were injected with combinations of nonapeptide (NP) + LHRHa, NP + HPE, NP + hCG, LHRHa + HPE, LHRHa + hCG and HPE + hCG. Of the three tested doses of NP, the median dose of 10 μg/kg was most effective (80% fish ovulated). The highest dose of 20 μg/kg was comparatively less effective (40% fish ovulated) whereas the lowest dose of 1 μg/kg was completely ineffective. The efficacy of nonapeptide at the dose of 10 μg/kg was similar to that of LHRHa, HPE and hCG. Treatment of fish with combination of above did not increase the ovulatory response except in case of LHRHa + HPE where all the injected fish ovulated (100%). Mean fertilization, hatching success rate and survival of hatchlings was highest in LHRHa + HPE treated group followed by NP + LHRHa and NP treated fish with other groups showing comparatively lesser but still significant response. Results presented are the first report on the successful induction of ovulation and spawning of rice-field eel using any of these agents.

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THE CONTROL OF PROSTAGLANDIN PRODUCTION IN THE FISH OVARY.

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A role for prostaglandins (PG) in fish ovulation has been demonstrated by various in vivo and in vitro investigations. In vitro ovulation studies have also shown that E and F PGs may have different effects on ovulation. Steroids, such as 17α,20ß-dihydroxy-4-pregnen-3-one stimulate ovarian PG production in yellow perch (Perca flavescens). However, the exact mechanism by which PG synthesis is controlled during fish reproduction is not clear. In the present study, this question was addressed by studying the effects of several second messenger agonists on in vitro PG production in brook trout (Salvelinus fontinalis) ovarian tissue at different maturational stages. Following incubation, PGE and PGF levels in the incubation media were measured by specific radioimmunoassays. The production of PGs by extrafollicular and follicular tissues was significantly stimulated by sodium orthovanadate, phorbol 12-myristate-13-acetate and the calcium ionophore, A23187. These data indicate that the signal transduction pathway by which PG synthesis in the trout ovary is controlled during reproduction, may involve activation of G-proteins, the phosphatidylinositol cycle, protein kinase C and calcium mobilization. Further, experiments utilizing actinomycin and cycloheximide also indicate that the production of PGE and PGF by brook trout ovarian tissue is modulated by distinct translational or non-translational pathways. Besides the stimulation of PG synthesis, the same agents also stimulate in vitro ovulation of trout follicles. However, it is also clear from studies utilizing indomethacin that this PG synthesis is not required for the stimulation of ovulation by these agents.

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VITELLINE ENVELOPE PROTEINS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Studies of the vitelline envelope in teleosts show that the vitelline envelope consists of two to four major proteins. Recently, it was shown that estradiol-17ß induced the major vitelline envelope proteins in three different species, the rainbow trout, brown trout (*Salmo trutta*) and turbot (*Scophthalmus maximus*). In the present study, antibodies directed against vitelline envelope proteins from rainbow trout were used to investigate the appearence of the vitelline envelope during oocyte development, and the presence of vitelline envelope proteins in plasma. In maturing females, the three major vitelline envelope proteins were detected in plasma more than six months before ovulation. During the same time period, it could be observed that the vitelline envelope started to grow from the inside. When estradiol-17ß levels increased, the amount of vitelline envelope proteins found in plasma increased. In juvenile rainbow trout treated with estradiol-17ß, the vitelline envelope proteins were first detected in the liver. The present study supports the hypothesis that the major protein constituents of the vitelline envelope in teleosts are synthesized in the liver under the endocrine control of estradiol-17ß.

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CARBOHYDRATE-POOR GONADOTROPIN

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At the 1987 symposium, we described an electrophoretically homogenous carbohydrate-poor gonadotropin (CP-GtH) (Rf 0.7) from chum salmon pituitaries. The CP-GtH stimulated ovarian incorporation of ^{131}I-vitellogenin (Vtg) and ovarian growth and its circannual plasma levels correlated with vitellogenesis in Atlantic salmon.

Stimulation of ovarian uptake of *in vivo* labelled ^{3}H-Vtg by CP-GtH has now been demonstrated. Intraperitoneally injected ^{3}H-Vtg was incorporated (44%) into the ovary of CP-GtH treated vitellogenic fish with a Vtg uptake rate of 73 ng/mm^2/hr, as compared to 52 ng/mm^2/hr in the control (25%). Gel filtration and HPLC reverse phase C-18 chromatography revealed that chum CP-GtH had a M_r of *ca*. 20 kDa and gave two peptides with M_r at 10.5 and 9 kDa. Preliminary sequencing on the less hydrophobic peptide indicated that it was not homologous with GtH I or GtH II (carbohydrate-rich GtH). The chemical characteristics of the CP-GtH will be discussed.

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SEASONAL OLFACTORY SENSITIVITY OF WILD MISSISSIPPI RIVER CARP TO SEX STEROIDS WITH PUTATIVE PHEROMONAL ACTIONS.

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The steroidal hormone 17α,20ß-hydroxy-4-pregnen-3-one (17,20ßP) functions as a potent sex pheromone in goldfish, *Carassius auratus*. Carp, *Cyprinus carpio*, and goldfish have similar reproductive systems and strategies, their periovulatory plasma steroid profiles are similar, and they are known to hybridize. We hypothesized that if sex hormones and their metabolites commonly function as sex pheromones in fish, then 17,20ßP or a structurally similar steroid should function as an olfactory stimulant and sex pheromone in carp. Seasonal olfactory sensitivity of wild Mississippi River carp to a number of steroids was measured by electro-olfactogram recording. The maturation inducing hormone 17,20ßP was found to be the most stimulatory of 26 steroids tested. Its threshold was 10^{-13} molar (M), with a maximal response at 10^{-8}M. Data were grouped in order to compare responses of males and females during and after spawning, and of immature (young-of-year) fish to 17,20ßP. The sensitivities of males and females were equivalent and significantly greater than the responses of immature fish. Responses to androstenedione (threshold 10^{-10}M) did not differ significantly between groups, however immature fish were the most responsive. The different relative sensitivities of mature and immature fish to 17,20ßP and androstenedione may reflect separate receptor mechanisms and functions for these steroids.

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IMPLICATION OF GABA IN THE CONTROL OF GONADOTROPHIN RELEASE IN THE GOLDFISH

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Following the observations by means of immunocytochemistry and high pressure liquid chromatography of high concentrations of GABA (gamma-aminobutyric acid) in the pituitary and in brain regions implicated in the regulation of gonadotrophin release in the goldfish, the effects of intraperitoneal injections of GABA or GABA transaminase inhibitors (4-aminohex-5-enoic acid; GVG) on plasma gonadotrophin (GTH) levels were studied. It was found that GABA (10µg/g) caused an increase of plasma GTH levels in sexually regressed or early maturing females, but not in maturing fish. GVG (100 and 300 µg/g) caused a large accumulation of GABA in the brain and pituitary at 24 and 48 hours postinjection together with a dose related increase of plasma GTH levels, suggesting that GABA stimulates GTH release. Neither GABA nor its agonists muscimol and baclofen had any direct effects on GTH release from dispersed pituitary cells in static incubation or perifusion. However, in vitro incubation of pituitary slices with GABA resulted in an increase of gonadotrophin-releasing hormone (GnRH) release. Finally, estradiol implantation, which decreases GABA concentrations in the telencephalon, was able to block the GABA induced GTH release. These results suggest that GABA stimulates GTH secretion by increasing GnRH release from the pituitary and that the negative feedback effects of estrogens could be mediated by GABA neurons.

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ADDITIONAL EVIDENCE FOR DUALITY OF FISH GONADOTROPHINS

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Two distinct glycoproteins related to chum salmon GTH 1 and GTH II were isolated from pituitary glands of a marine fish, the bonito (*Katsuwonus plelamis*), and characterised by amino acid sequence analysis in order to obtain additional evidence for duality of teleost GTHs. Glycoproteins were extracted with 35% ethanol-10% ammonium acetate from the pituitary glands, and intact GP I and GP II, consisting of two distinct subunits, were purified by ion-exchange chromatography on DEAE-cellulose, rpHPLC on C4, and gel filtration on Superdex 75. The association of the subunits was stable in GP I (36 kD) and unstable in GP II (30 kD). Immunoblotting revealed that antisera against beta subunits of chum salmon GTHs reacted with GP II, but not with GP I. In addition, none of the GPs was stained with antiserum against human TSH beta. Sequence identities of beta subunits are 43% between GP I and salmon GTH I, and 67% between GP II and salmon GTH II, but only 28% between the two GPs, Cystine residues of beta subunits of bonito GP I are located positions homologous to those of salmon GTH I, which differ from those of salmon GTH II, LH and FSH. Thus, it is evident that the bonito pituitary gland produces two chemically distinct glycoproteins related to chum salmon GTHs.

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THE EFFECTS OF PHOTOPERIOD AND TEMPERATURE ON THE ANNUAL REPRODUCTIVE CYCLE IN FEMALE STRIPED MULLET, *MUGIL CEPHALUS*

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In Hawaii, captive female striped mullet, *M. cephalus*, exhibit the following annual reproductive cycle: primary growth stage from April to August, cortical vesicle stage from August to October, vitellogenesis from October to March. We investigated the environmental control of this cycle during four 8 week trials. The protocol for each trial was similar and used 32-48 females placed into 4 different combinations of photoperiod and temperature (8 hrs light at 21°C, 8 hrs light at 30°C, 16 hrs light at 21°C, and 16 hrs light at 30°C. Trial 1 was started in April (just postseason), trial 2 in July (primary growth stage), trial 3 in September (cortical vesicle stage), trial 4 in February (vitellogenesis). The results from trial 1 indicate that this species has a true refractory period during which it is not responsive to either photoperiod or temperature. The results from trials 2 and 3 indicate that the onset of the cortical vesicle stage is stimulated under 8 hrs of light in either 21°C or 30°C while the onset of vitellogenesis is stimulated in 21°C under either 8 hrs or 16 hrs of light. The results from trial 4 indicate that vitellogenesis is terminated in 30°C under either 8 hrs or 16 hrs of light. This study suggests that in Hawaii, the onset of the cortical vesicle stage is primarily determined by photoperiod while the onset and duration of vitellogenesis is primarily determined by temperature.

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ATTEMPTS TO ASSESS OVARIAN MATURITY IN A MULTI-SPAWNING FISH, THE GUDGEON *GOBIO GOBIO* L., BEFORE INDUCED OVULATION.

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Several citeria were used in order to assess the ovarian maturity and the optimal time of hormonal induction of ovulation in a multispawning fish, the gudgeon *Gobio gobio* L. This small European cyprinid develops oocytes asynchronously, which renders it difficult to assess exactly ovarian maturity by macroscopic observation (swollen and soft abdomen, protuberant part genital, high condition factor). Ovulation and fecundation success after hormonal treatment (carp pituitary extract and/or 17 α-OH- Progesterone) are variable, all the more so as the duration of fecundability is very short. Ovarian maturity was determined by a morphometric and histological examination of oocytes sampled *in vivo* by catheterization just before hormonal treatment. The simultaneous presence of different maturation stage of oocytes leads to a high variability of oocyte size. A close relationship between these two parameters (size and maturity stage of oocyte was established histologically. The mean oocyte size, the minimal percentage of large oocytes (ø > 1 and 1.25 mm) and of oocytes with peripheric or subperipheric germinal vesicle required to obtain an ovulation rate higher than 50 to 75 % after hypophysation were determined by a probit analysis. Samplings carried out in injected but not ovulated females showed a strong *in ovario* resorption of large oocytes.

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STIMULATORY EFFECTS OF SEROTONIN ON GONADOTROPIN RELEASE IN THE ATLANTIC CROAKER.

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We have shown that the inhibitory dopaminergic control of gonadotropin (GtH) secretion demonstrated in a variety of teleost species is absent in Atlantic croaker, *Micropogonias undulatus*. We were interested, therefore, in studying other possible neuroendocrine regulatory mechanisms in this species. The effects of serotonin (5-HT), alone, and in combination with des Gly^{10} [D-Trp^{6}]-LHRHa on plasma GtH levels in yearling Atlantic croaker (60-70g BW) during their first reproductive cycle and also in 2- and 3-year old fish (300-1000g BW) were examined in the present study. Simultaneous administration of LHRHa (20ng/g) and 5-HT (20μg/g) to the first year group elicited a significant elevation in GtH levels over those induced by LHRHa alone, whereas administration of 5-HT alone failed to elevate plasma GtH levels significantly. Pretreatment of the yearlings with fluoxetine (10μg/g), a 5-HT reuptake inhibitor, potentiated the effect of 5-HT on GtH secretion, whereas pretreatment with ketanserin (10μg/g), a 5-HT receptor antagonist, completely inhibited 5-HT-induced GtH release. Administration of LHRHa (20ng/g) or 5-HT (20μg/g) significantly elevated GtH levels in the 2- and 3-year old fish, but the combined treatment failed to increase GtH levels above those induced by LHRHa alone. However, with a lower dose of LHRHa (5ng/g), the combination produced an additive effect. The results clearly indicate a stimulatory influence of 5-HT on GtH release in the Atlantic croaker.

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OVARIAN PROGESTOGENS IN CYPRINID FISH

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Progesterone and 17-hydroxyprogesterone are rapidly metabolised in vitro by carp ovaries to polar 7α-hydroxylated-5α-reduced compounds. These metabolites are also formed by goldfish ovaries, which in addition produce significant amounts of 17,20α-dihydroxy-4-pregnen-3-one (17,20αP). In ovaries of roach and rudd, the major metabolites of 17-hydroxyprogesterone are 17,20αP and testosterone glucuronide, while 7-hydroxylated metabolites are relatively minor. In rudd 5α-pregnane-3β,17,20α-triol and 17,20α-dihydroxy-5α-pregnane-3-one are also important metabolites. Progesterone is metabolised predominantly to 17,20αP and a 7-hydroxy-5α-reduced product. Priming and stage of migration of the germinal vesicle had no effect on the pattern of metabolites. When 17-hydroxyprogesterone substrate was increased from 42 ng to 1μg or 100 μg in incubations of roach or rudd ovaries (200 mg) the pattern of metabolites changed dramatically. Glucuronides and polar metabolites decreased from over 50% of metabolites to undetectable, while 17,20αP increased from 12-14% to 48-71%. The results suggest that some ovarian enzymes have very high activity but low capacity and that some of the effects previously attributed to gonadotrophin induced enzyme stimulation may in fact have been due to changes in endogenous precursor concentrations.

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OOCYTE GROWTH IN SPAWNING ATLANTIC COD

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The cod (Gadus morhua) is a marine multiple batch spawner (producing typically 15-20 batches of eggs in 5-7 weeks). There is no recruitment of oocytes to the vitellogenic oocyte mode during spawning (i.e. determinate fecundity). This paper deals with the growth of the oocytes between batches to give information to the mechanisms determining the final egg size, an important parameter in egg quality studies. Ovarian tissue (using catherization) and blood were sampled at different steps in the ovulatory rhythm. Each female spawned naturally and was studied separately. Normally there was no sign of handling stress. The size distribution of the vitellogenic oocytes became gradually narrower and more peaked as spawning progressed. The dry weight of the largest follicles of this mode was compared with the subsequent egg dry weight. The observed great variation in plasma estradiol-17β level between batches was correlated with the growth of the oocytes. It is concluded that the developing oocytes in the spawning cod sequester yolk, especially during final maturation producing the largest eggs in the beginning of the spawning period.

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STEROIDOGENESIS AND SPERM PRODUCTION IN THE EUROPEAN CATFISH, (SILURUS GLANIS)

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11-Ketotestosterone, 11β-hydroxytestosterone and 17,20α-dihydroxy-4-pregnen-3-one (17,20αP) were identified as the major metabolites of 17-hydroxyprogesterone in in vitro incubations of testes of the European catfish. Carp hypophysial homogenate (chh) stimulated in vitro production of testosterone, 11β-hydroxytestosterone, 17-hydroxyprogesterone and 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) from endogenuos precursors. Plasma concentrations of all the steroids measured were higher 24 h after chh injection than after saline treatment. Plasma concentrations of 17,20αP exceeded those of 17,20βP and were significantly correlated with the area of testicular cysts. Chh had no effect on sperm release or gonadal histology when administered either in vivo or in vitro.

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HORMONALY-INDUCED ARTIFICIAL PROPAGATION OF COMMON CARP FEMALES (Cyprinus carpio) BY MEANS OF SUPERACTIVE Gn-RH ANALOGUES AND DOPAMIN-ANTAGONIST ISOFLOXYTHEPIN.

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The single injection application of combination of one from two superactive Gn-RH analogues at the dose of 10 - 20 $\mu g.kg^{-1}$ with isofloxythepin at the dose of 1 - 2 $mg.kg^{-1}$ was demonstrated as a statistically effective technique for the hormonal induction of ovulation and for enabling the artificial propagation in four experimental series. The following two superactive Gn-RH analogues were used: (D-Ala^6) Gn-RH ProNHEt and (D-Tle^6) Gn-RH ProNHEt. In single experiments the ovulation was reached in 40 - 80 % of females injected with the combination of Gn-RH analogue with isofloxythepin. In single administration of Gn-RH analogues or isofloxythepin, the results were completely negative. In the control groups, where the carp pituitary was administered in two partial doses (1 + 3 $mg.kg^{-1}$), the ovulation was induced in 25 - 100 % of females. Viable eggs were obtained from both the experimental and control female groups.

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METABOLISM OF SPERMATOZOA AND COMPOSITION OF THE SEMINAL FLUID OF THE GRAYLING, *THYMALLUS THYMALLUS* (SALMONIDAE), AS A BASE FOR NEW METHODS FOR CRYOPRESERVATION OF SEMEN.

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Usual methods for cryopreservation of spermatozoa of fish derive from methods used in mammals. To find new ones which are more adequate to lower vertebrates, the metabolism of the spermatozoa and the composition of the seminal plasma need detailed investigations. For that purposes the activity of the following enzymes are demonstrated by means of enzymatic assays in spermatozoa and in the seminal fluid of the grayling: lipase, hydroxybutyrate dehydrogenase, lactate dehydrogenase, glycosidases, pyruvate kinase, adenylate kinase, phosphatases and proteinases. During the motility phase of the spermatozoa the changes of the concentration of metabolits (hydroxybutyrate, free fatty acids, glucose- and fructose-6-phosphate, lactate, pyruvate, phosphoenolpyruvate and adenosine triphosphate) were examined. Further the composition of the seminal plasma of *T. thymallus* is analysed by thin layer chromatography and quantified by biochemical tests.

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STEROIDOGENESIS IN THE OVARY OF THE EUROPEAN EEL, *ANGUILLA ANGUILLA* AT THE SILVER STAGE.

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Previous studies by Colombo and Quérat have demonstrated a high conversion of ^{3}H-pregnenolone into unknown polar steroids. Using GC-MS, we were able to identify these "unknowns". The polar steroids appeared to be 20α- and 20ß-hydroxylated products of mainly 17α-hydroxypregnenolone (70 %) and also of 17α-hydroxyprogesterone (10 %). This suggests a weak 3ß-HSD activity. Incubations, however, with δ5-steroids as precursors and with NAD as the only cofactor demonstrated a high 3ß-HSD activity, suggesting a "normal" pattern of steroid biosynthesis. Possibly, the addition of cofactors, especially NADPH, disturbed the normal steroidogenic patterns in the ovarian homogenate. Tissue incubations without cofactors but with radiolabeled precursors, however, were not successful because the enormous amounts of fat completely absorbed the precursors. Therefore a 24 hours tissue incubation was set up in the absence of exogenous precursors and the steroids released in the incubation medium were isolated, identified and quantified by GC-MS. From the steroids produced by the immature eel ovary, we have identified more than twenty. Besides a weak production of estrogens, it appeared that the main products were 5α-reduced androgens. But, testosterone, 11ß-hydroxyandrostenedione and 5ß-reduced androgens could also be detected. The polar 20α- and 20ß-hydroxylated pregnenes were hardly present.

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THE ISOLATION OF A PROTEIN WHICH BINDS VITELLOGENIN WITH HIGH AFFINITY FROM THE OOCYTE PLASMA MEMBRANES OF THE RAINBOW TROUT. Oncorhynchus mykiss.

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In the rainbow trout the majority of oocyte growth occurs during vitellogenesis and results predominantly from the sequestration of the yolk protein precursor, vitellogenin (VTG). Uptake of VTG into trout oocytes is selective and is thought to occur by receptor mediated endocytosis, involving specific cell surface receptors. The expression and regulation of such a receptor for VTG is likely to play a key role in the control of oocyte growth during vitellogenesis.

Membrane proteins from vitellogenic oocytes were solubilized using n-octyl β-D-gluco-pyranoside and subsequently separated on electrophoretic gels. Gels run under reducing conditions were silver stained to visualize the isolated proteins. Non-reducing conditions were used for ligand blotting using ^{125}I-VTG as the probe. The results clearly show that the plasma membrane of rainbow trout oocytes contains a protein which has a high affinity for VTG, and thus may well represent the vitellogenin receptor.

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CAPTIVITY ALTERS PHYSIOLOGY AND SEX REVERSES BEHAVIOR IN GNATHONEMUS PETERSII

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Little is known about the mechanisms through which captivity affects reproductive behavior in wild-caught vertebrates. Experiment 1 investigated the effects of captivity on sex differences in electric organ discharges (EODs) and on plasma hormone levels in adult G. petersii imported from Africa. On the day subjects were imported, males exhibited longer durations of phases 2 and 3 of the EOD and lower peak power spectrum frequencies (PPSFs) than females. After 7 days in captivity, the sex difference in the duration of phase 2 was abolished, and by day 14 the sex differences in phase 3 and the PPSF were eliminated. All sex differences in EODs were abolished or even reversed in individually- and group-housed subjects after 37 days in captivity, and females had higher testosterone (T) levels than males. Males exhibited the most dramatic changes in behavior. In Experiment 2, EOD data and blood were collected on day 0, and on days 5, 10 and 15 from newly imported adult individually-housed males, and blood collected on days 5 and 15 for group-housed males. Decreases in phase 3 of the EOD and increases in the PPSFs progressed over the 15 day captivity period, becoming statistically significant by days 10 and 15, respectively. Both T and 11-keto T dramatically decreased to near non-detectable levels by day 5 in the laboratory regardless of housing. Together with previous research indicating the sex differences in EODs in this species are androgen-dependent, these findings directly link captivity, hormones, and steroid-sensitive behavior, and show why most feral animals brought into captivity fail to exhibit sexual behavior.
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IN VITRO METABOLISM OF TESTOSTERONE BY GONADAL TISSUE OF A PROTANDRIC ANEMONEFISH AT VARIOUS SEXUAL STAGES.

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Gonadal tissues of the anemonefish Amphiprion frenatus, a facultative protandric teleost, were incubated with labeled testosterone. The gonads originated from animals which had been subjected to particular social stimuli by conspecifics in order to manipulate their sexual status. In males 11ß-hydroxylase-activity was high. The share of 11-oxigenated androgens was more than 60% in all cases with 11ß-hydroxytestosterone as the major metabolite. In females the actvity of this enzyme was low (<3%) or even undetectable. The more remarkable it is that the highest percentage of 11-oxigenated androgens (up to 83%) was found in 4 incubations from such fishes which were more or less advanced on the way from male to female. Their gonadal conditions were checked histologically. From these preparations it could be seen that the testicular part was reduced to small islets or virtually absent. It remains unknown in which cell type the 11ß-hydroxylase is located. Another noteworthy feature of the incubations from inverting specimens was a total lack of 5α- and 5ß-reductase-activity. Among the metabolites of female gonads no estradiol could be found.

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BINDING AND ACTION OF SALMON GROWTH HORMONE (s.GH) IN THE MATURE TROUT TESTIS.

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Stimulatory action of GH on testosterone and/or estradiol production has previously been reported in fish (Singh et al. 1988, Van der Kraak et al. 1990).

In trout we have shown that ^{125}I-s.GH binds to testicular membrane preparations with high affinity (Ka = 2 x 10 ^{9} M) and limited capacity (10 fmol/g fresh tissue). This binding is specifically inhibited by sGH and bovine GH, but not by s.gonadotropin and o.Prolactin.

In vitro: Salmon GH effects on male steroidogenesis were investigated in cultured cells (isolated from mature gonads). Salmon GH (0.05 to 1µg/ml) and bovine GH (10 µg/ml) increased accumulation of 17α20ßOHP in a time (2 to 6 days) and dose dependant way, while 11KT tended to be inhibited. The amplitude of sGH effects varied depending probably on the physiological stage of the gonads used. Involvement of somatomedin in this GH action is being investigated.

In vivo: The blood concentration of GH and 17α20ßOHP is low during spermiogenesis ; both hormone levels increase during spermiation, then decrease after, suggesting a trophic effect of higt GH concentration on the progestin.

Together, these observations support the idea of a physiological role for GH in testicular function.

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RECEPTOR MEDIATED ENDOCYTOSIS OF VTG IN FISH FOLLICLE

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Ultrastructural microscopy shows that during the rapid growth phase of the ovarian follicle in fish, vitellogenin (VTG) enters the oocyte by micropinocytosis.

This internalisation occurs on specialized areas of the oolemma leading to the formation of yolk vesicles as demonstrated by electron microscopy autoradiography.

Specific binding sites for VTG have been demonstrated using vitellogenic ovarian membranes. In direct binding studies with crude membrane preparations, the VTG receptor exhibited high capacity and low affinity (10^{-8} M.l $^{-1}$) characteristic of type II receptors. This receptor, a protein with an apparent MW around 100,000 Da, depending on the studied species, is visualized after solubilisation by ligand blotting with homologous iodinated VTG.

A study of the VTG receptor number in oocytes is described during the normal sexual cycle of the trout Onchorhynchus mykiss.

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THYROID FUNCTION IN FLUVIAL SEXUALLY MATURE LAKE ONTARIO COHO SALMON; EFFECTS OF RIVER TEMPERATURE AND CORRELATION WITH SEX STEROID HORMONE LEVELS

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Lake Ontario coho salmon were taken from the Credit River during October, November and December of the 1988, 1989 and 1990 spawning runs to follow changes in thyroid function during their prespawning residence in the river and to correlate these with changes in plasma levels of gonadal steroid hormones.

The 1989 season was marked by an early onset of winter; river water temperature fell to 0°C prior to the post-ovulatory period (mid November). The eggs taken that season had a lower than usual survival to yolk absorption, perhaps reflecting a reduced egg quality. This unusual season provided us with the opportunity to examine the effect of low ambient temperature on the thyroid function during the pre- and post-ovulatory stages.

In the 1988 and 1990 seasons, plasma thyroid hormone (TH) levels fell progressively between October and mid-late December (in females faster than in males) and hepatic 5'-monodeiodinase (MDI) activity declined rapidly; no such change in plasma TH levels was evident during the 1989 season. Plasma gonadal steroid concentrations exhibited a similar pattern in all three seasons. Regardless of month, the salmon did not respond to exogenous TSH stimulation, suggesting a down-regulation of the pituitary-thyroid axis during the pre-ovulatory period.

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THE EFFECTS OF TESTOSTERONE AND 17α-METHYLTESTOSTERONE CAPSULES ON MALE MATURATION IN THE MILKFISH, *CHANOS CHANOS*

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In a continuing effort to develop milkfish, *Chanos chanos*, hatchery technology, we investigated the effects of hormone implants on male testicular maturation. Forty adult males were implanted with silastic capsules containing either 10, 30, or 60mg of Testosterone (T) or 60mg of 17α-Methyltestosterone (MT). Another 10 males served as controls. Each treatment group was held in a separate tanks together with 10 females. On 4 week intervals, the males were anesthesized in order to check for milt production and to obtain a blood sample for T and 11-Ketotestosterone (KT) RIA analysis. T capsules sippressed milt production for the first 12 weeks following implantation. Increasing T dosages increased serum T levels but decreased serum KT levels. MT capsules also supressed milt production for the first 8 weeks and decreased serum levels of both T and KT for the first 20 weeks. Between week 12 and 32, milt production was higher in comparison to the first 8 weeks and in comparison to controls. The mean serum T and KT levels at 12 weeks were 1100 and 500 pg/ml respectively in comparison to the mean levels for controls of 7900 and 8800 pg/ml respectively. This study produced evidence for a negative feedback mechanism on endogenous production of T and KT and suggests that some other steroid other than T and KT is involved in milt production.

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cAMP IS INVOLVED IN THE MEDIATION OF GnRH ACTION IN TILAPIA; EVIDENCE FROM PERIFUSION OF PITUITARY FRAGMENTS AND PITUITARY DISPERSED CELLS.

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GnRH stimulation of gonadotropin (GTH) release in fish is transduced through the influx of Ca^{2+}, activation of protein kinase C, and arachidonic acid. As the antagonistic effect of dopamine involves D_2-type receptors known to inhibit adenylate cyclase, it was deemed necessary to examine the involvement of cAMP in GnRH signal transduction. Kinetic studies employing tilapia pituitary fragments showed that the response to dbcAMP occurs within less than 6 min. The response to GnRHa quadrupled in the presence of IBMX (0.2 mM), and activation of adenylate cyclase by forskolin resulted in an eightfold increase in GTH release. A sharp peak of cAMP production preceded the peak in GTH in the effluent medium. It can be argued that cAMP agonists act indirectly by their effect on the release of endogenous GnRH from the intact nerve terminals in the fragments. Therefore, the study was continued using short-term experiments with cultures of pituitary dispersed cells. Thirty min incubation in the presence of GnRHa resulted in a dose dependent increase in GTH output with an ED_{50} of 5 nM. Forskolin and 8-Br-cAMP stimulated the GTH release with an ED_{50} of 0.38 µM and 0.5 mM, respectively. GnRHa at a low dose of 1 nM stimulated the release by 145% and IBMX alone (0.2 mM) by 139%. However, when combined the release was nearly 700% of the basal level. These results support our previous suggestion that cAMP takes part in the transduction of GnRH signal leading to GTH release in fish.

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Reproductive pheromones in rainbow trout, *Oncorhynchus mykiss*, and kokanee salmon, *O. nerka*.

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Male rainbow trout in a Y-maze orientate to a chemical signal released by a recently ovulated female. However, an intact olfactory sense is not necessary for the maintenance of spawning behaviour - anosmic males spawned as readily as intact males when paired with ovulated, nesting females. We were unable to demonstrate orientation to conspecific female odour by kokanee males, but under field conditions males were less sexually active and less attentive to nesting females.

In both rainbow trout and kokanee, amounts of 'strippable' milt and plasma levels of testosterone and 17α, 20β-dihydroxy-4-pregnen-3-one were lower in anosmic males than in intact males paired with nesting females. In intact male rainbow trout available milt and plasma steroids increased within three hours of pairing with nesting females, compared with unpaired males, males paired with unovulated females, or males in all-male groups.

These findings confirm that in both rainbow trout and kokanee, females emit one or more sexual pheromone(s) with 'releasing' and 'priming' effects.

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INDUCTION OF GONADAL DEVELOPMENT AND MATURATION BY CHRONIC ADMINISTRATION OF TESTOSTERONE AND ANDROSTENEDIONE IN FEMALE JAPANESE SILVER EEL, ANGUILLA JAPONICA

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Serial implantation of testosterone pellets(50 ug/g b.w.) at 30 day intervals to day 75, or at 15 day intervals to day 105, stimulated the pituitary content of gonadotropin(GtH) to increase markedly; however, no significant changes in serum GtH levels were detected at the sampling times used. Vitellogenesis was stimulated in the ovary of all testosterone implanted fish, the gonadosomatic index(GSI) increased up to 11-12% or 21-22%, respectively; the ovary of at least one eel appeared to be at a preovulatory stage(GSI=39.8%).
Serial implantation of androstenedione pellets(50 ug/g b.w.) at 15 day intervals to day 105 stimulated pituitary GtH content; ovarian development was to the preovulatory stage, as demonstrated by the presence of large yolky oocytes and an average GSI of 34.8 ± 2.9%(greatest GSI was 42.1%). Implantation of LHRH-A (200 ug/fish) or injection of LHRH-A(0.1 ug/g b.w.) stimulated GtH release in the female eels treated with androstenedione.
These results demonstrate that chronic treatment with testosterone or androstenedione alone can stimulate the brain-pituitary-ovary axis of the Japanese silver eel to induce ovarian development to, or nearly to, the preovulatory stage; androstenedione appears to be more effective than testosterone in this regard.

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A NON-SALMONID MODEL FOR THE STUDY OF FISH REPRODUCTION

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Fundulus heteroclitus **in Florida show a group-synchronous ovarian developmental pattern and spawn with a semilunar periodicity from March through September. A routine husbandry procedure has been developed to extend the breeding season in the laboratory so as to have throughout the year a population of reproductively healthy fish with active pituitaries and responsive ovarian follicles. This experience has indicated that, contrary to conventional wisdom, neither temperature nor photoperiod play a dominant role in maintaining reproductive cycling, but rather food availability appears to be the ultimate environmental cue. A shift in the biosynthetic pathway from predominantly estrogen (E_2) to 17α-hydroxy,20β-dihydroprogesterone (DHP) production has been considered to be the norm for steroidogenesis during ovarian development. In *F. heteroclitus*, however, E_2 is secreted at all stages of follicle development, even by those follicles that are capable of producing DHP and able to undergo maturation in response to gonadotropin stimulation in vitro. Studies using isolated ovarian follicular preparations also indicated that the granulosa cells in *F. heteroclitus* possess all the enzymes necessary for the synthesis of DHP, testosterone and E_2. Thus the "two cell-type" model of steroidogenesis, originally promulgated from studies on salmonids, does not apply to this species. In conclusion, the complicated reproductive strategies and the diversity of ovarian developmental patterns in teleosts warrant additional experimentation using a broader range of fishes, including representatives of other non-salmonid species.**

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STIMULATION OF SPERMIATION IN TENCH (Tinca tinca L.) BY ANALOGUES Gn-RH AND CARP HYPOPHYSIS

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The effects of single injection of two Gn-RH analogues - (D-Tle^6) Gn-RH ProNHEt and (D-Ala^6) Gn-RH ProNHEt - and carp hypophysis on spermiation were studied from point of view of sperm volume per male, sperm volume per kg of body weight, spermatozoa concentration per ml of sperm, absolute number of spermatozoa per male and relative number of spermatozoa per kg of body weight. The sperm was collected in period 12 and 24 hours for 3.5 and 7 days, respectively. Dose 10 µg of Gn-RH analogue (D-Tle^6) Gn-RH ProNHEt per kg of body weight increased the relative number of spermatozoa per kg of body weight by 13.3 % ($P<0.05$), and the absolute number of spermatozoa per male by 78.7 % ($P<0.001$) in comparison with the dose 1 mg of carp hypophysis per kg of body weight. The lower doses 0.4 - 10 µg of Gn-RH analogue (D-Tle^6) Gn-RH ProNHEt per kg of body weight, and 1 mg of carp hypophysis per kg of body weight stimulated the spermiation better than higher doses 50 µg and 4 mg , respectively.

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PRESERVATION OF GAMETES IN EUROPEAN CATFISH (SILURUS GLANIS L.)

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Spermatozoa of European catfish were kept immotile after stripping directly in an immobilizing solution (200 mM NaCl, 30 mM Tris, pH 7) were successfully stored. This sperm was successfully stored in a liquid state at 5 - 6 °C with added antibiotics for 13 days, the fertilizing ability was similar to that of the freshly collected sperm ($P<0.1$). The method of cryopreservation was elaborated using the stepwise process of fast thawing procedure. Drops of sperm diluted in immobilizing medium including 12 or 15 % of glycerol,were held on an aluminum disc. Sperm was exposed to liquid nitrogen vapors (-80 to -85 °C) at 2 mm above the level of liquid nitrogen for 10 min and than transferred into liquid nitrogen. Eggs fertilized with cryopreserved thawed spermatozoa activated with distilled water. The yieldest 45.2 % of sac fry compared to 71 % with the intact sperm ($P<0.01$).

Non-fertilized eggs stored for a short time under aerobic conditions at 17 - 18 °C were still fertile 3 hours post spawning, the % of fertilizing was similar to the control eggs fertilized right after ovulating.

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SEASONAL CHANGES IN STEROIDS IN A PROTANDROUS TELEOST SPARIDENTEX HASTA VALENCIENNES

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Serum concentrations of 11-ketotestosterone, 11ß-hydroxytestosterone, testosterone, testosterone glucuronide, estradiol (E2) and 17,20ß-dihydroxy-4-pregnen-3-one (17,20ßP) were measured in male and female sobaity (Sparidentex hasta Valenciennes) at monthly intervals for 13 months. 11-Oxygenated androgens in male and estradiol in the female peaked in January-February during the spawning season. In both sexes testosterone concentrations were highest in the summer when other steroids were low. Testosterone glucuronide showed two peaks, one co-incident with the summer peak of testosterone, the other in the post-spawning period as estrogen and 11-oxygenated androgens were falling. 17,20ßP was found in only two fish in February. Serum 11-oxygenated androgens provide a reliable means of sexing the fish. A brief increase in estradiol in males in September-October may be indicative of the first stages of sex inversion.

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QUANTIFICATION OF VITELLOGENIN IN SEA BASS (*Dicentrarchus labrax*) MAINTAINED UNDER DIFFERENT PHOTOPERIOD USING AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA).

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A specific enzyme-linked immunosorbent assay (ELISA) for sea bass vitellogenin (Vg) was used to measure the changes of annual Vg levels in five groups of sea bass females held under the following photoperiod regimes: A)natural photoperiod (NP), B)all year around short photoperiod (SP, 9HL:15HD), C)two months (March-April) long photoperiod (LP, 15HL:9HD) in a short photoperiod year (SPY), D)one month LP (March) in a SPY and E)one month LP (September) in a SPY. In controls, spawning was recorded in February-March, whereas in B appeared slightly advanced (January-March); both groups started Vg synthesis in October. A three-four months spawning advance was recorded in groups C and D, respectively, and Vg levels started to rise in September. Finally an important delay of spawning time was observed in E (May) beginning Vg synthesis in December. In conclusion, levels of Vg were similar in all groups, but important differences were observed at surge and withdraw time of Vg in the blood. These changes follows the shift of spawning time induced by photoperiod manipulation.

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SEASONAL CHANGES IN SPERMATOCRIT, PLASMA SEX STEROIDS AND MOTILITY OF SPERM FROM ATLANTIC HALIBUT

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Captive male Atlantic halibut (Hippoglossus hippoglossus) from the Newfoundland region first mature at approximately 80 cm fork length. Milt was first released each year in January and February, just days before the first release of eggs by captive female halibut. Initial collections of milt were small in volume (< 1 ml) but increased considerably (> 60 ml / collection) 3-5 weeks after the initial appearance of milt. At the beginning of the male spawning season spermatocrits were low (< 60%) but sperm concentration increased linearly to greater than 90% by April and May. Spermatocrits of 80-100% were commonly observed from April to June when sperm was released as a ribbon-like paste that dissolved poorly in seawater. The first release of sperm occurred when the sex steroids, testosterone and 11-ketotestosterone reached maximum levels. The increase in spermatocrit was negatively correlated with a rapid decline in plasma levels of 11-ketotestosterone. Sex steroids remained low to nondetectable from April to June when spermatocrits were highest. Sperm motility was evaluated on a scale of I-V. Preliminary observations indicate that the best sperm motility was observed early in the spawning season; towards the end of the male reproductive season, sperm motility markedly declined.

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DEVELOPMENT OF RADIOIMMUNOASSAYS FOR TWO STURGEON GONADOTROPINS

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Radioimmunoassays (RIAs) for the measurement of two gonadotropins, stGTH 1 and stGTH 2, in the pituitary and plasma of sturgeon were developed using rabbit antiserum to purified sturgeon pituitary fractions that exhibited distinctly different gonadotropin activity both in vitro and in vivo. These purified fractions were used as the standards and radiolabelled competitors. The cross-reactivities of stGTH 1 in the stGTH 2 RIA and stGTH 2 in the stGTH 1 RIA were 2.0 and 9.3%, respectively. The cross-reactivity of both antibodies to sturgeon pituitary fractions containing growth hormone and prolactin were less than 1.0%. Cross-reactivities with gonadotropins from hake, salmon, tilapia, gillichthes and sheep were less than 0.01%. In females, plasma concentrations of stGTH 1 were elevated during the prematurational stages, dropping prior to spawning. In contrast, stGTH 2 plasma concentrations rose at ovulation. These data suggest that like salmonids, sturgeon have two gonadotropins controlling reproductive development.

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THE BEHAVIOURAL AND PHYSIOLOGICAL RESPONSES OF PRECOCIOUS MALE ATLANTIC SALMON (*SALMO SALAR* L.) PARR TO TESTOSTERONE.

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Testosterone is a potent odorant in precocious male Atlantic salmon parr. Electrophysiological recordings have demonstrated extreme olfactory sensitivity to this steroid with a threshold of detection as low as 10^{-14} M concentration. Recordings from the olfactory epithelium to testosterone, however, were only possible from spermiating males for a limited period of the year. Precocious parr were responsive only during a 3-4 week period during the month of October. Immature parr (male and female) did not respond to testosterone at any time.

Preliminary behaviour experiments have indicated that testosterone is also a potent attractant to spermiating male parr. Testosterone at concentrations as low as 10^{-10} M introduced into a behavioural chamber resulted in positive rheotactic and searching behaviour in the fish. Immature parr (male and female) did not respond to testosterone at any time.

The results of the work are discussed in relation to the role of testosterone in the physiology of the Atlantic salmon and its possible role as a behavioural pheromone.

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BLEACHED KRAFT PULP MILL EFFLUENT (BKME) ALTERS STEROID PRODUCTION, REGULATION AND METABOLISM IN WHITE SUCKER

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Our recent studies have demonstrated reproductive dysfunction in white sucker (*Catostomus commersoni*), longnose sucker (*C. catostomus*) and lake whitefish (*Coregonus clupeaformis*) populations exposed to BKME. These fish exhibit delayed sexual maturity, changes in fecundity, reduced secondary sexual characteristics and depressed circulating steroid levels relative to reference populations. The pituitary-gonadal axis of preovulatory white sucker affected by BKME-exposure was evaluated. Although BKME-exposed white sucker are capable of spawning viable eggs, injection of D-Arg^6, Pro^{9}N-Et sGnRH (0.1 mg/kg) failed to induce ovulation in any preovulatory fish, while 10 of 10 fish from the reference site ovulated within 6 h. As well, fish from the BKME-site did not exhibit an increase in testosterone or 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P) secretion in response to the GnRH analog. *In vitro* incubations of ovarian follicles obtained from fish at the BKME site revealed depressed basal secretion of testosterone and 17α20β-P and diminished responsiveness to human chorionic gonadotropin and forskolin relative to ovarian follicles from the reference site. Depressed levels of testosterone and 17,20β-P glucuronides in BKME exposed fish also suggest that there are effects on the peripheral metabolism of steroids. These studies demonstrate that BKME exposure affects reproduction by acting at multiple sites in the pituitary gonadal axis.

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CHARACTERIZATION OF GONADOTROPIN-RELEASING HORMONE (GnRH) ANTAGONISTS IN GOLDFISH (*CARASSIUS AURATUS*).

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In mammals it has been demonstrated that modification of the amino acid sequence of GnRH especially at positions 1, 2, 3 and 6 often results in antagonistic analogs, which spécifically bind to GnRH receptors with high affinity and inhibit the GnRH induced gonadotropin (GTH) release. In the present study we have tried to identify and characterize GnRH antagonists in goldfish using an *in vitro* pituitary fragment perifusion system. The antagonist [Ac-Δ-Pro^1, pFD-Phe^2, D-Trp^{3,6}]-mammalian GnRH(mGnRH-a) inhibited both salmon GnRH (sGnRH) and chicken-II GnRH (cGnRH-II) stimulated GtH secretion from the pituitary fragments. mGnRH-a inhibited 20 nM sGnRH induced GtH release in a dose-dependent manner with an ED_{50} value of 128.20 ± 82.51nM in sexually regressed fish. mGnRH-a also suppressed 20 nM cGnRH-II augmented GtH secretion in a dose-related fashion with an ED_{50} of 169.41 ± 17.47 nM. Antagonist [Ac-Δ-Pro^1, pFD-Phe^2, D-Trp^{3,6}]-salmon GnRH (sGnRH-a) inhibited sGnRH and cGnRH-II induced GtH secretion equally. mGnRH-a had a higher potency than sGnRH-a. These results imply that both sGnRH and cGnRH-II act through same population of receptors to enhance GtH secretion.

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INDUCED OVULATION OF BROWN TROUT USING GnRHa AND TRIIODOTHYRONINE AND THE EFFECTS OF THE HORMONE TREATMENT ON PROGENY QUALITY.

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The effectiveness of triiodothyronine (T_3) to potentiate the stimulatory effect of gonadotropin releasing hormone analogue (GnRHa) on final oocyte maturation and ovulation of brown trout (*Salmo trutta*) was evaluated. The minimum effective dose of GnRHa was 10 μg/Kg body weight, which significantly reduced mean time to ovulation by 9 days. A single T_3 injection at the time of the first GnRHa injection significantly elevated maternal plasma T_3 levels at the time of ovulation. Elevation of plasma T_3 levels, however, was not uniform in all T_3-injected fish and appeared to be influenced by GnRHa dose. In brown trout, T_3 did not potentiate GnRHa-induced maturation and ovulation. The naturally elevated maternal plasma T_3 levels during final oocyte maturation, as were determined by radioimmunoassay, could be responsible for the absence of a potentiating effect by exogenous T_3. The effects of maternal GnRHa and T_3 treatment of brown trout on the quality of the eggs and the development of the progeny were also evaluated. GnRHa treatment significantly reduced fertility and survival to the eye-pigment formation stage. This reduction in egg quality could be attributed to the effect GnRHa had on reducing the time to ovulation, rather than to a direct toxic effect, since even control fish that ovulated early showed reduced fertility. Maternal T_3 injection did not affect egg quality or *in ovo* development, but it did affect development of the larvae during absorption of the yolk. There was a significant correlation between maternal plasma T_3 levels at the time of ovulation and occurrence of skeletal abnormalities in the progeny. Skeletal abnormalities observed in this study were typical of exposure to pharmacological levels of T_3, suggesting direct transfer of T_3 from maternal blood into the developing oocytes.

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PLASMA PROTEIN INCORPORATION AND LOCALIZATION WITHIN THE DEVELOPING OVARY OF WINTER FLOUNDER.

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An assessment of the contributions of the homologous plasma proteins vitellogenin (VG) and very high density lipoprotein II (VHDL II) to the winter flounder (Pseudopleuronectes americanus) ovary have been made during the mid-vitellogenic period. Using tritium labelled VG and VHDL II preparations the in vivo ovarian uptake of these proteins were studied 2 weeks after intravenous administration. These experiments showed that VG incorporation was greater than VHDL II and internalized VG appeared chiefly as lipovitellin in a fraction of salt soluble ovarian protein making up 82% of the total. By comparison, VHDL II appears unprocessed by the ovary and contributes to a fraction of salt soluble ovarian protein yielding 12% of the total. The specific intracellular localization of VG and VHDL II within flounder oocytes were made using a protein A-gold immunocytochemical technique with the transmission electron microscope.

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CHARACTERIZATION OF THE PRIMARY STRUCTURE OF GONADOTROPIN-RELEASING HORMONE IN THE THAI CATFISH (*CLARIAS MACROCEPHALUS*)

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Two forms of gonadotropin-releasing hormone (GnRH) are present in the brain of the Thai catfish, *Clarias macrocephalus*. One form is novel and the other is identical to chicken GnRH II. The GnRH molecules were purified using reverse phase high performance liquid chromatography (HPLC) and radioimmunoassay (RIA). The presence of the N-terminal pGlu residue was established by digestion with bovine pyroglutamyl aminopeptidase. The amino acid sequences of the $GnRH_{2-10}$ fragments were determined using automated Edman degradation. The molecular mass of each GnRH molecule was established with ion spray mass spectral analysis. These data confirm that the proposed sequence is amidated.

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REPRODUCTIVE PHYSIOLOGY OF COLDWATER MARINE FISH: APPLICATIONS IN AQUACULTURE

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The establishment of new species, such as Atlantic cod (Gadus morhua) and Atlantic halibut (Hippoglossus hippoglossus), in aquaculture requires basic knowledge of various aspects of reproduction. This concerns regulation of ovarian growth and maturation as well as ovulatory rhythms.

Applications include general broodstock management, manipulation of spawning and optimalization of growth. While the Atlantic cod spawns spontaneously in sea cages and tanks, a stable halibut egg supply is acquired by stripping of females with careful surveillance of individual ovulatory rhythms. Fundamental differences in the pattern of oocyte growth appear to exist between periodic spawners, such as cod and halibut, and salmonids. These differences will have to be considered in attempts to control maturation and/or improve spawning performance.

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TELEOSTEAN EGGSHELL ZR-PROTEINS: ENDROCRINOLOGICAL AND PHYSIOLOGICAL ASPECTS

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Oogenesis in teleosts is ultimately controlled by two gonadotropins, which have been localized in specific pituitary cells. Release of these gonadotropins has been correlated to reproductive progress during sexual maturation of salmon, and to plasma levels of estrogens. These hormones control vitellogenesis and formation of the eggshell, which is of particular relevance for fish. While vitellogenesis is well understood, information on teleostean eggshell proteins is scarce due to their insoluble nature. Eggmembranes are often divided into primary membranes from the oocyte; secondary from the follicle and tertiary from the urogenital tract. While teleostean zona radiata is held to be synthesized by the oocyte, i.e. a primary membrane according to the above terminology, our data suggest a different scenario based on recent progress in protein biochemistry. Following oocyte activation, the zona radiata proteins are crosslinked by isopeptide bonds catalyzed by a transglutaminase-type enzyme, and protein monomers must consequently be isolated before polymerization. Synthesis of these proteins are induced by estradiol-17B, in vivo after i.p. administration of this steroid in both sexes of juvenile Atlantic cod (Gadus morhua) and Atlantic salmon (Salmo salar). Furthermore, in vitro studies of rainbow trout (Oncorhynchus mykiss) hepatocytes have demonstrated the ability of such cultures to synthesize and secrete zr-proteins after estradiol-17B treatment. In vivo studies of an Atlantic salmon population during an annual reproductive cycle, have revealed the physiological presence of salmon zr-proteins in plasma from maturing females. The levels of zr-proteins were measured by a specific semiquantitative ELISA-procedure. Using specific RIAs for estradiol-17B, GtH I and GtH II, it was demonstrated that levels of zr-proteins were individually correlated to both estradiol-17B and GtH I but not to GtH II. Our data support the hypothesis of estradiol-induced zr-protein liver synthesis, probably under the control of GtH I. The common egg-membrane terminology may not be applicable for the teleostean zona radiata.

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ORIGIN OF TELOSTEAN EGGSHELL PROTEINS AND THEIR SIGNIFICANCE DURING OOGENESIS: *IN VITRO* LIVER SYNTHESIS OF EGGSHELL PROTEINS INDUCED BY ESTRADIOL-17B.

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The regulation of the yolk precursor protein, vitellogenin, and its importance during fish and amphibian oogenesis, has been extensively studied. Vitellogenin is reported to be the major protein synthesized by the liver under the influence of estradiol-17β. However, the appearance of large amounts of unidentified mRNA isolated from estradiol-17β treated rainbow trout liver has been reported. Recently we reported that the *zona radiata* (the inner and major part of the eggshell) consists of three main protein components, *zr-proteins*, in Atlantic cod (*Gadus morhua*), rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). In addition, we have demonstrated that zr-proteins from Atlantic salmon appears in plasma, where their levels are correlated to plasma levels of estradiol-17β during an annual reproductive cycle. The origin of teleostean eggshell proteins has for a long time been quite controversial. The present study demonstrates the ability of hepatocytes from rainbow trout liver to synthesize large amounts of zr-proteins. Using *in vitro* cultures with added radioactive methionine, we have shown estradiol-17 β-dependent incorporation of this amino acid into proteins which crossreact immunologically with antibodies to zr-proteins. Furthermore, our data demonstrate that in such cultures the molar ratios of zr-proteins (α,β & γ) and vitellogenin synthesized and secreted by the hepatocytes are approx. 1:1:1:1. The data support a model of teleostean oogenesis in which zr-proteins and vitellogenin are coordinately regulated and synthesized in the liver for transport to the ovaries.

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PRIMER EFFECT OF 17_{α} 20_{β} DIHYDROXYPROGESTERONE (17,20B-P) AND ITS GLUCURONIDE (17,20B-P -G) ON EUROPEAN MINNOW (*Phoxinus phoxinus*) AND MULLET (*Mullus barbatus*).

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Recent studies on fish have established that besides their well-documented role as reproductive hormones, sex steroids and steroid glucuronides may also function externally as pheromones. The goal of the present work was to determine whether free or glucuronated forms of 17_{α}, 20_{β}-dihydroxy-4-pregnen-3-one (17,20B-P and 17,20B-P-G, respectively) are primer pheromones in european minnow (*Phoxinus phoxinus*) and mullet (*Mullus barbatus*). During the spawning period, monosex groups of fish were exposed to test solutions (10^{-10} M) over a 10 hour period. Milt volume and the volume of ovulated eggs were obtained by stripping. Milt volume increased in males of both species exposed to 17,20B-P, 17,20B-P-G, or conspecific ovarian fluid. Male minnows rendered anosmic by plugging the nares with vaseline and cotton wool were not stimulated by ovarian fluid or 17,20B-P. In female mullet, strippable egg volume underwent a striking increase after exposure to 17,20B-P or to aqueous extract of mullet testis, while 17,20B-P-G caused a decrease. Although preliminary, these results suggest that 17,20B-P and/or its glucuronide are primer pheromones in male minnow and mullet, species that differ markedly in ecology and systematic position. These findings not only suggest a widespread role for these compounds as primer pheromones in fish, but also underscore the need for further research on the components of sex pheromones (or of other modes of communication) that may be responsible for the specificity of their action.

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ENDOCRINE AND CHROMOSOME TECHNIQUES TO REGULATE SEX RATIO IN TILAPIA

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The treatment characteristics to ensure cent percent sex reversal in Oreochromis mossambicus using endocrine and chromosome manipulation techniques are described. Considering 17 -methyltestosterone (MT) as a model. The role played by the interfering factors like temperature photoperiod, period rate, stocking density on sex reversing potency of the steroid is explained: the role of other factors like solubility and purity of the steroid is described. Techniques to ensure cent percent meiotic and mitotic gynogens and all-female triploids are described: the survival of the treated zygotes is higher than the values reported by previous workers: the causative factors responsible for some of these are discussed.

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ENDOCRINE CONTROL OF SPAWNING BEHAVIOUR IN NESTING MALE DEMOISELLE (*CHROMIS DISPILUS*, POMACENTRIDAE).

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Previous studies have shown a strong correlation between plasma levels of testosterone (T) and 17α,20β-dihydroxy-4-pregnen-3-one (17,20βP), and spawning or courtship display in wild nesting male demoiselles. In contrast, plasma steroid levels were lower during egg-brooding behaviour, indicating that elevated steroids were not simply associated with possession of a territory. In order to examine the role of steroids in spawning or display behaviour more critically, brooding male fish were netted by SCUBA divers, injected with human chorionic gonadotrophin (hCG) and released back onto the nest sites. Behaviour was subsequently monitored by diving over the next 18h at the end of which time fish were recaptured and blood sampled. Treatment with hCG resulted in significant elevations in plasma T, 11-ketotestosterone and 17,20βP relative to saline treated fish. Thirty percent of hCG-injected fish also showed onset of display behaviour, whereas all saline-treated fish maintained brooding activity in phase with the rest of the population. Further studies examining the direct effects of exogenous steroids on reproductive behaviour are in progress. The underwater manipulation of nesting demoiselles appears to have considerable potential for the investigation of endocrine regulation of reproductive behaviour in wild fish populations.

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In vitro ESTROGENIC POTENCY OF PHYTOESTROGENS ON LIVER VITELLOGENIN SYNTHESIS IN THE RAINBOW TROUT (Oncorhynchus mykiss).

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The estrogenicity of both a soya-based diet and a commercial diet used in fish farming has been demonstrated by their ability to induce vitellogenin (VTG) synthesis when fed to Siberian sturgeon Acipenser baeri. Phytoestrogens, known to be present in soya extracts, were then synthesized and tested for their ability to induce vitellogenin synthesis by administration to yearling sturgeon. All of the compounds tested exhibited an estrogenic action except formononetin. In order to compare the estrogenic potencies of these phytoestrogens to estradiol, we developed a system for the primary culture of rainbow trout hepatocytes. A variety of steroids, including synthetic estrogens and phytoestrogens, were tested in this system. Our results demonstrate that all six phytoestrogens tested stimulate the synthesis of VTG in a dose-dependent manner. The phytoestrogens vary in potency, but all are considerable less potent than estradiol-17B. However, because phytoestrogens are present in many fish-meals at high concentrations, fish diets containing significant proportions of soya or alphalpha meal are likely to be able to induce VTG synthesis.

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PROOPIOMELANOCORTIN (POMC)- RELATED PEPTIDES IN THE PITUITARY AND OVARY OF EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX* L.) AND GILTHEAD SEA BREAM (*SPARUS AURATA* L.).

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POMC- related peptides are present in the brain and several other tissues of mammals where they appear to be involved in various physiological events. However, very little is known about the distribution and function of these peptides in non- mammalian vertebrates , even though their presence has been documented in the brain of cyclostomes, elasmobranchs and teleosts.Using immunocytochemical and biochemical techniques , we attempted to demonstrate the occurrence of a POMC- related opioid system in the pituitary and ovary of sea bass and sea bream. The ovaries were fixed in Bouin's solution and processed by the indirect immunofluorescence and PAP methods using polyclonal rabbit antisera against synthetic α-MSH and ß-endorphin (UCB , Belgium) , diluted 1:200 and 1:800 respectively with a PBS solution containing 1 mg BSA/ml, 0.1 % Na N3, and 0.03 % Triton X 100. Strong ß -endorphin - like immunoreactivity was localized in the cytoplasm of oogonia ; on the contrary, α-MSH - like immunostaining appeared to be localized in the granulosa and thecal layer of mature oocytes. Using HPLC combined with an immunological assay, ß -endorphin and related peptides , as well as α - MSH -like peptides were found in the pituitary , brain and ovary of sea bass and sea bream. In the ovary, ß-endorphin and Acetyl - ß - endorphin coexist , while in the pituitary only negligible amounts of ß -endorphin are found; similarly , α -MSH predominates over ACTH 1-13 . This suggests that ovarian and pituitary ß - endorphin undergo different fates , one independent of each other. An extensive acetylation of POMC- peptides occurs in the pituitary while authentic peptides are present in the ovary of both species.

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ACTIONS OF AMINES, PEPTIDES AND SEX STEROIDS IN REGULATION OF GONADOTROPIN-II SECRETION

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Gonadotropin-II (GtH-II) secretion in teleosts is under a combined stimulatory and inhibitory neuroendocrine regulation. It is well recognized that GtH-II secretion is stimulated by gonadotropin-releasing hormone (GnRH) and that, in a wide range of teleosts, GnRH-stimulated GtH-II release is inhibited by dopamine. Responsiveness to GnRH is presumed to vary with GnRH receptor capacity; GnRH receptors in the pituitary of goldfish vary during reproductive cycles, with the highest capacity occurring around spawning. In addition to the short-term dopamine inhibition of GnRH-stimulated GtH-II release, dopamine also can cause a decrease in GnRH receptor capacity. In intact goldfish, treatments with estradiol and testosterone cause increased responsiveness to GnRH agonist analog in stimulating GtH-II release. The primary metabolite of dopamine in goldfish is dehydroxyphenylacetic acid (DOPAC), indicating that degradation by monamine oxidase is primary. Catecholestrogens do not alter levels of dopamine in goldfish brain and pituitary, and have either no effect or a stimulatory influence on responsiveness to exogenous GnRH treatment. Neuropeptide Y is a potent stimulator of GtH-II release. Dopamine inhibits GnRH release in postpuberal goldfish by actions at the level of the pituitary, as well as the brain. Noreprinephrine has stimulatory effects on release of GnRH from brain, and it directly stimulates GtH-II release from the pituitary. Serotonin stimulates GtH-II release, and it also stimulates GnRH release from nerve fibers in the goldfish pituitary. In summary, dopamine has multiple inhibitory effects against an array of stimulatory inputs in the regulation of GtH-II secretion.

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DICHOTOMOUS EFFECTS OF PHORBOL ESTER ON OVARIAN STEROID PRODUCTION AND OOCYTE MATURATION IN *FUNDULUS HETEROCLITUS*

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Most evidence indicates that gonadotropic hormones induce ovarian steroidogenesis by a cAMP-mediated pathway. The specific roles and possible interactions of other signal transducing pathways and second messengers in regulating fish follicular functions are not well understood. In the present study we have investigated the actions of phorbol 12-myristate 13-acetate (PMA), a potent activator of protein kinase C (PKC), on somatic steroidogenesis and oocyte germinal vesicle breakdown (GVBD) by using prematurational ovarian follicles in vitro. PMA alone slightly increased basal 17α-hydroxy,20β-dihydroprogesterone (DHP) and 17β-estradiol (E_2) synthesis and significantly stimulated GVBD. Addition of FPE stimulated synthesis of DHP, testosterone (T) and E_2, and initiated GVBD (most likely by the DHP released). PMA inhibited FPE-stimulated steroidogenesis but increased the number of oocytes that underwent GVBD, probably by a mechanism independent of follicular steroid production. PMA also markedly impeded induction of steroidogenesis by dibutyryl cAMP, suggesting that the site(s) of action of PMA follows the generation of cAMP. In addition, PMA differentially affected the conversion of 25-OH-cholesterol, pregnenolone or progesterone to DHP, T and E_2: DHP and T production were reduced and E_2 synthesis increased (T aromatization was also increased). These results suggest an inhibitory role for the PKC pathway in ovarian steroidogenesis, but a marked stimulatory role for GVBD or oocyte maturation.

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EFFECTS OF DIETARY METHYLTESTOSTERONE ON ONE YEAR OLD SEA BASS

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The control of sex differentiation in sea bass (Dicentrarchus labrax) was attempted. The specific purposes of this study were to test: a) whether sea bass gonads are capable to respond to hormonal treatments just after sex differentiation, and b) the effects of 17α-methyltestosterone (MT) at two different doses on some tissues and body indexes. The ultimate goal is the production of all-female stocks by indirect feminization, since females appear to grow faster, and to avoid the precocious maturation of some males. Sea bass were treated with MT at either 10 or 30 mg/kg of food for 75 days. The results show that: a) treatment with 10 mg MT/kg slightly increased growth, while at 30 mg/kg growth was depressed, b) MT at 30 mg/kg resulted in 87% males, c) the hepatosomatic index, the size of the interrenal cells and the hematocrit level were increased in relation to the dose of MT, d) the visceral fat and the gonadosomatic index were reduced in relation to the dose of MT, and e) MT did not affect the external appearance, the carcass index and the survival of the treated fish.

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REGULATION OF TESTICULAR STEROIDOGENESIS BY COHO SALMON GONADOTROPINS, GTH I AND GTH II, *IN VITRO*. J. V. Planas, P. Swanson and W. W. Dickhoff. School of Fisheries, Univ. of Washington, Seattle, WA 98195 and Northwest Fisheries Center, National Marine Fisheries Service, Seattle, WA 98112 , USA.

The salmon pituitary produces two gonadotropins, GTH I and GTH II, which have similar activities on ovarian steroidogenesis *in vitro*. In order to determine the steroidogenic activity of GTH I and GTH II in maturing male salmonids, the effects of coho salmon GTH I and GTH II on the *in vitro* production of 11-ketotestosterone (11-KT), 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P) and cyclic AMP (cAMP) by testicular tissue from maturing coho salmon were investigated. Testicular fragments prepared from testes of fish in the mid- to late stages of spermatogenesis were incubated in the presence or absence of the test substances for 18 hr at 15º C and the secretion of 11-KT and 17,20β-P were determined by specific radioimmunoassays. The intracellular production of cAMP was measured using a commercial assay kit for cAMP. Both GTH I and GTH II stimulated the production of 11-KT and 17,20β-P in a dose- and time-dependent manner, but with slight differences in potency throughout the experimental period. While GTH I and GTH II were equipotent in stimulating the production of 11-KT and 17,20β-P in tissue from stage II testes, GTH II was more potent than GTH I in tissue from stage V testes. The steroidogenic activity of both GTH I and GTH II was mediated by cAMP as indicated by (1) the ability of dibutyryl cAMP and forskolin to mimic the steroidogenic actions of GTHs and (2) the similar ability of GTH I and GTH II to increase intracellular cAMP in the testicular preparations. Therefore, GTH I and GTH II do not appear to differ qualitatively in their ability to stimulate testicular 11-KT and 17,20β-P production, however GTH II appears to be more potent than GTH I in testes at the end of spermatogenesis.(Supported by NSF Grant DCB 9004332 to WWD and PS).

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ENVIRONMENTAL AND ENDOCRINE CONTROL OF BARBEL (*BARBUS BARBUS* L.) REPRODUCTION

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The influence of environmental (photoperiod, temperature, food, behaviour) and endocrine (estradiol-17B, testosterone, T3, T4) factors on barbel reproduction was investigated by tank experiments. Barbels were reared at constant temperature (20°C). They matured spontaneously and females were stripped of eggs at frequent intervals : 10-15 "spawnings" for each female were obtained at 15-day intervals during the reproductive period (from February to August). The distribution of intraovarian oocytes of females, just after ovulation, is quadrimodal (including primary oocytes, oocytes with vacuoles, vitellogenic oocytes and ova). A decreasing photoperiod (16.5Light / 7.5Dark -> 8L/16D), for an annual cycle contracted to 6 months duration, inhibited the spawning of both female and male fish. This allowed two periods of reproduction (February - May and September - November) within one year. The "spawning" of females and males (previously maintained under 10L/14D or 16.5L/7.5D) remained a longer time under 6L/2D/2L/14D or 6L/8.5D/2L/7.5D than under 8L/16D suggesting the existence of a daily rhythm of photosensitivity in the barbel. In natural water temperature (in tank) female barbels spawned twice during the reproductive period (June - July). In females the period between two succesive ovulations was characterized by high E2 levels (>300 pg/ml) and a sinusoidal variation of these E2 levels.There was a significant correlation between the testosterone levels and the gonadosomatic index in the males. The thyroxine (T4) concentrations of the males and females were low during reproduction. They were high outside reproductive periods. the opposite was noticed for the plasma triiodothyronine (T3) concentration. The barbels exhibited a spontaneous and synchronized expelling of sexual products if there was a spawning substrate.

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STRESS-INDUCED DISRUPTION OF THE LIVER-GONAD AXIS IN RAINBOW TROUT

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The complex of hormones responsible for production of the yolk precursor vitellogenin are disrupted by environmental stress. A receptor-mediated decline in hepatic vitellogenin production is demonstrated and the functional implications of a cortisol - sensitive estradiol receptor are discussed.

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REGULATION BY GONADAL STEROIDS OF THE EXPRESSION OF GONADOTROPIN (GTH-II) α AND ß SUBUNITS IN THE EUROPEAN EEL

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The gonadotropic function of the eel at the silver stage is very weak, resulting from a deficiency in GnRH secretion and from a dopaminergic blockade of the action of this neuropeptide. Estradiol was shown to stimulate the biosynthetic activity of pituitary gonadotropic cells, and to increase their content in GTH-II. Moreover, we demonstrated a stimulation by estradiol of cell-free mRNA directed synthesis of the α subunit precursor. In order to study further the molecular mechanism involved in this phenomenon, we cloned and characterized the cDNA corresponding to the α and ß subunits of the eel GTH-II. They were used as probes to measure their corresponding mRNA levels after different steroid treatments. Northern blot analysis of eel pituitary RNA showed that testosterone and estradiol implantation resulted in a strong increase in mRNA level for the ß subunit and, to a lesser extent, in mRNA level for the α subunit. These effects were detectable within 4 days and were maximum 4 weeks after steroid implantation. Co-implantation of testosterone and estradiol induced a strong potentiation of their effects on the ß subunit and only a cumulative effect on the α subunit. This suggests that the expressions of the two genes are regulated by different mechanisms.

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PHOTOPERIODISM AND MELATONIN RHYTHMS IN SALMONID FISH.

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The annual cycle of reproduction in salmonid fish appears to be controlled by an endogenous circannual rhythm which, under natural conditions, is entrained by the seasonal changes in daylength. Exposure to modified seasonal photocycles, constant 'long' or 'short' daylengths, or short periods of continuous light, can advance or delay maturation depending on the timing of exposure in relation to the phase of the reproductive cycle, and the photoperiodic history of the fish. These effects can be interpreted as corrective phase advances or phase delays of the circannual clock. Under laboratory conditions the most important determinant of these phase-shifts is the direction of change of photoperiod, rather than absolute daylength; maturation can be advanced even in fish which do not experience any increase in daylength in spring provided they receive a decrease to an even shorter photoperiod shortly before the summer solstice. The nature of the mechanism(s) responsible for the transduction of photoperiodic information to the reproductive axis in salmonids is unclear. In many vertebrates the pineal gland converts photic information into a circadian rhythm of melatonin secretion, and, in certain seasonally-breeding mammals, the duration of the night-time increase in this hormone determines the reproductive response. In the rainbow trout and Atlantic salmon there is a similar nocturnal elevation in melatonin secretion, which accurately reflects the duration of the dark period (e.g. longer under 8L:16D than 16L:8D), and hence provides the fish with information on daily and calendar time. In contrast to other vertebrates the melatonin rhythm in at least one salmonid, the rainbow trout, is not truly circadian (does not free-run in constant darkness). However, this is not inconsistent with the hypothesis that the photoperiodic entrainment of seasonal reproduction in salmonids is mediated by seasonal changes in patterns of melatonin secretion.

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SHORT PERIODS OF CONTINUOUS LIGHT CAN ADVANCE AND DELAY SPAWNING IN THE RAINBOW TROUT.

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Photoperiod regimes utilising continuous light (LL) and requiring no blackout facilities are potentially attractive for the commercial production of out-of-season eggs. A series of experiments were conducted to assess the ability of short periods of LL (in an otherwise ambient regime) to advance or delay maturation in November-January spawning rainbow trout maintained under naturally fluctuating water temperatures. Fish subjected to 2 month periods of LL from mid-September to mid-April (Sept-Nov, Oct-Dec, Nov-Jan, Dec-Feb, Jan-Mar, Feb-Apr) commenced spawning 6-7 months after first exposure to LL, but a high proportion of fish (>85%) attained maturity only in those groups maintained under LL from Dec-Feb and Jan-Mar. Although a few fish responded to only 2 weeks LL in a subsequent experiment at least 1 months exposure (Jan-Feb) was required to advance spawning in a majority (>80%) of the fish. In a commercial trial 96% of females subjected to 2 months LL from Jan-Mar spawned again in the summer (principally July and August) and milt was available from similarly treated males throughout this period. Conversely, spawning was delayed until February-April in 73% of females maintained under LL from late July to late September; the remaining 27% failed to mature during the experimental period. These results demonstrate that short periods of LL can both advance and delay spawning in the rainbow trout, and that the proportion of fish responding is dependent on both the duration of the light period and its position in relation to the phase of the reproductive cycle. It is proposed that short periods of LL influence spawning time in the rainbow trout by causing corrective phase-shifts (advance or delay) of an endogenous circannual rhythm. (Work supported by the NERC).

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PLASMA SEX STEROID BINDING ACTIVITY IN THE AFRICAN CATFISH, *CLARIAS GARIEPINUS*.

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The aim of the present study was to examine African catfish blood plasma for the presence of sex steroid binding activity and to determine the steroid binding characteristics of the presumed sex steroid binding protein (SBP). To this end, a binding assay was set up on the basis of the dextrane-coated charcoal technique. From the steroids tested, testosterone (T) was found to have the highest binding affinity and was used as tritiated ligand in all future experiments. In competition studies (1- to 1000-fold molar excess of radioinert steroids) the following steroids competed with tritiated T, in the order of decreasing affinity: T >> androstenedion > 17ß-estradiol > 11ß-hydroxytestosterone = DHT > 17α-hydroxy,20ß-dihydroprogesterone > 11-ketotestosterone >> 11-ketoandrostenedion > cortisol > 11ß-hydroxyandrostenedion. Omission of calcium and addition of 2 mM EDTA led to a decrease of specific binding (T - 3.5%, androstenedion - 32.2%, 17α-hydroxy,20ß-dihydroprogesterone - 69.3%), an effect which was more pronounced for steroids showing a low affinity to SBP. Scatchard analysis of saturation assays with plasma samples from mature males showed that T was bound by a single type of binding site with high affinity and relatively high capacity (K_D - 1.21±0.24 nM, capacity - 326±65 nM, equivalent to ca. 94 ng T/ml plasma; n = 6, mean and standard deviation). Similar results were obtained for the K_D when it was determined from the association and dissociation rate constants. Affinity and capacity data were similar in sexually mature male and female fish. Small changes were observed in binding capacity in plasma from males during ontogenetic development (before puberty until sexual maturity) or after castration; binding affinity appeared to remain constant.

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INFLUENCE OF DIET ON OVARIAN GROWTH AND STEROIDOGENESIS IN LARGEMOUTH BASS.

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Largemouth bass, Micropterus salmoides, are typically raised on diets of forage fish. The use of commercially available pelleted feeds has been anecdotally linked to impaired reproduction in bass. These studies were performed to compare patterns of ovarian growth and steroidogenesis in bass raised on forage (goldfish, Carassius auratus) and pellet (BioDiet grower) diets. Peak GSI values for pellet fed fish were significantly greater than in forage fed fish (6.98 ± 0.70 vs. 4.59 ± 0.39). In forage fed bass, serum testosterone (T) and estradiol (E2) titers increased rapidly during ovarian recrudescence; maximum steroid levels were correlated with maximum GSI. Despite producing large ovaries, no peak in serum E2 was observed in pellet fed bass during ovarian recrudescence. The E2 secreting capacity of ovaries from forage and pellet fed bass was assessed in vitro. Ovarian slices from pellet fed bass had higher basal E2 secretion, and a greater response to hCG stimulation, than tissue from forage fed fish. These results suggest that 1) low serum E2 titers in pellet fed fish are probably not due to ovarian dysfunction; 2) increased GSI in pellet fed bass may be the result of compensatory hypertrophy due to low serum E2 titers; and 3) diet can have important effects on ovarian growth in this species.

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HORMONAL PROFILE ASSOCIATED WITH BREEDING BEHAVIOUR IN *OREOCHROMIS NILOTICUS*.

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Seven phases have been defined in the breeding cycle of the mouthbrooding tilapia, *Oreochromis niloticus*, maintained as families comprising one male and 7-10 females. These were: quiescence, initial gonadal growth, inert behaviour, nuptial coloration, pairing, spawning and brooding. Fish formed a hierarchy and the status of each female corresponded with her breeding phase with the males always being dominant. In females, estradiol level increased from the quiescent phase through the initial ovarian growth and the inert phases, reached a peak of *ca.* 30 ng/ml in nuptial coloration and decreased soon after the completion of spawning. Testosterone in females increased from about 4 ng/ml in the quiescent phase to about 30 ng/ml in inert and nuptial coloration stages but decreased to about 4 ng/ml during pairing. The highest testosterone level (56 ng/ml) occurred in fish brooding eggs or larvae in the mouth. Brooding females exhibited the most aggressive behaviour, attacking other females and even the dominant male. The immunoreactive progestin, 17α, 20β-dihydroxy-4-pregnene-3-one was less than 6 ng/ml in all phases tested. In two females sampled during actual spawning gonadotropin (taGTH) level was above 140 ng/ml; at all other phases taGTH level ranged from 10.4 to 14.3 ng/ml. In the two males participating in the above spawning taGTH was *ca.* 30 ng/ml while in males reared separately from females it did not exceed 10 ng/ml.

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INTER-SEXUAL VIBRATIONAL COMMUNICATION DURING SPAWNING BEHAVIOUR IN THE HIMÉ SALMON (LANDLOCKED RED SALMON, ONCORHYNCHUS NERKA).

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Synchronous spawning between the sexes is essential for many fishes to succeed in fertilization. Here, we report (1) recording experiments of electromyographic activity and accompanying body vibration during spawning behaviour in the himé salmon. The results showed that the himé salmon have an elaborate communication system in which characteristic vibrational signals are exchanged. These vibrational signals are produced by body vibration due to trunk muscle activity related to the spawning, transmitted between the sexes with an accurate timing, and act as cues to synchronize the gamete release. We also report (2) experiments to determine what mode of sense is used to detect the vibrational signals. Co^{2+} ion which blocked the response from the lateral line nerve to vibrational stimuli also blocked the spawning behaviour elicited by a vibrating "model". This suggests that the lateral line sense is involved in the inter-sexual vibrational communication during spawning in the himé salmon.

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EFFECT OF AGE AND STAGE OF SPAWNING SEASON ON OUTPUT, FERTILIZING CAPACITY AND FREEZABILITY OF RAINBOW TROUT (Oncorhynchus mykiss) SPERM

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First spawning rainbow trout milters (n = 41) were stripped at two week intervals. Volume, density and fertilizing capacity of fresh and frozen semen were recorded. Avg. length of spawning season was 5.5 (1.5 to 8) mo with > 90% of males supplying semen from early December to mid March. Volume, sperm number/stripping and freezability of semen were lower in the 1st third of the season than in the remaining part (0.9 vs 3.8 ml; 17 vs 46 $X10^9$; 26 vs 43% eyed eggs, all $P < 0.01$). Fertilization rate of fresh semen did not differ (76 vs 75% eyed eggs). Ten milters were followed through 2 consecutive seasons. Avg. values for 1st and 2nd season were: volume/stripping: 3.3 vs 6.5 ml ($P < 0.01$); number of sperm/stripping: 46 vs 72 $X10^9$ ($P < 0.01$); fertilization rate of fresh semen: 78 vs 80% eyed eggs; fertilization rate of frozen semen: 45 vs 34% ($P < 0.01$). Seasonal trends were similar for both years. The effect of individual fish was highly significant for all parameters except for the fertilization rate of unfrozen semen.

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STEROIDS: DEVELOPMENTAL CONTINUUM BETWEEN MOTHER AND OFFSPRING

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Evidence from several lines of research leads us to theorize that sex steroids of maternal origin are important in affecting sexual development and perhaps differentiation in embryonic or very young salmonid fishes. This premise is based on inference from data we have demonstrating that the ovulated egg has similar androgen, estrogen and progestin loads as ovarian fluid and maternal plasma, and that these concentrations decline following fertilization until the time of hatching at which time the animal initiates de novo steroid synthesis. However, the central control of steroidogenesis is established prior to this time, being present in the yolk sac fry where we have found that gonadotropin can stimulate at least androstenedione production. Hypothalamic and pituitary protein hormones are also present during early development. The chronology of these events is such that the timing of sexual development could be under control of higher centers or interrenals and that the steroids of maternal origin could play a priming or permissive role.

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SEX STEROIDS IN THE RAINBOW TROUT: PLASMA LEVELS AND TESTICULAR SECRETION IN VITRO.

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Circulating sex steroid levels vary in the course of the reproductive cycle in male rainbow trout (*Oncorhynchus mykiss*). Changes in steroid secretion of testicular explants in response to a pituitary extract (PE) from *Oncorhynchus* sp. also follow a cyclic pattern. Maximum PE sensitivity and steroid secretion capacity was observed during the spawning season. In mammals, LH is an important factor for regulating testicular LH sensitivity. Thus, changes in the PE sensitivity of trout testicular explants may reflect the 'stimulatory history' *in vivo*. To test this, male trout at the end of spermatogenesis and/or during the spawning season were treated with SGA-GTH (Syndel Ltd., Canada; 30 µg/kg body wt), or were passively immunized against SG-G100 by antiserum injections. Control groups received BSA or non immune serum, respectively. Testicular explants were then incubated with increasing amounts of PE. SGA-GTH treatment increased plasma steroid levels and up-regulated testicular steroid secretion *in vitro*, i.e. greater steroid amounts were secreted in response to the same PE dose. Passive immunization decreased plasma steroid levels and also led to an up-regulation of PE stimulated testicular steroid secretion. In addition, we observed an increased sensitivity for PE. Thus, the PE responsiveness of the already rather active steroidogenic system of advanced trout testis is up-regulated following an increase and a decrease of circulating GTH bioactivity. This may help understanding that both, high steroid and high maturational GTH levels are recorded in the blood of mature male trout, a situation apparently conflicting with the negative feedback concept.

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MEASUREMENT OF THE OOCYTE MATURATION-INDUCING STEROID IN TELEOST URINE.

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Urine from sexually mature male and female plaice, female Dover sole and female Atlantic salmon contains sulphated 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P; the maturation-inducing steroid). The amounts (500 to 3000 ng/ml) of this novel compound far exceed those of free and glucuronidated 17,20β-P (Scott, A.P. and Canario, A.V.M. 17α,20β-dihydroxy-4-pregnen-3-one 20-sulphate: a major new metabolite of the teleost oocyte maturation-inducing steroid. *Gen. Comp. Endocrinol.*, in press). Injection of mature female plaice (*Pleuronectes platessa*) with Human Chorionic Gonadotrophin significantly increased 17,20β-P-sulphate levels in urine. This compound did not appear to be produced, however, by plaice ovaries or testes incubated with tritiated 17α-hydroxy-4-pregnen-3-one. Other sulphated steroids were produced, however, including, in the male, 17α,20α-dihydroxy-4-pregnen-3-one 20-sulphate.

Perhaps the most interesting finding we have made is that 17,20β-P-sulphate is a major product of rainbow trout gonads. Nobody has previously suspected the existence of this compound. We have established that it does not induce oocyte maturation. It might, however, be a pheromone.

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NEUROPEPTIDES AND THEIR GENES IN FISH

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Salmon is a useful model for the study of neuropeptides that control reproduction, growth and water balance. To enable us to understand the control of these functions we need to study the molecular basis of the genes that encode the relevant neuropeptides.

The gonadotropin-releasing hormone (GnRH) family, which controls reproduction, is now known to contain seven distinct family members. Salmon possess two forms of GnRH. One of these is widely distributed in vertebrates, whereas the other is found mainly in teleosts. Salmon GnRH is 80% similar to human GnRH in contrast to salmon growth-hormone releasing hormone (GHRH) which has only 41% sequence identity with the human form. However, sequence analysis of the cDNA for GHRH shows that the biologically active part of the peptide (amino acids 1-29) is more highly conserved during evolution.

This same principle also applies to vasotocin. The two cDNAs for salmon vasotocin both code for identical forms of the peptide, which is 89% similar to human vasopressin. In both the GHRH and vasotocin cDNAs, the gene-associated peptides have not been conserved during evolution suggesting that these portions of the molecules do not have a physiological function.

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ISOLATION AND CHARACTERIZATION OF THE LIPID COMPOSITION OF VITELLOGENIN FROM THREE TELEOSTS

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Vitellogenin (VTG), the hepatically derived glycolipophosphoprotein that serves as the macromolecular precursor to the egg yolk proteins, has been isolated from estradiol-treated fish. A Mono Q anion-exchange column connected to a fast protein liquid chromatography (FPLC) system was used. The method is an effective and rapid one-step procedure, which gives a pure preparation of VTG as assessed by electrophoresis, ^{32}P-phosphate incorporation and amino acid composition. Lipid class and fatty acid analyses of purified VTG from turbot, cod and rainbow trout show that teleost VTG has a total lipid content around 20 %. The most abundant lipid class is phospholipids followed by triacylglycerols. The major fatty acids of VTG were 22:6 (n-3) > 16:0 > 18:1 (n-9) > 20:5 (n-3). In the investigated species the polyunsaturated fatty acids (PUFA) accounted for more than 40 % of the total fatty acids. The fatty acid composition of VTG shows large similarities to the fatty acid composition of the egg in the same species, which indicates the importance of VTG as a lipid source for the developing oocytes.

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MECHANISM OF STIMULATORY ACTION OF GROWTH HORMONE ON OVARIAN STEROIDOGENESIS IN SPOTTED SEATROUT.

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We have previously shown that recombinant salmon growth hormone and bovine growth hormone (bGH) stimulate gonadal steroidogenesis in *Fundulus*. In present study the mechanism of bGH's action on steroidogenesis in spotted seatrout (*Cynoscion nebulosus*) was examined using an *in vitro* ovarian fragment incubation system and compared to that of gonadotropin (hCG). bGH was effective in stimulating estradiol and testosterone production and accumulation of cAMP in ovarian follicles even at a low concentration (50 ng/ml). Similar responses were observed with hCG and forskolin. The response to a combined treatment with bGH and hCG was almost the same as the sum of the stimulatory effects of the two hormones, possibly indicating that bGH does not potentiate the action of gonadotropin. bGH, hCG and forskolin stimulated aromatase activity. Cyanoketone abolished the bGH stimulation of estradiol production but did not affect the bGH stimulation of aromatase activity. Similar results were obtained with hCG and forskolin. These results indicate that bGH, like hCG and forskolin, has direct stimulatory effects on aromatase activity. The addition of cycloheximide or actinomycin D to the media abolished both bGH- and hCG-induced estradiol production as well as the stimulatory effects of the two hormones on aromatase activity. These results suggest that the stimulatory effect of bGH on aromatase activity is mediated by cAMP and depends on the synthesis of new RNA and a regulatory protein.

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EFFECT OF ISOLATION, PROXIMATION AND INTERACTION BETWEEN MALE AND FEMALE CATFISH, CLARIAS BATRACHUS ON CIRCULATING SEX STEROIDS AND GONADOTROPIN

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Changes in sexual behaviour following hormonal treatment have been well studied but effect of social stimuli on hormonal profile is least studied. Therefore, effect of isolation, proximation and interaction between males and females catfish, Clarias batrachus on circulating testosterone (T), estradiol (E2) and gonadotropin (GtH) have been studied employing radioimmunoassay (RIA). Experiments were conducted on two age groups (2 and 14 months old) maintained at 12L/12D photoperiod and 30±1^C. Isolation of sex resulted in the decline of hormone levels in both sexes when compared to unseperated fish. The decrease in male and female was identical. Exposure of male for 2 weeks to water from female aquarium after 240 days of isolation raised the levels of T and E2, but had no effect on GtH level. Response from female, when exposed for 2 weeks to water from male tank was significantly greater in terms of elevation of T and E2 levels in circulation than males. But GtH concentration in plasma did not register significant change, and the behaviour was similar to that of male GtH. When males and females were put in same tank but seperated by perforated partition, the level of all hormones (T, E2 and GtH) increased appreciably in both sexes. The magnitude of increase in level of circulating hormones was maximum when isolated males and females fish were put together in the tank without partition.

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MITOCHONDRIAL MALIC ENZYME FROM HERRING TESTICULAR AND OVARY TISSUES

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In the present study was examined the tissue specificity of malic enzyme in mitochondria from herring testicular and ovary for the presence or absence of the NADP-dependent and NAD(P)-dependent forms of malic enzyme. The activity of malic enzyme per gram wet weight or per miligram of mitochondrial protein in herring testicular and ovary tissues showed significant tissue specificity. Malic enzyme was present in relatively high activity in herring testicular mitochondria with the NAD(P)-dependent form dominating, by contrast, activity was extremely low in herring ovary mitochondria. The specific activity of herring malic enzyme per mg of mitochondria from testicular tissue was 30 times greater than that of malic enzyme found in mitochondria of ovaries.

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PHOTOPERIOD CONTROLS SPAWNING TIME IN THE ATLANTIC HALIBUT (HIPPOGLOSSUS HIPPOGLOSSUS)

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Although the spawning of the turbot, dab and sole is thought to be timed by photoperiod, no corresponding information exists for the halibut. The possible influences of light were investigated in the present work in which 10 year old 15kg halibut (Shetland stock) were maintained in 3.6m diam. ($13m^3$) covered tanks supplied with artificial light controlled by a Sangamo timer and, beginning in June, over a 3 year period, exposed to the same 12 month seasonal light cycle (lat. 56.5°N) advanced by 2 months so that the longest and shortest days occurred in April and Oct respectively. The timing of the first spawning was unaffected by photoperiod, occurring at the same time as controls over the period Feb-April, presumably because fish were not exposed to the altered photoperiod until mid-cycle. Subsequently, spawning was advanced by 1 and then 2 months at the end of the 2nd and 3rd years respectively. These results indicate that photoperiod manipulation provides a useful means of producing all-year-round supplies of eggs for the farming of halibut. The phase-delay in spawning at the end of year 2 and the spawning of fish in year 3 on exactly the same daylength as controls, albeit advanced by 2 months, suggests that reproduction in the halibut, as in a number of other fish, is timed by an endogenous rhythm.

This work was suppored by HIE and the MAFF (CSG).

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GROWTH AND SEXUAL MATURATION OF ATLANTIC SALMON UNDER TWO TEMPERATURE REGIMES IN FLOATING ENCLOSURES.

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Individual tagged Atlantic salmon postsmolts, with initial mean weight of 0.85 kg, were reared in four floating enclosures and in two conventional sea cages from November 1989 to May 1990, when all the fish were transferred to a common cage. To establish two different temperature regimes, water was supplied from 5 m depth to two of the enclosures and from 25 m depth to the two others. In February, groups of fish were transferred between these two water temperatures. Between November and January the temperature was approximately the same at 5 and 25 m depth. Later the temperature was 1 to 2°C higher at 25 m. In the cages the temperature was 1°C higher than at 5 or 25 m until December. From January to May the temperature was 3 to 6°C lower. The fish were slaughthered in July 1990, when sex and gonadosomatic index were determined. Studies based on growth pattern of immature fish indicate that the group in the open cages had higher growth rate than the other groups as long as the temperature in the cages was higher, and that their growth rate decreased with decreasing temperature. From January to March the groups in the enclosures with water from 25 m depth had the best growth, but in the last part of the experiment their growth rate was lower. Groups with low growth rates during the first part of the experiment had increased growth rates in late spring and early summer. In the enclosures, the proportion that matured as grilse varied between 14.9 and 18.0% for males and 0 and 2.7% for females. In the conventional cages 22.3% of the males and 4.5% of the females matured as grilse. There were no significant differences in maturation among the groups in the enclosures. However, the percentage maturation in the cages was significant higher than the overall maturation in the enclosures.

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RECENT STUDIES OF THE GOLDFISH INDICATE BOTH UNMODIFIED AND MODIFIED HORMONES FUNCTION AS SEX PHEROMONES.

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The goldfish sex pheromone system now appears to be comprised of at least four components. The first component is the steroid hormone 17α,20β-dihydroxy-4-pregnen-3-one (17,20P) which is released unmodified to the water by ovulatory goldfish where it functions as an olfactory stimulant triggering gonadotropin release in conspecifics. A second component appears to be an unmodified androgen, perhaps androstenedione, and it inhibits the actions of 17,20P. In both instances it is clear the pheromone is a free steroid. The detection threshold for free 17,20P as determined by electro-olfactogram recording (EOG) is 1000 times lower than that of glucuronated 17,20P, and when the olfactory organ is continuously adapted to 17,20P it is no longer responsive to 17,20P glucuronide, suggesting the olfactory receptors are specific for free 17,20P. However, although glucuronated steroids are clearly inactive in goldfish, recent EOG experiments suggest the existence of a third steroidal sex pheromone in goldfish which is conjugated, but not with glucuronic acid, and whose actions are independent of free 17,20P. The fourth goldfish pheromone, released by ovulated and sexually receptive females, is derived from prostaglandin $F_{2\alpha}$. Recent studies of this pheromone using radioactive tracers, HPLC analysis, and EOG recording strongly suggest that it is comprised of at least three components, all of which are unknown metabolites of $PGF_{2\alpha}$. As we learn more about the goldfish pheromone system its complexity becomes increasingly apparent and with this understanding the realization that many fish are likely to use complex, often species-specific, mixtures of compounds as pheromones comprised of unmodified hormones as well as modified hormonal metabolites and conjugates.

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EFFECT OF STOCKING DENSITY ON SEX DIFFERENTIATION OF OREOCHROMIS NILOTICUS (L.)

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The onset of sexual differentiation is one of the important factors effecting the efficacy of sex inversion in all-male tilapia production. This period is reported to be 20 (at 20 °C) and 33 days (at 25-26 °C) for O. mossambicus and O. niloticus respectively. This experiment was conducted to determine the influence of stocking density on the timing of sexual differentiation in O. niloticus. Fry were initially stocked at 2 (low), 10(medium) and 20(high) per liter and fed in excess with ground trout pellet (54%protein) 5 times a day. The fry were sampled at 3-4 day intervals for 42 days after hatching for histological analysis. Sexual differentiation in fry stocked at medium and high densities commenced 3 days earlier when compared with fry at low density. At all densities sexual differentiation was completed within 14 days of hatching (27±2 °C).

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ASSESSMENT OF EGG QUALITY AND EARLY LIFE HISTORY TRAITS IN ATLANTIC SALMON TREATED WITH TESTOSTERONE.

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The consequences of hormone treatment on egg quality and early life history traits have not been thoroughly examined. In the present study, Atlantic salmon (Salmo salar) were treated with silastic pellet implants containing testosterone (200 μg/fish) four times to stimulate ovarian growth and development. Eggs collected from control and testosterone treated fish were fertilized and comparisons of egg quality parameters made up until exogenous feeding. Weight and diameter, as well as protein, lipid, carbohydrate, amino acid, energy, dry matter and ash content of eggs collected from testosterone treated fish were significantly lower than eggs collected from controls. Embryonic development and survival of treatment fish were lower than controls, but higher growth and development of alevins from treatment groups were observed after hatching. This higher growth and development of alevins was possibly due to the anabolic effect of testosterone being transferred from mother to eggs during the period of oocyte growth. The stimulatory effect of testosterone on ovarian growth and development suggests possible applications in aquaculture.

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HORMONAL PHEROMONES IN FISH: STATUS AND PROSPECTS

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Although many teleost species are known to use sex pheromones, the impact of this information on the study of fish reproductive physiology has been minimized by two practical impediments: the lack of chemically identified sex pheromones which would permit controlled physiological experiments, and the fact that pheromone research generally has involved non-commercial species. Recent studies, suggesting that sex pheromones of a variety of teleosts (Cyprinidae, Clariidae, Gobiidae, Cottidae, Salmonidae) are released hormones (steroids, prostaglandins) and their metabolites, have begun to remove both impediments. These findings are exciting not only because they demonstrate that sex pheromone research can be facilitated by the use of commercially available *hormonal pheromones*, but also because the pheromonal effects (ovarian growth, ovulation, spermiation, sex behavior) are of general interest at both pure and applied levels. This paper briefly covers three aspects of fish hormonal pheromones. (1) *a review of the evidence.* (2) *consideration of areas where research has yet to be undertaken.* Of theoretical interest is the question of how reproductive ecology and strategy are related to hormonal pheromone evolution, and the stages this evolution might involve. This issue is closely related to that of the species-specificity of hormonal pheromones, a potential problem considering the chemical conservatism of the endocrine system. On a functional level, many questions are unanswered: e.g. by what route is the pheromone released, and what temporal exposure patterns result? is responsiveness to hormonal pheromones related to reproductive condition and, if so, by what agents and at what levels? (3) *suggestions of ways that an understanding of hormonal pheromones can contribute to related aspects of fish reproductive physiology.* Apart from obvious applications to controlled reproduction of cultured fish, hormonal pheromones can be readily applied to studies of olfactory receptor function, and of brain mechanisms regulating sex behavior and pituitary function.

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THE DEVELOPMENT OF THE GONAD IN THE PROTANDRIC ANEMONEFISH AMPHIPRION FRENATUS

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Gonadal development and social control of sex differentiation in the protandric anemonefish Amphiprion frenatus has been investigated histologically. Primary gonads of juvenile fish are immature ovaries in all individuals. Further gonadal development is socially controlled by chemical cues. Immature fish, which succeed in taking up the α-position in a group develop immediately into functional females without passing through a male phase. α-females induce the differentiation of spermatogenic activity in lower ranking fish. Experimental changes of the social hierarchy alter gonadal development. Animals ascending from the (male) β- to the (female) α-position change sex and develop into functional females. The gonadal transformation is accompanied by marked changes of urinary bladder morphology. It is suggested, that this organ may be involved in the social control of sex.

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EFFECT OF PHOTOPERIOD DURING PARR-SMOLT TRANSFORMATION ON SEXUAL MATURATION IN ATLANTIC SALMON.

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Altough of considerable economic importance for the salmon farming industry, the relatioship between growth rate and time of parr-smolt transformation in freshwater, and growth rate and maturation in seawater is poorly understood in Atlantic salmon. In the present study, potential 1+ smolts of Atlantic salmon were reared under three experimental light regimes, either simulated natural photoperiod (LDN), continuous light (LD24:0) or a combination of a continuous, low intensity background illumination and a superimposed simulated natural photoperiod (dual photoperiod, LDD). LD24:0 and LDD enhanced growth rate in freshwater and stimulated earlier development of smolt characters compared with LDN. The smolts from LD24:0 and LDD were larger than those from LDN on transfer to seawater in May 1988. In seawater all groups were held under natural light. Water temperature was 10+/-1°C. In seawater the fish from LDN had a higher overall growth rate, and after 16 months of rearing in seawater, there were no significant size differences among the groups. However, the incidence of sexual maturation as post-smolts, after 6 months in seawater was significantly higher in the LD24:0 and LDD groups (5.7 and 3.9%, respectively) than in the LDN group (0.9%), whereas the incidence of grilsing, after 18 months in seawater was higher in the LDN group (16.8%) compared to LD24:0 and LDD (10.6 and 9.1%, respectively). The results indicate a relationship between photoperiod manipulation in freshwater, smolt size and the timing of sexual maturation. The higher incidence of males maturing as post-smolts may be a consequence of the larger smolt size, whereas the higher proportion of grilse in the LDN group may reflect the higher growth rate in seawater. This work was supported by the Nordic Fund for Technology and Industrial Development (NI88.107).

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VITELLOGENIN AND VITELLOGENESIS IN STRIPED BASS BROODSTOCK.

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A rabbit antiserum raised against blood plasma from mature female striped bass (Morone saxatilis) and then adsorbed with mature male plasma recognized only mature female-specific plasma proteins (anti-FSPP). It was used in a single radial immunodiffusion assay (RIDA) to monitor fractions collected during vitellogenin (Vg) purification. Phosphoprotein phosphorus (PP) was also measured. Immature striped bass given subdermal silastic tubing implants containing 15 mg of estradiol (E_2) were bled, the plasma was collected, mixed with aprotinin (1 TIU/ml), and chromatographed on a DEAE-agarose column. A single symmetrical peak was collected that was induced by E_2, reacted with anti-FSPP, contained most of the PP, and appeared as a single band (~170 Kd m.w.) after SDS-gradient PAGE. In Western blots the anti-FSPP recognized only this band. The peak fraction gave a single band in native gradient PAGE which stained positively for lipid (Sudan black-B) and phosphorus (methyl-green). Sepharose-6B chromatography of native Vg revealed a single band of 500-600 Kd m.w., suggesting that the intact molecule may be a homotetramer. The RIDA was used to measure changes in circulating Vg through two full reproductive cycles in female striped bass broodstock. Plasma Vg levels rose rapidly from < 0.1 mg/ml in October to peak levels (0.5 - 1.0 mg/ml) in November through March. They then declined continuously during the spawning period in April and May. Vg was undetectable (< 0.05 mg/ml plasma) only in summer (July-September). These results indicate that the Vg assay can be used to identify maturing females up to seven months prior to the spawning season. It has potential application for use like a clinical "pregnancy test" in striped bass aquaculture.

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Actions of GtH I and GtH II on ovarian steroidogenesis in the rainbow trout, *Oncorhynchus mykiss*.

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Gonadotrophin (GtH) is a major endocrine effector in the control of ovarian development in teleosts. In this study the action of two structurally distinct gonadotropins, GtH I and GtH II, were investigated on steroidogenesis in cultured ovarian follicles of the rainbow trout, *Oncorhynchus mykiss*. Follicles at various stages of development throughout vitellogenesis were incubated with or without GtH and the culture media subsequently analysed for oestradiol-17β and testosterone. In the absence of gonadotrophin, production rates of these steroids varied during the study mimicing the well established seasonal patterns that occur *in vivo*. In culture, GtH I, but not GtH II stimulated oestradiol-17β production, especially during early and mid-vitellogenic development. Similarly, GtH I, but not GtH II, stimulated testosterone production in follicles approaching ovulation. The steroidogenic action of GtH I provides further evidence that GtH I plays an integral role during the major growth phase of oocyte development, paralleling the function of FSH in tetrapods.

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RECENT PROGRESS IN SALMON GONADOTROPIN I and II RESEARCH: RECONCILING OLD AND NEW IDEAS.

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Although it is clear that two chemically different gonadotropins, GTH I and GTH II, that are homologous to tetrapod FSH and LH are present in the salmon pituitary, the physiological significance of these two GTHs has not been firmly established. In salmonids, immunocytochemical studies have shown that GTH I and GTH II are produced by different cell-types in the proximal pars distalis. GTH I can first be detected in the pituitary of coho salmon 50-54 days post-fertilization whereas GTH II appears much later, near the time of the onset of spermatogenesis and vitellogenesis. Blood plasma levels of GTH I increase during the period of vitellogenesis and spermatogenesis, but decline at the time of final maturation. In contrast, plasma levels of GTH II are low or non-detectable until final maturation. These data suggest that GTH I may be involved in regulation of gametogenesis prior to final maturation whereas GTH II may be involved in regulating the final stages of maturation. This idea is supported by data showing that GTH I is 100-times more potent that GTH II in stimulating uptake of vitellogenin by trout ovarian follicles *in vitro*. Studies of the steroidogenic actions of GTH I and GTH II have shown that during salmonid vitellogenesis and spermatogenesis, both GTHs stimulate ovarian estradiol and testicular 11-ketotestosterone production *in vitro* with similar potency. One consistent finding is that GTH II is clearly more potent than GTH I in stimulating *in vitro* production of 17α,20β-dihydroxy-4-pregnen-3-one by either ovarian or testicular tissue from salmon near final maturation. Recent studies of gonadal receptors using both autoradiographic localization and radioreceptor assay techniques suggest that GTH I and GTH II have different binding sites. Data from physiological studies of GTH I and GTH II in salmonids will be discussed with a view toward previously published data on various GTH preparations.

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TWO GONADOTROPIC GLYCOPROTEINS IN THE RED SEABREAM (PAGRUS MAJOR)

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Two gonadotropic glycoproteins (PmGTH I and II) were purified from pituitaries of red seabream, a marine teleost which has an asynchronous-type ovary and spawns every day during the spawning season, by ion-exchange chromatography, gel filtration and preparative SDS-PAGE. They were composed of two distinct subunits and their molecular weights were 32 kD for PmGTH I and 38 kD for PmGTH II, respectively. Both PmGTH I and II have activities to induce oocyte maturation of the red seabream *in vitro*.

Homologous radioimmunoassay for the measurement of PmGTH II was developed using a rabbit antiserum against the β subunit of PmGTH II and intact PmGTH II as standards and radioactive competitors. Competition curves for red seabream plasma and pituitary extract were parallel to the standard curve, while PmGTH I showed low cross-reactivity with the antibody. This specific RIA system revealed that plasma levels of PmGTH II began to increase at migratory nucleus stage and reached a peak of 3.7 ng/ml at oocyte maturation stage, followed by a rapid decrease to minimum levels at time of spawning. These results suggest that the female red seabream possesses diurnal rhythm of GTH secretion during spawning season.

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PHOTOPERIODIC CONTROL OF GROWTH, INCIDENCE OF SEXUAL MATURATION AND OVULATION IN ADULT ATLANTIC SALMON.

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Growth, incidence of sexual maturation and timing of ovulation is of major importance in farming of Atlantic salmon. The present study investigated the possibility to use photoperiodic manipulation to control these processes. Atlantic salmon kept 18 months in netpens in brackish water at Matre Aquaculture Station, Western Norway, were distributed into three netpens. Three different photoperiodic regimes were used during the spring: natural light (NL), natural light + 24L:0D additional light from March 13 (ALM), and natural light + 24L:0D additional light from January 23 (ALJ). The fish were transferred from the netpens to three raceways on July 13, and distributed evenly in the raceways making a total of 9 subgroups. The raceways were covered with lightproof tents and illuminated with fluorescent tubes. The photoperiods used were: 8L:16D (8L), simulated natural photoperiod (SNP) and continuous light (24L). Growth rate increased after onset of additional light in immature fish both in January and March. Incidence of maturation among the females decreased from 90% maturing in the NL group to 66% and 11% in the ALM and ALJ groups respectively. A similar reduction was observed among the males with 83% maturing in the NL group and 56% and 16% in the ALM and ALJ groups respectively. Ovulation was accelerated in the 8L groups compared to the SNP groups and delayed in the 24L groups compared to SNP. Additional light from March accelerated ovulation compared with natural light. The results indicate a sesonal growth pattern influenced by photoperiod in immature adult salmon. It is also demonstrated that the decision to mature or not, is influenced by photoperiod and, finally, that ovulation can be controlled by photoperiodic manipulation.

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ENTRAINMENT OF THE SEMILUNAR REPRODUCTIVE CYCLE OF FUNDULUS HETEROCLITUS

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Spawning in F. heteroclitus occurs cyclically with peaks on new and full moon spring tides. Entrainment of the cycle was studied by daily collection of eggs in groups of fish maintained in 200 L. aquaria. Fish exposed to constant dimlight (0.1 Lux) spawned with a period of 14.3± 0.9 days, confirming that the cycle is endogenous. Superimposition of three day pulses of artificial moonlight (0.1 lux) on an LD 15:9 light cycle entrained spawning to the light pulses. Bright moonlight superimposed on constant dimlight also entrained spawning, but the nadirs of the cycles occurred during the artificial moonlight. Fish maintained on 21 hour days (LD13:8) spawned with periods of approximately 12.5 days compared to 15-16 days for fish maintained on 24 hour days. Entrainment to 27 hour days (LD19:8) was less consistent, resulting in a 25 day cycle in one experiment and a 14.3 day cycle in a repeat experiment. Locomotor activity entrained very precisely to 24 hour days but less clearly to the experimental daylengths, especially the 27 hour days. Activity increased with spawning on all three daylengths. When a tidal period cycle in water level was superimposed on the daily light cycle, spawning occurred with a semilunar periodicity and was concentrated on days when a "flooding" tide occurred near the time of "lights off". A one-week shift in the timing of this coincidence resulted in a similar shift in the occurrence of spawning peaks. These results indicate that the spawning cycle of F. heteroclitus can be entrained by moonlight, day-night cycles and the tidal period of water movement.

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CHANGES IN 17α, 20β, 21-TRIHYDROXY-4-PREGNEN-3-ONE MEMBRANE RECEPTOR CONCENTRATIONS IN THE OVARIES OF SPOTTED SEATROUT DURING FINAL OOCYTE MATURATION.

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A high affinity membrane receptor for 17α, 20β, 21-trihydroxy-4-pregnen-3-one (20β-S), the maturation-inducing steroid in several species of sciaenid fishes, has been identified in the ovaries and testes of spotted seatrout (*Cynoscion nebulosus*). In the present study changes in the concentration and dissociation constant of the 20β-S receptor in seatrout ovaries were investigated during final oocyte maturation. 20β-S membrane receptor concentrations were increased two- to three-fold in seatrout ovaries after induction of final oocyte maturation with an injection of LHRHa. The dissociation constant of the receptor was not significantly altered, however, following this treatment. Ovarian samples will be obtained from spotted seatrout collected on their spawning grounds in order to determine whether similar changes in receptor concentrations occur during a natural spawning cycle. We have shown that full-grown seatrout oocytes require gonadotropin stimulation in order to become responsive to 20β-S. The possible role of gonadotropin in the regulation of 20β-S receptor synthesis is being investigated using an *in vitro* ovarian incubation system.

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FREE AMINO ACIDS AND K^+ AS OSMOTIC EFFECTORS DURING PREOVULATORY SWELLING OF MARINE FISH EGGS.

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Oocytes of marine fishes undergo a rapid swelling by hydration during final maturation prior to ovulation and spawning. The volume of the ripe pelagic eggs is typically increased by a factor of 3-5 relative to that of its originating oocyte and water content typically rises from about 70% to 90-92% in the mature egg. The hydration of the oocyte decreases the specific weight of the yolk, so that the spawned egg becomes boyant in sea water. In demersal eggs the volume increase is usually less and the water content in the ripe egg is lower.

Our results on 7 species with pelagic eggs shows that the amount of FAA (free amino acids) increases with a factor between 18 and 8 times during hydration and reaches a final concentration of 130 to 200 mM in the ripe egg. In the tested demersal eggs FAA concentration in the mature egg is low (between 13 and 64 mM) and K+ seems to be much more important as an osmoefector during hydration than FAA. In the tested pelagic eggs we find a significant decrease in protein content during hydration whereas in the tested demersal eggs we find a significant increase in protein content during hydration. We therefore conclude that a large amount of the FAA pool present in the ripe pelagic egg is created by hydrolysis of yolk protein. In the tested demersal eggs however this process (if existing) seems relatively unimportant relative to preovulatory swelling.

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RELATIONSHIPS BETWEEN FECUNDITY, EGG SIZE, EGG VOLUME AND FISH WEIGHT IN FOUR FARMED STOCKS OF ATLANTIC SALMON (SALMO SALAR).

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Fecundity has been shown to be dependent on strain and age in farmed rainbow trout and a number of wild salmonid stocks. There is, however, a paucity of information on factors affecting fecundity in farmed salmon. Total egg number, egg size and total egg volume data were collected from individual 2 and 3-sea winter spawning hens of 4 different farmed Atlantic salmon stocks. Relationships between these parameters and post-strip weight were analysed using regression and covariance techniques to evaluate differences in reproductive performance between the broodstock groups. Egg size was found to be poorly related to fish weight, with coefficients of determination (r^2) of 0-19%. Regressions of total fecundity on fish weight yielded r^2 values of between 9 and 62%, with all groups showing an increased fecundity with increased fish size. The most significant regressions on fish size were described by total egg volume with r^2 ranging from 32-73%. The relationships between the 4 stocks, the two year classes and the implications of these on broodstock management are discussed.
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IDENTIFICATION OF PERITUBULAR CELLS IN THE TESTIS OF THE COMMON CARP (CYPRINUS CARPIO L.), USING AN ANTI-DESMINE ANTIBODY.

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Spermatogenesis in fish occurs within tubules or lobules in which the descendants of stem cells are located within cysts lined by cells of Sertoli. However, conflicting ideas were held concerning the presence of peritubular, or perilobular, cells in the interstitial tissue of male fish gonads. Therefore, in connection with our work on gonad differentiation, we have undertaken a study for these cells in the testis of carp, using methods which have been shown to characterize peritubular myoid cells in mouse.
In our ultrastuctural study we observed long and thin cells, located against the basal membranes around the spermatogenic tubules. Their location is similar to that of myoid boundery cells in species of the pike.
Using indirect immunocytochemistry at the light microscopical level we were able to demonstrate the presence of cytoskeletal filaments of desmine around the testis tubules, in accordance with a similar localisation in mouse testis. However, contrary to the findings in mouse, activity of alcaline phosphatase was restricted to blood vessels, it was not observed around testis tubules.
We conclude that in carp testis peritubular cells are present, similar to those in mouse and that desmine is a marker for these cells.

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AGE-DEPENDENT SEXUAL MATURATION IN THE FEMALE COMMON GOBY, *POMATOSCHISTUS MICROPS* AND THE INFLUENCE OF PHOTOPERIOD AND TEMPERATURE

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The common goby, *Pomatoschistus microps*, is a small estuarine teleost of limited longevity (1.5-2 years), growing over much of its lifespan. Size therefore approximates to age. Immature female gobies (in mixed sex tanks) were subjected to lighting regimes of 16L/8D, 12L/12D, 10L/14D, or 8L/16D from autumn 1989 to spring 1990 all at 18°C. Fish were deemed to have responded if they had achieved any degree of vitellogenesis. Samples taken after three months demonstrated a size-dependent increased sensitivity to photoperiod, the largest fishes in each regime maturing first. The mean response-size decreased with increasing photoperiod, implying that size does not restrict sexual maturation in this fish. These results may be viewed as an age-dependent decrease in photorefractoriness, at least at 18°C. At the end of the experiment in February, most fish had responded, even those on short days(8L/16D).

This age-dependent response is influenced by temperature. An explanation is offered for the varied breeding seasons and maturation times experienced by this fish at both extremes of its natural range.

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INTERACTION OF SEX STEROIDS WITH DOPAMINE (DA) AND GONADOTROPIN RELEASING HORMONE (GnRH) IN THE CONTROL OF GONADOTROPIN (GTH) SECRETION IN THE GOLDFISH

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Gonadal steroids may interact with stimulatory (i.e. GnRH) and inhibitory (i.e. DA) factors to exert feedback effects on GTH secretion in teleosts. In goldfish, intraperitoneal (i.p.) implantation for 5 days with silastic pellets containing 100 μg/g estradiol (E_2) or testosterone (T) potentiates the serum GTH response to GnRH, but does not affect basal GTH levels. In sexually regressed females, injection of a DA antagonist, domperidone (DOM; 10 μg/g) caused a slight increase in serum GTH. The GTH response to DOM was enhanced in E_2 and T treated fish. In sexually recrudescent females, DOM caused a dramatic increase in serum GTH; this response was enhanced in T-implanted fish. DA turnover rates (TOR) were determined following tyrosine hydroxylase inhibition with alpha-methyl-para tyrosine (MPT; 200 μg/g). DA contents in the telencephalon (TEL), hypothalamus (HYP) and pituitary (PIT) were determined by HPLC. E_2 and T increased DA TOR in TEL and PIT, and enhanced the GTH response to MPT. *In vivo* treatment with E_2 and T potentiates the *in vitro* GTH response of pars distalis fragments in perifusion to salmon GnRH (sGnRH). Exposure to increasing doses of the DA agonist LY17155 (LY) progressively inhibited GTH responses to 100nM sGnRH. ED_{50} estimates of LY inhibition were not affected by E_2 and T, demonstrating that DA sensitivity of gonadotroph cells is not affected by steroids. Injection of recrudescent male fish with MPT caused a 70 % depleteion of PIT DA content for 7 days and increased the GTH response to sGnRH analog. T treatment also potentiated sGnRH analog-stimulated GTH release. MPT-induced depletion of DA prior to T treatment did not affect the positive action of T on GTH release. The results demonstrate that gonadal steroids act at multiple sites to regulate GTH secretion.

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VITELLOGENESIS IN SALMONIDS.

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A feature common to all salmonids is their large egg size. In the complex co-ordinated assembly of the developing egg the majority of oocyte growth occurs during vitellogenesis and results from the uptake of selected proteins from the maternal circulation. Sequestering and packaging of an hepatically derived plasma precursor, vitellogenin (VTG) into yolk protein appears to account for the greatest proportion of this growth. The induction and synthesis of VTG have received considerable study, however, far less is known about the uptake of VTG into the oocyte and the mechanisms controlling this uptake in salmonids, or indeed in any oviparous vertebrate.

This paper reviews the current knowledge on vitellogenic development of oocytes in salmonids, focusing on the uptake of VTG into the oocyte, the mechanisms controlling this uptake and the overall dynamics of the oocyte growth process during vitellogenesis.

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MECHANISMS BY WHICH CALCIUM IONOPHORE AND PHORBOL ESTER MODULATE STEROID PRODUCTION BY GOLDFISH PREOVULATORY OVARIAN FOLLICLES.

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My recent studies have shown that agents which increase intracellular calcium content (calcium ionophore A23187) and activate protein kinase C (phorbol ester PMA) modulate basal and cAMP-stimulated steroid production by goldfish preovulatory ovarian follicles. This report describes studies evaluating sites in the steroidogenic cascade (pre- and post-adenylyl cyclase) influenced by calcium and protein kinase C. PMA (12.5-400 nM) inhibits hCG stimulated cAMP production. PMA also inhibits dibutyryl cAMP and forskolin stimulated testosterone production suggesting a second site of action distal to cAMP formation. This effect appears to be exerted prior to cholesterol side-chain cleavage as metabolism of 25-OH cholesterol to testosterone was not inhibited by PMA. Calcium has both stimulatory and inhibitory effects on cAMP and steroid accumulation. Full expression of the stimulatory effects of hCG on cAMP and testosterone production was dependent on the presence of calcium in the incubation medium. However, a high dosage of A23187 (4000 nM) attenuated hCG stimulated cAMP and testosterone production. A23187 appears to influence the cholesterol side chain cleavage enzyme by enhancing conversion of 25-OH cholesterol to testosterone; A23187 did not affect pregnenelone stimulated testosterone production. These data demonstrate specific regulatory actions of calcium and protein kinase C at multiple sites in the steroidogenic cascade both proximal and distal to cAMP generation.

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THE MALE GONAD AS A SOURCE OF PHEROMONES STIMULATING OVARIAN GROWTH IN THE AFRICAN CATFISH CLARIAS GARIEPINUS

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Recent investigations with the African catfish Clarias gariepinus showed that ovarian growth is influenced by conspecific male stimuli. Male pheromonal cues are involved, which enhance vitellogenesis. Steroids and steroid glucuronides of male origin, known to act as pheromones in later phases of the reproductive cycle of this species in mediating attraction of ovulating females, could be demonstrated by GCMS in ovarian-growth-stimulating holding water. Extirpation of the male gonads (testis and/or seminal vesicle) did not abolish the stimulatory effect of "male holding water", possibly due to unrecognized gonadal remnants or non-gonadal sources of the pheromone(s) or their precursors (1). Recent results are presented of an alternative approach to elucidate the male gonad role. The effect of fluid from homogenized testes and seminal vesicles on the ovarian development of pubertal Clarias gariepinus was tested.
References: (1) Van Weerd et al (1991) Aquac., in press.

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STEROID METABOLISM AND SYNTHESIS OF HIGHLY POLAR 7-HYDROXYLATED STEROIDS BY OVARIAN FOLLICLES AND EXTRAFOLLICULAR TISSUE OF THE GUPPY (*Poecilia reticulata*) DURING OOCYTE GROWTH AND GESTATION

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Metabolism *in vitro* of various radiolabeled steroid precursors by ovarian follicles at various stages of development (vitellogenic, postvitellogenic, early gestation, late gestation and postparturition), and extrafollicular tissue (EFT) of the guppy was investigated. While estradiol-17ß was one of the end products of metabolism in vitellogenic follicles, 17α,20ß-P and several 5-reduced metabolites were synthesised by postvitellogenic follicles. The yield of 17α,20ß-P was much lower than some 5ß-reduced metabolites synthesised by postvitellogenic follicles. Gestation stage follicles rapidly converted the precursors almost exclusively into 5-reduced and very polar 7-hydroxylated steroids, and their glucuronides. Postpartum follicles showed very poor potential for steroid metabolism. These results demonstrate distinct changes occurring in the steroidogenic potential of the follicles during the reproductive cycle. Unlike in other viviparous vertebrates, no particular steroid seems to be involved in maintaining gestation in the guppy; all the steroid precursors are converted into highly polar metabolites and their conjugates during gestation, thereby facilitating their excretion. The EFT comprising stromal tissue, special thecal cells, chromatin-nucleolus and peri-nucleolus stage oocytes also synthesised very polar 7-hydroxylated steroids and their glucuronides, providing evidence for the first time that the teleost ovarian EFT plays a role in steroidogenesis. The possible physiological significance of the synthesis of the novel polar steroids by the follicles and the EFT is discussed.

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GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC ANALYSIS OF GONADAL STEROIDS IN THE MALE AFRICAN CATFISH, *CLARIAS GARIEPINUS*.

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The aim of the present research is to identify steroid hormones involved in the feedback mechanism of the hypothalamo-hypophysial-gonadal axis. Our first step therefore was to identify and to quantify steroids, by GC-MS, in testis tissue, testis incubation medium and in blood plasma before and 48 hours after castration. Previous incubation studies with radiolabeled steroid precursors showed that at least 24 steroids could be synthesized by catfish testis in vitro. Most of these steroids were also detected by GC-MS in testicular tissue fragments and in testis incubation medium. The quantitatively dominating steroids in the incubation medium were 11ß-hydroxyandrostenedione, 11ß-hydroxytestosterone, etiocholanolone and 5ß-pregnane-3α,17α,20α-triol. In blood plasma 16 out of the 24 steroids mentioned above were identified. From the androgens testosterone and 11-ketotestosterone were found at levels above 5 ng/ml. Androstenedione, 11ß-hydroxyandrostenedione, 11-ketoandrostenedione and 11ß-hydroxytestosterone concentrations ranged between 2 and 5 ng/ml. Two days after castration, the concentrations of these androgens were decreased dramatically.

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GROWTH AND SEXUAL MATURATION IN REARED ATLANTIC SALMON.

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Sexual maturation after one year in the sea is a problem of great economic importance in Norwegian fish farming. The aim of our investigation is to describe growth patterns of different sex and maturation groups, and to study what effects different light regimes in freshwater have on growth in seawater. Individually tagged Atlantic salmon parr were reared under three different light regimes from September, 1987 until smoltification in spring 1988. The light regimes used were: 12 hours light, 12 hour darkness (LD12:12), simulated natural photoperiod (LDN) and 24 hours light (LD24:0). In early May the individuals were transferred to a common tank with seawater for subsequent growth under natural photoperiod until September, 1989, when sex was determined and gonad weigths recorded. The results indicate that maturing salmon are no larger than immatures at the time the process of maturation is starting. We also find that light strongly affects the rate of growth under natural photoperiod and relativly high temperature. Photoperiod treatment in freshwater strongly affects the quality and timing of the parr/smolt transformation and thereby the growth rate in the sea. The LD24:0 smolts were larger on transfer to seawater, but the smolts from LD12:12 had the highest growth rate in seawater, even higher than in the LDN group.

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PRODUCTION AND REGULATION OF 17α20ßOHP IN IMMATURE MALES OF RAINBOW TROUT.

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Although 17α20ßOHP is produced by the mature testes of salmonids (particularly by spermatozoa) it is not clear whether the 20ß oxido reductase (20ßOR) is present and if this steroid is produced and submitted to regulation in the immature stage. The following experiments were performed on immature male rainbow trout (6 to 13 months), stage I of spermatogenesis (gonias only) as determined histologically. Immature testes, homogenized in phosphate buffer (50 mM, pH 6.3, CaCl2 0.5 mM, saccharose 0.25 M) incubated (1 to 4 hrs) with 3H-17αOHP and NADPH as cofactor produced 3H-17α20ßOHP as detected by TLC and HPLC. In vivo : 17α20ßOHP plasma levels were detectable by RIA (0.61±0.23 ng/ml) and was found to be elevated (4.85±2.6 ng/ml) 24 h following a single injection of partially purified gonadotropin (GtH) (5 ng/g body weight). In vitro : immature testicular explants (11-20 mg/well) incubated for 24 h in culture medium produced 17α20ßOHP (76±20 pg/mg tissue) and this production was stimulated by purified GtH (166±15 pg/mg tissue). In these experimental conditions testosterone, 11keto-testosterone, oestradiol (0.5-500 ng/ml) had no significant effect on the progestin secretion. These results demonstrate that 17α20ßOHP is produced and can be stimulated by GtH at a very early stage of testicular development. At this stage 20ßOR appears to be localised in a cell type other than spermatozoa.

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THE ROLE OF ARGININE VASOTOCIN IN THE CONTROL OF STEROID HORMONE PRODUCTION IN GOLDFISH TESTES.

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Our previous studies have identified a role for the products of phosphatidylinositol (PI) hydrolysis in the control of steroid hormone production by goldfish testes in vitro. Recent studies have shown that the neurohypophysial peptide, arginine vasopressin (AVP), modulates steroidogenesis in rat Leydig cells via effects on PI hydrolysis. The present study examined the effects of arginine vasotocin (AVT), an endogenous neurohypophysial hormone of fish, on in vitro testosterone (T) production by goldfish testes. AVT, while ineffective alone, caused a dose related inhibition of human chorionic gonadotropin (hCG) stimulated T production, with maximum inhibition occurring at an AVT dosage of 10^{-6} M. This effect was of the same magnitude as the inhibition in response to protein kinase C activator PMA and calcium ionophore A23187. The relative potency of neurohypophysial peptides at this effect are as follows: AVT > AVP >> oxytocin = isotocin suggesting that this response is AVT specific. These data suggest that AVT may play a role in the control of testicular steroidogenesis in teleost fish and, as such, mechanisms controlling testis function are conserved between mammals and fish.

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MEMBRANE CONDUCTANCE CHANGES DURING OOCYTE MATURATION IN THE TELEOST *ORYZIAS LATIPES*.

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Oocyte maturation in a number of species is accompanied by changes in the membrane potential. This seems to be due to modification of membrane conductance to particular ions. There is a paucity of such knowledge in fish. This report concerns an electrophysiological study of oocyte maturation in the medaka, *Oryzias latipes*.

All oocytes were removed from decapitated females and retained their follicular cell layer intact. Three to four oocytes, immobilised by a gold wire grid, were each impaled by a glass microelectrode. A second microelectrode was inserted into one oocyte in some cases to allow current injection.

Membrane potentials of immature oocytes were markedly more negative and relatively stable compared with mature unovulated oocytes and eggs. Continuous recording of the membrane potential in some oocytes for a number of hours revealed a gradual depolarization as maturation proceeded. Current/voltage curves indicated that this depolarization was associated with a decrease in the membrane conductance.

The role of the follicle cells in this conductance change remains to be determined.

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IN VITRO BIOACTIVITY OF VARIOUS FORMS of GnRH IN RELATION TO THEIR RESISTANCE TO DEGRADATION IN THE RAINBOW TROUT

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The aim of the present work was to study the biological activity of different GnRH (mammalian and salmon) in relation to their resistance to degradation. This information is essential for the selection of efficient analogs for inducing or synchronizing spawning.

The work was performed *in vitro* by using cultured pituitary cells. The efficiency of the different peptides as well as the kinetics of their degradation and the origin of the possible proteases involved in this process were studied.

The native LHRH is the less potent, followed by the native sGnRH. Different mammalian and piscine analogs have an higher but equal *in vitro* potency while the piscine analog DAla^{6}Pro^{9}sGnRH is the more efficient. A very weak degradation was monitered only for the native molecules while the analogs were not degraded ; the proteases involved in this process are secreted from the cells into the incubation medium. These results indicate that differences in *in vivo* bioactivities of the studied peptides probably reflect differences in their binding affinities to the pituitary GnRH receptors and/or differences in their resistance to degradation in tissues other than the pituitary such as kidney and liver.

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EVIDENCE OF A MEMBRANE RECEPTOR FOR 17α,20ß-DIHYDROXY-4-PREGNEN-3-ONE (DHP) IN SALMONID OOCYTES

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Putative DHP receptor activity was isolated from the cytosol and membrane fraction of brook trout oocytes but none was detected in the nucleus. Preliminary results also failed to demonstrate testosterone binding activity in the nuclei of non-vitellogenic oocytes. Although the molecular weights of the cytosolic and membrane fractions are different the subunit structure on SDS PAGE is identical. The movement of DHP into the oocyte is by passive diffusion and in low quantities as compared to Xenopus oocytes but the movement into the zona radiata fraction of the follicle was by facilitated diffusion presumably by binding to putative membrane receptors. Addition of DHP to ovarian follicles of rainbow trout oocytes with central germinal vesicles caused all oocytes to undergo germinal vesicle breakdown (GVBD) except those follicles that had been photoaffinity labelled with R5020. Follicles incubated with testosterone or R5020 alone failed to undergo GVBD. *(Supported by NSERC A0781 to MW; NSERC A6732 to DRI & a NSERC post-grad scholarship to AM.)*

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UTILIZATION OF YOLK FATTY ACIDS BY GOLDFISH EMBRYOS AND LARVAE

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Although there is a growing body of knowledge of the lipid chemistry of teleost yolk, there is little information on the utilization of yolk lipids by the embryos and larvae, especially for non-salmonid freshwater species. Of particular interest is the utilization of yolk fatty acids during early development, since yolk fatty acid composition can be influenced by the diet of the vitellogenic female. Goldfish eggs were fertilized in a Tris-saline solution (pH 9.1) in Petri dishes (214 ± 47 (SD) eggs/dish). Two dishes were sampled each day for six days, along with the ovulated eggs. Fertility was 89.1% ± 3.7 and 89% of the fertile eggs survived for the rest of the experiment. Hatching occurred on Day 4 and the frequency of abnormal development was 1.6%. The eggs contained 37.7 µg lipid/egg; this was depleted by 7.7% on day 4 and 12.4% on day 6. Regression analysis indicated significant decreases in proportions of 16:1 (n-7), 18:1 (n-9), 18:1 (n-7), 18:2 (n-6), 20:3 (n-6) and 20:5 (n-3) and an increase in 18:0 to day 6. The results are consistent with the hypothesis that monoenes are a preferred substrate for catabolism by fish embryos and larvae. Several fates for the depleted (n-6) fatty acids are possible.

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ATRESIA IN THE OVARY OF WESTERN MACKEREL (Scomber Scombrus L.)

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Spawning stock biomass estimates of the western mackerel (Scomber scombrus) are made by dividing the annual egg production of the population by the average annual fecundity. Fecundity estimates are made prior to spawning and do not take account of follicular atresia of vitellogenic eggs which occurs during spawning. Random samples of spawning fish were collected during four survey periods covering the spawning area to the south west of Ireland; atretic follicles were then identified from histological sections using morphological criteria based on the breakdown of the zona pellucida. The prevalence (presence or absence of altretic follicles in individual ovaries) varied between 28 and 50% and the intensity (abundance of atretic follicles within an ovary) varied between 0.3 and 59%. In order to subtract the atretic follicle production from the potential fecundity estimate it is necessary to calculate the turnover rate. Preliminary experiments studying the rate of degradation of atretic follicles suggests a duration of between 3 and 15 days.

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DOPAMINE MAY INTERRUPT THE TRANSDUCTION OF GnRH SIGNAL IN THE PITUITARY OF TILAPIA AT MORE THAN ONE SITE.

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The mediation of GnRH effect on GTH release in fish involves the influx of Ca^{2+}, activation of phospholipase C and protein kinase C (PKC) and the formation of arachidonic acid. Data derived from perifusion of tilapia pituitary fragments and from short exposure of dispersed cells in static culture to cAMP agonists suggest that adenylate cyclase-cAMP are also involved as an additional or interconnected transducing system for GnRH in fish. In the presence of dopamine (DA; 1 µM) the basal secretion of tilapia GTH from perifused pituitaries was suppressed, and the surge of GTH in response to a superactive analog of GnRH (0.4 nM) was abolished. Similarly, DA inhibited the stimulatory effects of Ca^{2+} ionophore (A23187; 0.1 mM), OAG (0.2 mM), dbcAMP (3 mM) or forskolin (10 µM) but not that of arachidonic acid (50 µM). The presence of DA (1 µM) did not affect the formation of cAMP by the pituitary fragments in response to either GnRHa or forskolin, but totally abolished the surge of GTH in response to these agents. These results suggest that the inhibitory effect of DA on stimulated GTH release is exerted on the Ca^{2+}-Diacylglycerol-PKC route at a site distal to the Ca^{2+} influx but proximal to the formation of arachidonic acid. Along the other route of adenylate cyclase-cAMP system, the inhibition possibly occurs at a site distal to the formation of the nucleotide.

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THE LOCALIZATION OF GABA IN THE PITUITARY OF THE AFRICAN CATFISH, CLARIAS GARIEPINUS, AND THE EFFECT OF GABA ON THE GTH RELEASE.

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Gamma-aminobutyric acid (GABA) is one of the major inhibitory neurotransmitters within the central nervous system of vertebrates. Apart from mammals, little attention has been paid to the role and localization of GABA in other classes of vertebrates. In the teleost *Carassius auratus* GABA immunoreactive fibers have been demonstrated in the anterior and the neurointermediate lobes of the pituitary (Kah et al 1987) and in several areas of the forebrain (Martinoli et al. 1990).

Our aim was to investigate the role of GABA in the GTH release and the localization of GABA in the pituitary of the African catfish, *Clarias gariepinus*. For the ultrastructural localization, the tissue was fixed, cryosubstituted and embedded in Lowicryl HM20. Sections were treated with anti-GABA in a dilution of 1:5000 for 45 minutes and detected with goat anti-rabbit gold (GAR 10nm). Numerous immunoreactive fibers were located in the proximal pars distalis of the pituitary, containing the GTH, STH and TSH cells. In addition, pituitary fragments were placed in a perifusion system to measure the GTH-release; untill now treatment with GABA did not result in a clear effect on the GnRH- stimulated GTH-release.

Ref.: Kah et al. , Gen. Comp. Endocrinol. 67, 324-332 (1987)
Martinoli et al., Cell Tissue Res.,260, 77-84 (1990)

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ENDOGENOUS CIRCANNUAL RHYTHMS AND THE CONTROL OF REPRODUCTION IN THE SEA BASS (DICENTRARCHUS LABRAX)

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Under simulated natural conditions in eastern Spain (lat. 40°N long. 0°) spawning of female sea bass occurs over a 6-8 weeks period in February and March. Exposure of fish to one or two months of constant long days (LD 15:9) in March, April or May in an otherwise constant short day (LD 9:15) photoperiod, i.e. long days in advance of the time when the fish would receive long daylengths under natural conditions, produces advances in spawning with some groups commencing spawning as early as October. By contrast 1 or 2 months or continuous long days later in the cycle delays spawning, in some groups by up to 4 months. Exposure of fish to constant short or long daylengths throughout the year, starting in February, initially produced 2 month advances and 1½ month delays in the timings of spawning respectively. However, maintenance of the fish under the same short and long photoperiods for subsequent cycles produced further spawnings at approximately yearly intervals in the two groups with the fish under short days spawning earlier and those under long days later than the controls. Collectively, the data establish that reproduction in the sea bass is controlled by an endogenous circannual mechanism which under natural conditions is entrained by the seasonally-changing pattern of day-length.

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