REPRODUCTIVE PHYSIOLOGY OF FISH

5TH INTERNATIONAL SYMPOSIUM

JULY 2-8, 1995
THE UNIVERSITY OF TEXAS
AUSTIN, TEXAS

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Fifth International Symposium on the Reproductive Physiology of Fish
The University of Texas at Austin

Program Schedule

Sunday, July 2, 1995

Noon - 6 pm	Check-in and Registration (Jester Center West Lobby)
7:30 pm - 10:30 pm	Social (Alumni Club)

Monday, July 3, 1995

7:00 am - 8:40 am	Breakfast (Jester Center Dining Room, 2nd floor)[a]
7:00 am - 8:40 am	Registration (Symposium Office, Jester Center West Lounge, 2nd floor)
8:40 am - 9:00 am	Opening Remarks (Jester Center Auditorium)[b]
9:00 am - 11:50 am	Session I - Pituitary/Gonadotropin
11:50 am - 1:30 pm	Lunch
1:30 pm - 5:00 pm	Session II - Hypothalamus/Brain
5:00 pm - 6:30 pm	Dinner
7:00 pm - 9:30 pm	Techniques Workshops[c] (University Teaching Center)

Tuesday, July 4, 1995

7:00 am - 8:30 am	Breakfast
8:30 am - 12:00 pm	Session III - Aquaculture
12:00 pm - 1:30 pm	Lunch
2:30 pm - 5:00 pm	Poster Session I[d]
5:00 pm - 6:30 pm	Dinner
6:30 pm - 10:30 pm	Optional Tour, River Front July 4th Celebration[e]

Wednesday, July 5, 1995

7:00 am - 8:30 am	Breakfast
8:30 am - 12:00 pm	Session IV - Environmental Influences on Reproduction
12:00 pm - 1:00 pm	Lunch
1:00 pm - late evening	San Antonio Tour (dinner included)

Thursday, July 6, 1995

7:00 am - 8:30 am	Breakfast
8:30 am - 12:00 pm	Session V - Reproductive Life History
12:00 pm - 1:30 pm	Lunch
1:30 pm - 5:00 pm	Session VI - Behavior
5:00 pm - 6:30 pm	Dinner
7:00 pm - 9:30 pm	Poster Session II

Friday, July 7, 1995

7:00 am - 8:30 am	Breakfast
8:30 am - 12:00 pm	Session VII - Gonadal Physiology
12:00 pm - 1:30 pm	Lunch
1:30 pm - 5:00 pm	Session VIII - Gametogenesis
5:00 pm - 5:10 pm	Concluding Remarks
6:00 pm - 11:00 pm	End of Meeting Barbecue (dinner and music included)

[a] All meals will be served in the Jester Center Dining Room, 2nd floor

[b] All scientific sessions will take place in the Jester Center Auditorium

[c] On Monday evening, July 3, three different technique seminars/workshops will be held concurrently. We would like to give all participants the opportunity to attend each workshop and to keep the attendance at each workshop at a reasonable level. Thus, each workshop will be given three times. So that attendance is distributed evenly at each session, we will ask participants to sign up for the workshops Sunday and Monday. Please look for the sign-up sheets at the Symposium Registration desk. We would like to stress that these seminars will be information (not product) driven. The companies that will give these talks will have commercial displays at other times during the meeting.
Workshops will be conducted on the following topics:

1. Controlled release technology of bioreactive compounds, presented by AquaPharm Technologies, Inc. (Maryland, USA)
2. RNAse protection assays and other methods for RNA transcription quantification, presented by Ambion, Inc. (Texas, USA)
3. Enzyme immunoassay systems for hormone quantificiation presented by Cayman Chemical Co. (Michigan, USA)

[d] All poster sessions will take place in the Jester Center West Lounge, 2nd floor

[e] All tour buses will pick up at Speedway Avenue outside Jester Center

Scientific Program

Monday, July 3, 1995

Session I - Pituitary/Gonadotropin

Chairs: H. Habibi, and H. Goos

9:00 - 9:30 a.m. — R.W. Schulz, J. Bogerd, P.T. Bosma, F.E.M. Rebers, M.A. Zandbergen, and H.J.Th. Goos
Physiological, morphological, and molecular aspects of gonadotropins in fish with special reference to the African catfish, *Clarias gariepinus* (OR-1)

9:30 - 9:50 a.m. — B. Querat
Structural relationships between fish and tetrapod gonadotropins (OR-2)

9:50 - 10:10 a.m. — H. Tanaka, H. Kagawa, and K. Hirose
Steroidogenic activities of two distinct gonadotropins in red seabream, *Pagrus major* (OR-3)

10:10 - 10:30 a.m. — Break

10:30 - 10:50 a.m. — A. Elizur, I. Meiri, H. Rosenfeld, N. Zmora, W.R. Knibb, and Y. Zohar
Seabream gonadotropins: temporal profile of gene expression (OR-4)

10:50 - 11:10 a.m. — C. Weil, M. Bougoussa-Houadec, C. Gallais, S. Itoh, S. Sekine, and Y. Valotaire
Variation of GtH 1 and GtH 2 mRNA levels at different stages of gonadal development in rainbow trout, *Oncorhynchus mykiss* (OR-5)

11:10 - 11:30 a.m. — D. Huggard, and H.R. Habibi
Effect of testosterone on growth hormone and maturational gonadotropin subunit gene expression in the cultured goldfish pituitary, *in vitro* (OR-6)

11:30 - 11:50 a.m. — Z. Yaron, G. Gur, P. Melamed, B. Levavi-Sivan, A. Gissis, D. Bayer, A. Elizur, C. Holland, Y. Zohar, and M.P. Schreibman
Blocks along the hypothalamo-hypophyseal-gonadal axis in immature black carp, *Mylopharyngodon piceus* (OR-7)

Session II - Hypothalamus/Brain

Chairs: R. Peter, and L.H. Ran

1:30 - 2:00 p.m. — V.L. Trudeau, and R.E. Peter
Functional interactions between neuroendocrine systems regulating GTH-II release (OR-8)

2:00 - 2:20 p.m. — B. Linard, I. Anglade, S. Bennani, G. Salbert, J.M. Navas, T. Bailhache, F. Pakdel, P. Jégo, Y. Valotaire, C. Saligaut, and O. Kah
Some insights into sex steroid feedback mechanisms in the trout (OR-9)

2:20 - 2:40 p.m. Y. Gothilf, A. Elizur, J.F.F. Powell, N.M. Sherwood, and Y. Zohar
Three forms of gonadotropin-releasing hormone in gilthead sea-bream and striped bass: physiological and molecular studies (OR-10)

2:40 - 3:00 p.m. P.T. Bosma, S.M. Kolk, S. Van Haren, W. Van Dijk, R.W. Schulz, and H.J. Th. Goos
Combined autoradiographic and immunohistochemical localization of pituitary GnRH receptors in male African catfish (OR-11)

3:00 - 3:40 p.m. Break

3:40 - 4:00 p.m. J.A. Flores
Detection and measurement of gonadotropin II (GTH II) secretion from individual trout pituitary cells using a reverse hemolytic plaque assay (RHPA) (OR-12)

4:00 - 4:20 p.m. F. Van Goor, J.I. Goldberg, and J.P. Chang
Dopamine action on calcium currents in identified goldfish (*Carassius auratus*) gonadotropin cells (OR-13)

4:20 - 4:40 p.m. D. Marcano, H.Y. Guerrero, N. Gago, E. Cardillo, M. Requena, L. Ruiz, and G. Robaina
Monoamines metabolism in the hypothalamus of the juvenile teleost fish, *Chaetodipterus faber* (PISCES:Ephippidae) (OR-14)

4:40 - 5:00 p.m. K.P. Joy, and B. Senthilkumaran
A serotonergic control of pituitary-gonadal activity in the female catfish, *Heteropneustes fossilis*, under normal and long photoperiods (OR-15)

Tuesday, July 4, 1995

Session III - Aquaculture

Chairs: B. Jalabert, and C. Sullivan

8:30 - 9:00 a.m. K.J. Rana
Cryopreservation of aquatic gametes and embryos: recent advances and applications (OR-16)

9:00 - 9:20 a.m. T. Weismann, F. Lahnsteiner, and R.A. Patzner
A uniform cryopreservation method for semen of salmonid fishes and its adaption for practical application (OR-17)

9:20 - 9:40 a.m. K.B. Davis, C.A. Goudie, and B.A. Simco
The plasticity of sex determining genotypes in channel catfish (OR-18)

9:40 - 10:00 a.m. J.L. Specker, L.C. Woods III, L. Huang, and M. Kishida
Application of a non-invasive sex test in the aquaculture of striped bass (OR-19)

10:00 - 10:40 a.m. Break

10:40 - 11:00 a.m. A. Goren, H. Gustafson, and D. Doering
Commercial field trials demonstrate efficacy and economic benefit of a controlled release GnRHa implant (OR-20)

11:00 - 11:20 a.m. B. Breton, Y. Roelants, T. Mikolajczyk, P. Epler, and F. Ollevier
Induced spawning in teleost fish after oral administration of GnRH-A (OR-21)

11:20 - 11:40 a.m. M. Harel, A. Tandler, G.W. Kissil, and S.W. Applebaum
The role of dietary protein in vitellogenin synthesis and oocyte development, and its effects on reproductive performance and egg quality in gilthead seabream, *Sparus aurata*, broodstock (OR-22)

11:40 - 12:00 noon M. Carrillo, J.M. Navas, M. Thrush, J. Ramos, M. Bruce, S. Zanuy, and N. Bromage
The effect of seasonal alteration in the lipid composition of broodstock diets on egg quality in the European sea bass (*Dicentrarchus labrax* L.) (OR-23)

Poster Session I
2:30 pm - 5:00 pm

Pituitary/Gonadotropin

Agulleiro, B., M.P. García Hernández, A. López Ruiz, and A. García Ayala
Isolation and morphological identification of gonadotropic cells from the Mediterranean yellowtail (*Seriola dumerilii* Risso 1810) pituitary (PI-1)

Bogerd, J., J.J.L. Jacobs, and H.J.Th. Goos
Modification of 3'-RACE (Random 3'-RACE): partial cloning of the estrogen receptor of the African catfish (PI-2)

Chan, Y.H., K.W. Cheng, K.L. Yu, and K.M. Chan
Cloning of two prolactin complementary DNA from goldfish, *Carassius auratus* (PI-3)

Dickey, J.T., and P. Swanson
Development of RNAse protection assays for quantification of gonadotropin (GTH I and GTH II) subunit transcript levels in coho salmon (*Oncorhynchus kisutch*) (PI-4)

García-García, A., M.C. Sarasquete, J.A., Muñoz-Cueto, M.L. Gonzales de Canales, and R.B. Rodriguez
Isoforms of GTHs from tuna (*Thunnus thynnus*) pituitaries isolated by chromatofocusing and ion exchange chromatography (PI-5)

Garcia-Hernandez, M.P., Y. Koide, A. García Gómez, and H. Kawauchi
Isolation and characterization of two distinct gonadotropins from Mediterranean yellowtail (*Seriola dumerilii*, Risso 1810) pituitary glands (PI-6)

Goos, H.J.Th., J. Bogerd, M. Ter Bekke, and N. Van der Spoel
Molecular cloning of the cDNAs encoding the Prl, GH, SL and POMC precursors of the African catfish, *Clarias gariepinus* (PI-7)

Gur, G., P. Melamed, B. Levavi-Sivan, C. Holland, A. Gissis, A. Elizur, Y. Zohar, and Z. Yaron
Long-term testosterone treatment stimulates GTH II synthesis and release in the pituitary of the black carp, *Mylopharyngodon piceus* (PI-8)

Law, M.S., K.W. Cheng, T.K. Fung, Y.H. Chan, K.L.Yu, and K.M. Chan
Cloning of two growth hormone complementary DNA from goldfish, *Carassius auratus* (PI-9)

Limesand, S.W., Y-W.P. Lin, D.A. Price, and R.A. Wallace
***Fundulus heteroclitus* gonadotropin. 4. Cloning and sequencing of gonadotropic hormones (GTH) (PI-10)**

Lo, A., J. Emmen, H.J.Th. Goos, and J.P. Chang
Direct positive effects of testosterone on GnRH-stimulated gonadotropin release from dispersed goldfish pituitary cells (PI-11)

Mahmoud, S.S., M.M. Moloney, and H.R.Habibi
Cloning of the goldfish growth hormone cDNA (PI-12)

Mañanós, E., P. Swanson, J. Stubblefield, and Y. Zohar
Purification of striped bass (*Morone saxatilis*) gonadotropin II and development of an enzyme immunoassay for its measurement (PI-13)

Meiri, I., Y. Gothilf, N. Zmora, H. Rosenfeld, W.R. Knibb, Y. Zohar, and A. Elizur
Preovulatory changes in gonadotropin gene expression and secretion in the gilthead seabream, *Sparus aurata* (PI-14)

Melamed, P., G. Gur, B. Levavi-Sivan, A. Elizur, and Z. Yaron
Intracellular mediation of the GnRH effect on transcription of the tilapia GtH IIβ gene (PI-15)

Vissio, P.G., G.M. Somoza, M.C. Maggese, and D.A. Paz
Localization of pituitary cells of immunocytochemistry in the "Argentine silver side" *Odonthestes bonariensis* (PI-16)

Weber, G.M., and E.G. Grau.
Changes in serum and pituitary levels of prolactin and growth hormone with reproduction and fasting in the tilapia, *Oreochromis mossambicus* (PI-17)

Yoshiura, Y., M. Kobayashi, Y. Kato, and K. Aida
Molecular cloning of cDNAs encoding two gonadotropin β subunits (GTH Iβ and IIβ) from goldfish (PI-18)

Zhu, Y., and P. Thomas
Plasma somatolactin concentrations in Atlantic croaker during gonadal recrudescence (PI-19)

Hypothalamus/Brain

Barannikova, I.A., and B.I. Feldkoren
Cytosol sex steroids binding in brain and its levels in blood of female sturgeon (*Acipenser gueldenstaedti* Br.) during anadromous migration (PI-20)

Cardillo, E., N. Gago, H.Y. Guerrero, M. Requena, L. Ruiz, G. Robaina and D. Marcano
Serotonin metabolism in the brain of the juvenile marine teleost, *Chaetodipterus faber* (PISCES: Ephippidae) (PI-21)

Chow, M., Y. Gothilf and Y. Zohar
Molecular characterization of the seabream gonadotropin-releasing hormone gene isolated from striped bass (*Morone saxatilis*) (PI-22)

Christoforov, O.L., I.G. Murza, and B.I. Feldkoren
Brain sex steroid binding in immature, pre- and post-spawning trout, *Salmo trutta* L. (PI-23)

Dyubin, V.P.
The effects of superactive Gn-RH analog on maturation and serum sex steroids levels in wolffish (*Anarchichas lupus* L.) (PI-24)

Feldkoren, B., and I. Barannikova
Sex steroid binding in brain cytosol of stellate sturgeon (PI-25)

Khan, I.A., and P. Thomas
Neuroendocrine control of gonadotropin II release in the Atlantic croaker: involvement of γ-amino butyric acid (PI-26)

Lo Nostro, F., M. Ravaglia, G. Guerrero, M.C. Maggese, and G. Somoza
Characterization of molecular variants of GnRH in the brain of protogynous "swamp eel", *Synbranchus marmoratus* (PI-27)

Peter, R.E., C.S. Nahorniak, V.L. Bastos, C.K. Murthy, P.D. Prasada Rao, R.C. de L. Milton, R.P. Millar, and J.R. Rivier
Structure-activity relations of gonadotropin-releasing hormone in goldfish: origins of superactive analogs (PI-28)

Porter, M.J.R., C.F. Randall, and N. Bromage
The effect of pinealectomy and enucleation on circulating melatonin levels in Atlantic salmon parr (PI-29)

Rebers, F.E.M., P.Th. Bosma, P.H.G.M. Willems, R.W. Schulz, and H.J.Th. Goos
GnRH-induced calcium fluxes in primary cultures of African catfish (*Clarias gariepinus*) gonadotropes (PI-30)

Schreibman, M.P., L. Magliulo-Cepriano, M. Pennant, Z. Yaron, and G. Gur
Observations of the brain-pituitary axis in immature black carp (PI-31)

Sperry, T., and P. Thomas
Characterization of an androgen receptor in the brain of the Atlantic croaker, *Micropogonias undulatus* (PI-32)

Stefano, A.V., O. Fridman, and G.M. Somoza
Chromotographic and immunological evidence for a third form of GnRH in addition to cIIGnRH and sGnRH in the brain of *Odonthestes bonariensis* (Atheriniformes) (PI-33)

Subhedar, N., J. Cerdá, and R.A. Wallace
Distribution of neuropeptide Y-like immunoreactivity in the forebrain and retina of the killifish, *Fundulus heteroclitus* (PI-34)

Tchoudakova, A.V., and G.V. Callard
Evidence for tissue-specific aromatase transcripts in goldfish brain and ovary (PI-35)

Wang, D.S., H.R. Lin, and H.J.Th. Goos
The neuroendocrine regulation of gonadotropin secretion in the bagrid catfish, *Mystus macropterus* (PI-36)

Youson, J.H., M. Docker, and S.A. Sower
Concentration of gonadotropin-releasing hormones in brain of larval and metamorphosing lampreys of two species with different adult life histories (PI-37)

Aquaculture

Bart, A.N., D.F. Wolfe, and R.A. Dunham
Effects of cryoprotectant, sperm density and straw size on long-term cryopreservation of blue catfish, *Ictalurus furcatus*, sperm (PI-38)

Borode, A.O., and A.A. Salami
Induction of spawning using non-conventional gonadotrophins (PI-39)

Carnevali, O., F. Centonze, S. Brooks, J. Sumpter, and N. Bromage
mRNA expression and enzymatic activity of cathepsin D related with seabream egg quality (PI-40)

Castrejón-Osorio, M.T., and S. Escárcega
Control of reproduction in grass carp *Ctenopharingodon idella* (PI-41)

Clearwater, S.J., and L.W. Crim
Milt quality and quantity produced by yellowtail flounder (*Pleuronectes ferrugineus*) following GnRH-analogue treatment by microspheres or pellet (PI-42)

Dréanno, C., M. Suquet, J. Cosson, C. Cibert, H. Huignard, and R. Billard
CO_2 effects on flagella of native and demembranated turbot spermatozoa (PI-43)

Estay, F.J., N.F. Díaz, and L. Valladares
Ovarian morphological changes and plasmatic sex steroid profiles in two cultured salmon (*O. kisutch* and *S. salar*) broodstock populations in Chile (PI-44)

Freund, F. , G. Hörstgen-Schwark, and W. Holtz
Plasma steroid hormones in adult triploid tilapia (*Oreochromis niloticus*) (PI-45)

Galbreath, P.F., K.J. Adams, P.A. Wheeler, and G.H. Thorgaard
Production of clonal lines of Atlantic salmon x brown trout hybrids by gynogenesis (PI-46)

Gale, W.L., M.S. Fitzpatrick, and C.B. Schreck
Immersion of Nile tilapia in 17α-methyltestosterone and mestanolone for the production of all-male populations (PI-47)

Goudie, C.A., B.A. Simco, and K.B. Davis
Failure of gynogenetically-derived male channel catfish to produce all-male offspring (PI-48)

Gwo, J.-C.
Ultrastructural study of osmolality effect on spermatozoa of three marine teleosts (PI-49)

Holland, C.H., S. Hassin, E.L. Mañanós, Y. Zohar
The effects of sustained administration of testosterone and GnRHa on GtH-II levels and gametogenesis in immature striped bass, *Morone saxatilis* (PI-50)

Joss, J., and G. Joss
Breeding Australian lungfish in captivity (PI-51)

Kaeriyama, M., S. Urawa, and M. Fukuwaka
Reproductive variation in hatchery-released sockeye salmon (PI-52)

King, H., and G.Young
Increased milt production by gonadotropin releasing hormone analog (GnRHa)-treated Atlantic salmon after injection of 17α-hydroxyprogesterone (PI-53)

Labbé, C. , G. Maisse, and J.H. Crowe
Interaction of cryoprotectants with trout sperm plasma membrane during freeze-thawing: a biophysical study (PI-54)

Lahnsteiner, F., B. Berger, T. Weismann, and R.A. Patzner
Evaluation of physiological and biochemical parameters for estimation of semen fitness for cryopreservation and for quality determination of deep frozen semen in the rainbow trout (*Onchorynchus mykiss*) (PI-55)

Linhart, O.
Ova management and optimization of artificial insemination in the European catfish (*Silurus glanis* L.) (PI-56)

Mager, R.C., S.I. Doroshov, J.P. Van Eenennaam
Reproduction of delta smelt (*Hypomesus transpacificus*) in captivity (PI-57)

Magnus, Y., A. Ar, and E. Lubzens
Permeability of ornamental carp fertilized eggs to ^{3}H-DMSO and its toxicity to developing embryos (PI-58)

Mahmoud, S.S., M.M. Moloney, and H.R.Habibi
Production of recombinant carp growth hormone molecule (PI-59)

Mims, S.D., W.L. Shelton, and J.A. Clark
Steroid induced sex reversal of paddlefish (PI-60)

Mylonas, C.C., and Y. Zohar
Preparation and evaluation of GnRHa-loaded, polymeric delivery systems for the induction of ovulation and spermiation in cultured fish (PI-61)

Navas, J., E. Mañanós, M. Thrush, J. Ramos, S. Zanuy, M. Carrillo, Y. Zohar, and N. Bromage
Effect of the lipid composition of the diet on the hormonal levels and spawning performance of sea bass (*Dicentrarchus labrax*) (PI-62)

Neira, R., F.J. Estay, N.F. Diaz, and X. García
Characterization of SO reproductive cycle for coho salmon (*Oncorhynchus kisutch*) in Chile (PI-63)

Ohta, H., and T. Izawa
Cool storage of the Japanese eel (*Anguilla japonica*) spermatozoa (PI-64)

Okoko, M., and R.P. Phelps
Effect of methyltestosterone concentration on sex ratio, growth and development of Nile tilapia (PI-65)

Perchec, G., J. Cosson, F. André, C. Paxion, and R. Billard
Alteration of carp spermatozoa motility by urine contamination during sampling (PI-66)

Phelps, R.P., D.C. Neu, K.L. Veverica, and M.S. Fitzpatrick
Interactions of feeding rate, weight gain, reproductive characteristics and spawning of male channel catfish (PI-67)

Richardson, G.F., L.W. Crim, Z. Yao, and C. Short
Cryopreservation of yellowtail flounder (*Pleuronectes ferrugineus*) semen (PI-68)

Ritar, A.J., and M. Campet
Cryopreservation of sperm from striped trumpeter *Latris lineata* (PI-69)

Rosenblum, P., H. Horne, G. Garwood, T. Brandt, and B. Villarreal
Delayed ovarian development and reduced fecundity in largemouth bass raised on a pelleted feed containing high levels of steroids and low levels of arachidonic acid (PI-70)

Rothbard, S., Y. Hagani, B. Moav, W.L. Shelton, and I. Rubinshtein
Gynogenesis in albino grass carp, *Ctenopharyngodon idella* (Val.) (PI-71)

Sato, N., I. Kawazoe, Y. Suzuki, and K. Aida
Characterization of emulsion prepared with lipophilized gelatin and its application for inducing vitellogenesis in Japanese eel (PI-72)

Schoore, J.E., R. Patiño, K.B. Davis, B.A. Simco, and C.A. Goudie
Gonadal sex differentiation in channel catfish (PI-73)

Shangguan, B., and L.W. Crim
Effects of stripping frequency on the seasonal spemiation response and sperm quality in male winter flounder, *Pleuronectes americanus* (Walbaum) (PI-74)

Shelton, W.L., and S. Rothbard
Gonadal differentiation of black carp (PI-75)

Solar, I.I., J. Smith, H.M. Dye, D. McKinley, Y. Zohar, and E.M. Donaldson
Induced ovulation of Chinook salmon using a GnRHa implant: effect on spawning, egg viability and hormone levels (PI-76)

Stefansson, S.O., J. Duston, and R.L. Saunders
Post-smolt maturation in Atlantic salmon fed different ration levels (PI-77)

Steinberg, H., A. Hedder, R. Baulain, and W. Holtz
Cryopreservation of rainbow trout (*Onchorhynchus mykiss*) sperm in straws (PI-78)

Tiersch, T.R.
Cryopreservation of fish sperm: laboratory, hatchery and field studies of twenty species (PI-79)

Vizziano, D., J.R. García-Alonso, D. Carnevia
Effect of cations, pH and osmolality on sperm motility of male white croaker, *Micropogonias furnieri* (PI-80)

Yao, Z., L.W. Crim, G.F. Richardson, and C.J. Emerson
Cryopreservation, motility and ultrastructural changes of sperm from the ocean pout (*Macrozoarces americanus* L.), an internally fertilizing marine teleost (PI-81)

Zanuy, S., C.C. Mylonas, L.A. Sorbera, M. Carrillo, and Y. Zohar
Sustained administration of GnRHa increases sperm volume without altering sperm counts in the sea bass (*Dicentrarchus labrax*) (PI-82)

Zheng, W., J.R. Cardwell, and N.E. Stacey
Sex pheromone-enhancement of fertility in male cyprinids: application in goldfish and carp (PI-83)

Environmental Influences on Reproduction

Bon, E., J. Nunez Rodriguez, and F. Le Menn
The effect of photoperiod on vitellogenin synthesis and oocyte endocytosis in rainbow trout (*Oncorhynchus mykiss*) (PI-84)

Bornestaf, C., E. Antonopoulou, and B. Borg
Effects of aromatase inhibitors on sexual maturation in three-spined stickleback, *Gasterosteus aculeatus* (PI-85)

Brooks, S., T.G. Pottinger, C.R. Tyler, and J.P. Sumpter
Does cortisol influence egg quality in the rainbow trout, *Oncorhynchus mykiss*? (PI-86)

Brown, N.P., N.R. Bromage, and R.J. Shields
The effect of spawning temperature on egg viability in the Atlantic halibut, (*Hippoglossus hippoglossus*) (PI-87)

Burton, M.P.M.
Links between nutrition and reproduction in fish (PI-88)

Contreras-Sanchez, W.M., C.B. Schreck, and M.S. Fitzpatrick
Effect of stress on the reproductive physiology of rainbow trout, *Oncorhynchus mykiss* (PI-89)

Coward, K., and N.R. Bromage
Density-dependent inhibition of spawning in the substrate spawning cichlid *Tilapia tholloni* (Sauvage) (PI-90)

Dabrowski, K., R.E. Ciereszko, A. Ciereszko, G.P. Toth, S.A. Christ, and J. Ottobre
Off-season maturation and factors influencing gamete production in yellow perch (*Perca flavescens*) (PI-91)

Davies, B., P. Swanson, and N.R. Bromage
The effects of photoperiod and temperature on serum GTH I, GTH II and the timing of maturation in the female rainbow trout (PI-92)

Hansen, T., O.S. Kjesbu, J.C. Holm and Ø. Karlsen
Growth, gonadal development and spawning time of Atlantic cod (*Gadus morhua*) reared under different photoperiods (PI-93)

Idler, D.R., Y.P. So, G.L. Fletcher, and J.F. Payne
Depression of blood levels of reproductive steroid glucuronides in male winter flounder exposed to small quantities of hibernia crude, crankcase oil, oily drilling mud and harbour bottom sediments in the few months prior to spawning (PI-94)

Johnson, L.L., S.Y. Sol, D.P. Lomax, and T.K. Collier
Effects of endocrine-disrupting chemicals on marine flatfish reproduction: an approach to environmental risk assessment (PI-95)

MacLatchy, D.L., X. Yao, L. Tremblay, and G.J. Van Der Kraak
The hormone mimic β-sitosterol alters reproductive status in goldfish (PI-96)

Ungerer, J.R., and P. Thomas
Transport and ovarian accumulation of o,p'-DDT in the Atlantic croaker (*Micropogonias undulatus*) during gonadal recrudescence (PI-97)

Wednesday, July 5, 1995

Session IV - Environmental Influences on Reproduction

Chairs: C. Richter, and L. Johnson

8:30 - 9:00 a.m. — C.A. Strüssmann, and R. Patiño
Temperature manipulation of sex differentiation in fish (OR-24)

9:00 - 9:20 a.m. — J.F. Baroiller, and E. Geraz
Temperature sex determination in two tilapias species, *Oreochromis niloticus* and the red tilapia (Red Florida strain): effect of high or low temperatures (OR-25)

9:20 - 9:40 a.m. K. Aida and M. Amano
Salmon GnRH gene expression following photoperiod manipulation in precocious male masu salmon (OR-26)

9:40 - 10:00 a.m. N.R. Bromage, C.F. Randall, and M.J.R. Porter
How do photoperiod, the pineal gland, melatonin, and circannual rhythms interact to co-ordinate seasonal reproduction in salmonids? (OR-27)

10:00 - 10:40 a.m. Break

10:40 - 11:00 a.m. B. Norberg, B.Th. Björnsson, and C. Haux
Photoperiod controls the timing of reproduction in Atlantic cod (OR-28)

11:00 - 11:20 a.m. I. Berglund
Effects of water temperature in spring on sexual maturation in male Atlantic salmon (*Salmo salar* L.) parr. (OR-29)

11:20 - 11:40 a.m. G. Van der Kraak, and M. McMaster
What is the significance of subtle changes in the reproductive performance in response to environmental disturbances? (OR-30)

11:40 - 12:00 noon. J.G.D. Lambert, and P.A.H. Janssen
A long term study of the effects of polluted sediments on the annual reproductive cycle of the female flounder, *Platichthys flesus* (OR-31)

Thursday, July 6, 1995

Session V - Reproductive Life History
Chairs: N. Pankhurst, and M.S. Fitzpatrick

8:30 - 9:00 a.m. I.P. Callard
The reproductive endocrinology of elasmobranch oviparity and viviparity (OR-32)

9:00 - 9:20 a.m. S.A. Sower
Neuroendocrine control of reproduction in lampreys (OR-33)

9:20 - 9:40 a.m. M. Matsuyama, S. Morita, N. Hamaji, M. Kashiwagi, and Y. Nagahama
Diurnal rhythm in testicular activity in the secondary male of a protogynous wrasse, *Pseudolabrus japonicus* (OR-34)

9:40 - 10:00 a.m. P. Kestemont, J. Rinchard, and R. Heine
A comparative study of the vitellogenesis dynamic and reproductive ecology in single and multispawner cyprinids (OR-35)

10:00 - 10:40 Break

10:40 - 11:00 a.m. J.S. Rhodes, R.D. Fernald, and R.C. Francis
Plasticity of sexual expression in an African cichlid (OR-36)

11:00 - 11:20 a.m. H. Ueda, M. Kaeriyama, A. Urano, K. Kurihara, and K. Yamauchi
Homing mechanisms in salmon: roles of vision and olfaction (OR-37)

11:20 - 11:40 a.m. P.M. Lokman, and G. Young
Plasma sex steroids in female New Zealand freshwater eels (*Anguilla spp.*) before and at the onset of the natural spawning migration (OR-38)

11:40 - 12:00 noon. L.W. Crim, Z. Yao, and Z. Wang
Reproductive mechanisms associated with internal fertilization of eggs in a benthic marine teleost, the ocean pout *Macrozoarces americanus* (OR-39)

Session VI - Behavior

Chairs: S. Scott, R. Bjerselius, and J. Cardwell

1:30 - 2:00 p.m. N.E. Stacy, and J.R. Cardwell
Hormones as sex pheromones in fish (OR-40)

2:00 - 2:20 p.m. E.L.M. Vermeirssen, and A. P. Scott
A pheromone in female rainbow trout urine (OR-41)

2:20 - 2:40 p.m. P.W. Sorensen, L. Bowdin, A.R. Brash, R. Kellner, and F.W. Goetz
Origins and functions of F prostaglandins as hormones and pheromones in the goldfish (OR-42)

2:40 - 3:00 p.m. C. Waring, and A. Moore.
F-series prostaglandins have a priming pheromonal effect on mature male Atlantic salmon parr (OR-43)

3:00 - 3:40 p.m. Break

3:40 - 4:00 p.m. A.H. Bass
Alternative life history strategies and dimorphic males in an acoustic communication system (OR-44)

4:00 - 4:20 p.m. P. Poncin, M. Ovidio, G. Skoufas, C. Mélard, K. Mol, D. Desprez, B. Cuisset, E.R. Kühn, and J.C. Ruwet
Behavioural and endocrine study of *Oreochromis aureus*, with special reference to sex-reversed males (OR-45)

4:20 - 4:40 p.m. H. Zakon
Behavior, brains, and biophysics: steroidal modulation of communication signals in electric fish (OR-46)

4:40 - 5:00 p.m. J.G. Dulka
Androgen-induced changes in electrocommunicatory behavior are correlated with changes in Substance P-like immunoreactivity (SPI-ir) in the brain of the weakly electric fish, *Apteronotus leptorhynchus* (OR-47)

Poster Session II
7:00 pm - 9:30 pm

Environmental Influences on Reproduction

McMaster, M.E., G.J. Van Der Kraak, and K.R. Munkittrick
Differential modes of hormonal disruption in fish exposed to various organic contaminants (PII-1)

Mosconi, G., A. Gallinelli, F. Facchinetti, and A. Polzonetti
Chronic stress activates opioid-adrenal system in gilthead seabream *Sparus auruta* (PII-2)

Nash, J., D.E. Kime, W. Holtz, and H. Steinberg
Slow release melatonin implants elevate daytime plasma melatonin but do not affect the reproductive seasonality of the female rainbow trout, *Oncorhynchus mykiss* (PII-3)

Okimoto, D.K., and M.H. Stetson
Effect of light on melatonin secretion *in vitro* from the pineal of the hammerhead shark, *Sphyrna lewini* (PII-4)

Pankhurst, N.W., G. Van Der Kraak, and R.E. Peter
Evidence that inhibition of reproduction by stress is not mediated by the action of cortisol on ovarian steroidogenesis (PII-5)

Randall, C.F., M.J.R. Porter, and N.R. Bromage
Preliminary observations on the effects of melatonin implants and pinealectomy on the timing of reproduction in rainbow trout (PII-6)

Shimizu, A.
Effects of photoperiod and temperature on reproductive activity of the mummichog *Fundulus heteroclitus* during various seasons (PII-7)

Short, C.E., L.W. Crim, and M.J. Morgan
The effects of stress on spawning performance and larval development in Atlantic cod, *Gadus mohura* (PII-8)

Singh, T.P., N. Sinha, B. Lal, and K. Acharia
Seasonal shifts in daily cycles of free T4 and T3 and role of sex steroids in binding ability of T4 and T3 to their thyroid binding proteins in *Clarias batrachus* (PII-9)

Taranger, G.L., H. Daae, K.O. Jørgensen, and T. Hansen
Effects of continuous light on growth and sexual maturation in sea water reared Atlantic salmon (PII-10)

Tate, A.E., and L.A. Helfrich
Phase-shifted photothermal cycles advance gametogenesis and sexual maturation of F_1 hybrid striped bass (PII-11)

Tremblay, L., and G. Van Der Kraak
Interactions of the environmental estrogens nonylphenol and β-sitosterol with liver estrogen receptors in fish (PII-12)

Reproductive Life History

Barnett, C. W., and N.W. Pankhurst
Population density effects gonadal steroids levels in both territorial and non-territorial male damselfish, *Chromis dispilus* (PII-13)

Barron, B.R., W.M. Tsang, S. Larsen, and P.M. Collins
An evaluation of rockfish (*Sebastes* spp.) as models for the study of reproduction and development in viviparous marine fish (PII-14)

Connaughton, M.A., and M.H. Taylor
Seasonal changes in weakfish sonic muscle fiber morphology and metabolic substrate concentrations (PII-15)

Degani, G.
Control of reproductory cycle in female *Trichogaster* (PII-16)

Grier, H.J., R.G. Taylor, and R. Reese
The reproductive cycle of the common snook, *Centropomus undecimalis* (PII-17)

Hines, G.A., K.M. Wasson, W.T. Waggoner, and S.A. Watts
One fish, two fish,...girl fish, guy fish? Social environment and gonadal steroidogenesis in tilapia (PII-18)

Jackson, L.F., W. King V, E. Monosson, and C.V. Sullivan
The white perch, *Morone americana*: a laboratory model for reproduction of temperate basses (PII-19)

Johnson, A., and P. Thomas
Seasonal changes in gonadal histology and sex steroid hormone levels in the protogynous hermaphrodite, *Epinephelus morio* (PII-20)

Kasimov, R.Y., and S.Y. Mikalilova
Sturgeons of Caspian Lake: Biology, ecology ways of reproduction and quantity maintenance (PII-21)

Khanam, S.F., S. Ali, and S. Khan
Plasma levels of gonadotropin and steroid hormones in the Indian major carp, *Labeo rohita* during sexual maturation (PII-22)

Kishida, M., W.A. Tyler III, and J.L. Specker
Changes in plasma estradiol and testosterone concentrations during a brooding cycle of female mouthbrooding tilapia, *Oreochromis mossambicus,* and male mouthbrooding tilapia, *Sarotherodon melanotheron* (PII-23)

Koya, Y., T. Matsubara, T.Ikeuchi, N. Okubo, S. Adachi, and K. Yamauchi
Reproductive cycle and embryonic growth during gestation of the vivparous teleost, *Zoarces elongatus* (PII-24)

Manning, A.J., and L.W. Crim
Variability in egg quality and production in a batch-spawning flounder, *Pleuronectes ferrugineus* (PII-25)

Marcano, D., H.Y. Guerrero, and G. Caceres-Dittmar
Seasonal changes in plasma levels of sexual hormones in the tropical freshwater teleost, *Pygocentrus notatus* (TELEOSTEI: Characidae) (PII-26)

Rey-Vásquez, G., M.C. Abel, J.C. Vilardi, and M.C. Maggese
Allozymes variation in two morphological types of the swam eel, *Synbranchus marmoratus*, Bloch (PII-27)

Rinchard, J., P. Kestemont, R. Heine, and J.C. Micha
A multidisciplinary approach of the reproductive ecology of single and multiple spawner cyprinid fish in river (PII-28)

Salami, A.A., and A. Borode
Reproductive biology of wild *C. gariepinus* from two sites in south western Nigeria (PII-29)

Salinas-Torres, D., and M.T. Castrejón-Osorio
Reproductive biology in Texas cichlid *Cichlasoma cyanoguttatum* (PII-30)

Saxena, A.
Young murrels are less fatty (PII-31)

Tan-Fermin, J.D., S. Adachi, H. Ueda, K. Aida, Y. Nagahama, and K. Yamauchi
Annual changes in reproductive parameters and plasma steroid hormones in female catfish *Clarias macrocephalus* (Gunther) (PII-32)

Thompson, R.L., and P. Thomas
Steroid concentrations and reproduction in the Bluegill sunfish (PII-33)

Wiegand, M.D., and K. Charleson
Depletion of fatty acids during embryonic development in the medaka (PII-34)

Behavior

Appelt, C.W., and P.W. Sorensen
Female goldfish release pheromonally active F-prostaglandins via two routes and may control their release (PII-35)

Bjerselius, R.. W. Li, and P.W. Sorensen
Spermiated male sea lamprey release a potent sex pheromone (PII-36)

Cardwell, J.R., and N.E. Stacey
Olfactory hypersensitivity to sex pheromones in blind cave fish (PII-37)

Moore, A., and C. Waring
Seasonal changes in olfactory sensitivity of mature male Atlantic salmon (*Salmo salar* L.) parr to prostaglandins (PII-38)

Murphy, C.A., J.R. Cardwell, and N.E. Stacey
Characterization of steroidal sex pheromones in the round goby, (*Neogobius melanostomus*) (PII-39)

Oliver, A.S., P. Thomas, and C.V. Sullivan
Gametogenesis or behavior? The role of sex steroids in hermaphrodite reproduction (PII-40)

Tacon, P., A. Fostier, P.-Y. Le Bail, P. Prunet, and B. Jalabert
Relations between maternal behaviour, ovarian development, and endocrine status, in the mouthbrooding female of *Oreochromis niloticus* (PII-41)

Watts, S.A., G.A. Hines, K.M. Wasson, and W.T. Waggoner
A sexual paradox: Androgen and estrogen synthesis in tilapia (PII-42)

Gonadal Physiology

Andersson, E., C.P. Tensen and J. Bogerd
Characterization of a cDNA encoding a putative testicular gonadotropin receptor from the African catfish (PII-43)

Antonopoulou, E., S. Jakobsson, I. Mayer, and B. Borg
Effects of a 17α,20β-dihydroxy-4-pregnene-3-one on testicular androgens in Atlantic salmon mature parr *in vivo* and *in vitro* (PII-44)

Bhattacharya, S., and N.R. Jana.
Thyroid hormone induces the synthesis of a 52k protein in perch Leydig cell which stimulated androgen release (PII-45)

Condeça, J.A.B., and A.V.M. Canário
Steroidogenesis during estrogen-induced sex inversion in the sea bream, *Sparus aurata* (PII-46)

Dittman, A.H., L. Yan, and P. Swanson
Cloning and functional expression of coho salmon (*Oncorhynchus kisutch*) gonadotropin II receptor (PII-47)

Fitzpatrick, M.S., W.L. Gale, C.H. Slater, and C.B. Schreck
Gonadal androgen receptors in fishes (PII-48)

Ghosh, S., and P. Thomas
Binding characteristics of 20β-S to Atlantic croaker sperm membranes (PII-49)

Goetz, F.W., M.A. Garczynski, and S.Y. Hsu
Expression of Kallikrein gene family mRNAs in the trout ovary (PII-50)

Inbaraj, R.M., A.P. Scott, and E.L.M. Vermeirssen
5β-pregnane-3α,17α,20β-triol: a major metabolite of the oocyte maturation-inducing steroid in plaice, *Pleuronectes platessa* (PII-51)

Jakobsson, S., R.W. Schulz, M.A. Blankenstein, I. Mayer, and B. Borg
Androgen binding in the stickleback kidney (PII-52)

Kime, D.E., M.A.S. Abdullah, and K.P. Lone
Effects of substrate concentrations on steroidogenesis in ovaries and testes of three species of ambisexual fishes (PII-53)

King V, W. , S. Ghosh, P. Thomas, and C.V. Sullivan
Ovarian receptors for 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S) in striped bass (PII-54)

Laidley, C.W., and P. Thomas
Purification and characterization of a plasma sex-steroid binding protein in the spotted seatrout (*Cynoscion nebulosus*) (PII-55)

Lee, S.T.L., A.M.C. Tan, D.E. Kime, T.M. Chao, H.S. Lim, R. Chou, T.J. Lam, and C.H. Tan
***In vitro* steroidogenesis by the gonads and spermatozoa of the grouper (*Epinephelus tauvina*) implanted with 17α-methyltestosterone (PII-56)**

Lehrter, J.M., and J.M. Trant
Identification of ovarian steroids produced during the reproductive season in channel catfish (*Ictalurus punctatus*) (PII-57)

López-Rivas, R.M., and M.T. Castrejón-Osorio
Spawn induction on rosy barb *Barbus conchonius* with $F_{2\alpha}$ prostaglandin (PII-58)

MacDougall, T., and G. Van Der Kraak
Epidermal growth factor enhances ovarian prostaglandin synthesis in the goldfish (PII-59)

Maebayashi, M., B.M. Amiri, N. Omoto, T. Kawakita, T. Yamaguchi, S. Adachi, and K. Yamauchi
***In vitro* steroid biosynthesis by gonads of a hybrid sturgeon, Bester, at different developmental stages (PII-60)**

Medler, K.F., and J.M. Trant
Gonadotropic and cAMP control of steroidogenic enzymes in male channel catfish, *Ictalurus punctatus* (PII-61)

Mercure, F., and G. Van Der Kraak
Peroxisome involvement in ovarian steroidogenesis in teleosts (PII-62)

Modesto, T., and A.V.M. Canario
Steroid levels during ovulation and spermiation in toadfish (*Halobatrachus didactylus*) (PII-63)

Morrey, C., M. Nakamura, T. Kobayashi, Y. Nagahama, and E.G. Grau
Immunolocalization of steroidogenic cells throughout gonadal restructuring in the protogynous hermaphrodite *Thalassoma duperrey* (PII-64)

Mugnier, C., J.L. Gaignon, E.Lebegue, C. Fauvel, and A. Fostier
Maturation inducing steroid in turbot, *Scophthalmus maximus* L. (PII-65)

Nunez, B.S. and J.M. Trant
Regulation of steroidogenesis in the stingray interrenal (PII-66)

Riebe, J.D., T.P. Barry, J.J. Parrish, and J.A. Malison
17α,20β-dihydroxy-4-pregnen-3-one stimulates cortisol production by rainbow trout interrenal tissue *in vitro*: mechanism of action (PII-67)

Scott, A.P., E.L.M. Vermeirssen, C. Mylonas and Y. Zohar
Radioimmunoassay of 5β-pregnane-3α, 17α, 20β-triol in spermiating male plaice, *Pleuronectes platessa* (PII-68)

Slater, C.H., M.S. Fitzpatrick, and C.B. Schreck
Characterization of an androgen receptor in salmonid leukocytes: link to androgen induced immunosuppression (PII-69)

Trant, J.M.
Isolation and characterization of the cDNA encoding the spiny dogfish shark (*Squalus acanthias*) form of cytochrome P450c17 (PII-70)

Vília, P., and A.V.M. Canario
Effect of LHRH on sex reversal and steroid levels in gilthead seabream (*Sparus aurata*) (PII-71)

Gametogenesis

Benfey, T.J.
Ovarian development in triploid brook trout (*Salvelinus fontinalis*) (PII-72)

Berlinsky, D.L., Y. Tao, and C.V. Sullivan
Isolation and characterization of a vitellogenin receptor in white perch, *Morone americana* (PII-73)

Bidwell, C.A., and S.H. Danetz
Vitellogenin receptor expression in the medaka, *Oryzias latipes* (PII-74)

Carnevali, O., G. Mosconi, D.S. Zanuy, and A.M. Polzonetti-Magni
***In vitro* hormonal control of vitellogenin synthesis in two marine species *Dicentrarchus labrax* and *Sparus aurata* (PII-75)**

Cavaco, J.E.B., J.G.D. Lambert, R.W. Schulz, H.J.Th. Goos
Will puberty be regulated by sexual steroids in male African catfish (*Clarias gariepinus*)? (PII-76)

Cerdá, J., T.R. Petrino, Y.-W.P. Lin, and R.A. Wallace
Inhibition of *Fundulus* oocyte maturation *in vitro* by serotonin (PII-77)

Chang, C.-F., E.L. Lau, and B.Y. Lin
The effects of exogenous estradiol-17β in juvenile males of protandrous black porgy, *Acanthopagrus schlegeli* (PII-78)

Heppell, S.A., N.D. Denslow, L.C. Folmar, and C.V. Sullivan
Universal vertebrate vitellogenin antibodies (PII-79)

Hiramatsu, N., and A. Hara
Three egg yolk proteins are derived from salmon vitellogenin (PII-80)

Korsgaard, B.
Influence of natural vitellogenesis and estradiol-treatment on hepatic protein synthesis and gluconeogenesis in *Zoarces viviparus* (PII-81)

Khan, M.N., R. Renaud, and J.F. Leatherland
Correlation between plasma and egg steroid hormone content of Arctic charr (PII-82)

Krasnow, H.L., and G.V. Callard
Analysis of differentially expressed mRNAs during spermatogenic development in the shark testis (PII-83)

Lal, B., T.P. Singh, H.N. Singh, and S. Harikrishnan
Regulation of hydromineral balance and spermiation by mGtH and prolactin in freshwater catfish, *Heteropneustes fossilis* (PII-84)

Larsson, D.G.J., S.J. Hyllner, H. Fernández-Palacios Barber, and C. Haux
Estradiol-17β induces vitelline envelope proteins in 14 teleost species (PII-85)

Lin, F., K. Dabrowski, and L.P.M. Timmermans
Early gonadal development and sex differentiation in muskellunge (*Esox masquinongy*) (PII-86)

Lund, E.D., C.V. Sullivan, and A.R. Place
Lipid contents of female striped bass plasma and oocytes exhibit seasonal changes associated with oocyte maturation (PII-87)

Matsubara, T., and Y. Koya
Proteolytic cleavage of yolk proteins during oocyte maturation in barfin flounder (PII-88)

Pati, D., and H.R. Habibi
Effect of GnRH peptides on histone H-I kinase activity in the follicle-enclosed goldfish oocytes, *in vitro* (PII-89)

Redding, J.M., and G.V. Callard
Negative regulation of DNA synthesis by phosphodiesterase inhibitors in testes of the shark *Squalus acanthias* (PII-90)

Silversand, C., B. Norberg, J.C. Holm, Ø. Lie, and C. Haux
Dietary influence on the fatty acid composition of vitellogenin and the subsequent effect on the egg composition in cod (*Gadus morhua*) (PII-91)

Thomas, P., and S. Ghosh
Regulation of the maturation-inducing steroid receptor in spotted seatrout ovaries (PII-92)

Toth, G.P., S.A. Christ, R.E. Cicreszko, and K. Dabrowski
Spermatogenesis in the yellow perch (*Perca flavescens*) -Comparison of relative germ cell types in 2 subpopulations of young-of-year fish (PII-93)

Tripathi, V., and T.P. Singh
Effect of some steroids and prostaglandins on GVBD and ovulation in catfish, *Heteropneustes fossilis* (PII-94)

VanPutte, C.L.M., and D.S. MacKenzie
Effects of estrogen on red drum (*Sciaenops ocellatus*) thyroid function (PII-95)

Wang, Z., and L. Crim
Aspects of spermatogenesis and spermiogenesis in ocean pout *Macrozoarces americanus* (PII-96)

Friday, July 7, 1995

Session VII -Gonadal Physiology

Chairs: D. Kime, and J. Leatherland

8:30 - 9:00 a.m. — M.A. Maestro, J.V. Planas, P. Swanson, and J. Gutiérrez
Insulin-like growth factor I (IGF-I) in the fish ovary (OR-48)

9:00 - 9:20 a.m. — T. Miura, C. Miura, K. Yamauchi, and Y. Nagahama
Activin B is a major mediator of hormone-induced spermatogonial proliferation in the Japanese eel (OR-49)

9:20 - 9:40 a.m. — S.-Y. Hsu, and F.W. Goetz
Ovulation of specific transcription an antileukoproteinase-like mRNA in the brook trout ovary (OR-50)

9:40 - 10:00 a.m. — A.V.M. Canario, E. Couto, P. Vília, D.E. Kime, S. Hassin, and Y. Zohar
Sex steroids during the ovulatory cycle of gilthead seabream (*Saprus aurata*) (OR-51)

10:00 - 10:40 a.m. — Break

10:40 - 11:00 a.m. — A. Fostier
Regulation of the aromatase activity in rainbow trout, *Oncorhynchus mykiss*, ovarian follicles (OR-52)

11:00 - 11:20 a.m. — J.V. Planas, J. Athos, and P. Swanson
Regulation of ovarian steroidogenesis *in vitro* by gonadotropins during sexual maturation in coho salmon (*Oncorhynchus kisutch*) (OR-53)

11:20 - 11:40 a.m. — M. Ebrahimi, P.B. Singh, and D.E. Kime
Steroidogenesis by milt and testis of roach is affected by substrate concentration (OR-54)

11:40 - 12:00 noon — J. Pinter, and P. Thomas
Studies on a nuclear progestogen receptor in the ovary of the spotted seatrout, *Cynoscion nebulosus* (OR-55)

Session VIII -Gametogenesis

Chairs: G. Young, and C. Laidley

1:30 - 2:00 p.m. — S.J. Hyllner, and C. Haux
Vitelline envelope proteins in teleost fish (OR-56)

2:00 - 2:20 p.m. G.J. LaFleur, Jr., B.M. Byrne, R.M. Greenberg, C. Haux, and R.A. Wallace
Liver-derived cDNAs: vitellogenins and vitelline envelope proteins (OR-57)

2:20 - 2:40 p.m. C.R. Tyler, and K. Lubberink
Towards the development of genetic probes to the rainbow trout vitellogenin receptor (OR-58)

2:40 - 3:00 p.m. G. Yoshizaki, W. Jin, R. Patiño, and P. Thomas
Connexin genes, gap junctions, and ovarian maturational competence (OR-59)

3:00 - 3:40 p.m. Break

3:40 - 4:00 p.m. H. Kagawa
Effects of insulin-like growth factor-I on final maturation of oocytes of red seabream, *Pagrus major, in vitro* (OR-60)

4:00 - 4:20 p.m. M. Yoshikuni, Y. Oba, and Y. Nagahama
A pertussis toxin sensitive GTP-binding protein is involved in the signal transduction pathway of the maturation-inducing hormone (17α, 20β-dihydroxy-4-pregnen-3-one) of rainbow trout (*Oncorhynchus mykiss*) oocytes (OR-61)

4:20 - 4:40 p.m. F. Piferrer, and G. Callard
Inhibitory regulation of spermatogenesis in shark testis (OR-62)

4:40 - 5:00 p.m. F. LeGac, and M. Loir
Insulin-like growth factor expression and action in trout testis (OR-63)

Abstracts

OR-1

PHYSIOLOGICAL, MORPHOLOGICAL, AND MOLECULAR ASPECTS OF GONADOTROPINS IN FISH WITH SPECIAL REFERENCE TO THE AFRICAN CATFISH, *CLARIAS GARIEPINUS*.

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This keynote lecture will first briefly review the concept of the duality of GTHs in fish. Attention will then focus on the biological activities of the GTHs, including the (partial) overlap regarding steroid production, the ligand binding characteristics, and the cellular localisation of GTH receptors. Also, recent results will be presented on the characterisation of a cDNA encoding a putative GTH receptor from catfish testis. The potential of molecular biological techniques is further exemplified by studies on GTH gene expression, showing that key players in regulating the gonadotrope's activity, GnRH and sex steroids, also modulate the GTH mRNA levels. Moreover, cell biological aspects will be dealt with, including differential functions of GTH storage organelles in gonadotropes. After shortly reviewing the large assembly of compounds modulating GTH secretion, we will present recent results on the actions and interactions of cfGnRH and chicken GnRH-II on GTH secretion in catfish. Finally, as there is no evidence yet for the presence of a GTH I-like hormone in the African catfish, it is suggested that certain species may form an exception regarding the duality of GTHs.

OR-2

STRUCTURAL RELATIONSHIPS BETWEEN FISH AND TETRAPOD GONADOTROPINS

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The gonadotropins (LH and FSH in Tetrapods, GTH1 and GTH2 in Fish) and the thyrotropin (TSH) are members of the glycoprotein hormone family. All these hormones are composed of two subunits α and ß, the latter bearing the hormonal specificity. The GTH1 ß subunit differs from the other gonadotropin ß subunits by its cystein arrangement. The GTH2-type gonadotropin has been well characterized in Chondrostei and Teleostei, whereas the presence of the GTH1-type is so far evidenced only in the modern teleostean species, all belonging to the Clupeocephali. What are the parental relationships existing between these "Fish" gonadotropins and those from the Tetrapods, and how are they linked to thyrotropins?
A tentative phylogenetic tree is presented based on data obtained by mean of two different algorythms, PAUP (a maximum parsimony method) and Neighbor-Joining (a distance matrix method), together with a critical analysis of the sequences available. It appears that GTH2 and LH are derived from a common precursor. It seems very likely that GTH1 has evolved from the same precursor as FSH but the time of divergeance between these two hormones cannot be determined. Therefore, it is still unpredictable whether the Chondrostei and the ancient Teleostei have either a FSH-type or a GTH1-type gonadotropin. It also remains unclear whether the TSH branch diverged before the separation between the GTH1-FSH and the GTH2-LH lineages or from a (GTH1-FSH)-TSH precursor after the GTH2-LH lineage had branched out.

OR-3

STEROIDOGENIC ACTIVITIES OF TWO DISTINCT GONADOTROPINS IN RED SEABREAM, *PAGRUS MAJOR*

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We have previously shown the presence of two distinct gonadotropins, PmGTH I and II, in red seabream pituitary. The aim of the present study was to clarify the *in vitro* steroidogenic activities of PmGTH I and II in the ovarian follicles. Fish, which were spawning daily, were sacrificed at different times of day. Ovaries were removed and cut into small pieces. Oocytes with their surrounding follicular layers intact were dispersed from the ovarian pieces by pipetting and oocytes at various developmental stages were collected by sieving. About 20-30 mg (wet weight) of oocytes were incubated in 24-well culture plates containing 1 ml L-15 medium (pH 7.4) with or without various doses of PmGTH I and II. After incubation for 20 h at 18 ℃, the concentration of estradiol-17β (E_2) in media was measured by radioimmunoassay. Both PmGTH I and II stimulated E_2 production by vitellogenic stage ovarian follicles (oocytes 300-500 μm in diameter). E_2 production by vitellogenic follicles incubated with 900 ng/ml PmGTH II was 3-5 times higher than those incubated with 900 ng/ml PmGTH I. Stimulation of E_2 production by PmGTH II quickly decreased when oocytes reached about 500 μm in diameter. E_2 production in follicles of oocytes at the migratory nucleus stage and the mature stage was not stimulated by PmGTH I nor II. These results indicate that both PmGTH I and II stimulate *in vitro* E_2 production in vitellogenic follicles, and PmGTH II is more potent than PmGTH I.

OR-4

SEABREAM GONADOTROPINS: TEMPORAL PROFILE OF GENE EXPRESSION

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The gilthead seabream, *Sparus aurata*, is a protandrous hermaphrodite. All individuals are males by the end of the first year and subsequently most change into females which have asynchronous development of oocytes. We have isolated and cloned the full length cDNAs encoding the ß subunit of GtHI and GtHII from the seabream, using RACE PCR. The cloned sequences were used as probes to study the temporal profile of gene expression of these two subunits throughout the stages of gametogenesis, sex reversal, final gonadal maturation, spawning and post spawning. For each month, during a 28 month period, 10 fish were sacrificed and pituitary RNA extracted. The levels of ßGtHI and ßGtHII mRNA were measured by dot blot hybridization and analyzed by computing densitometer (values standardized using individual ß-actin mRNA levels). The results showed that both genes were expressed throughout the year, however levels were higher for ßGtHII. During the spawning season (which lasts for about three months), the RNA expression levels of both subunits increased dramatically. Expression of ßGtHI peaked at the start of the spawning season when most of the oocytes undergo active vitellogenesis, whereas expression of ßGtHII peaked later in the spawning season. This presents a unique model where GtHI and GtHII are required at the same time and increase simultaneously.

OR-5

VARIATION OF GtH 1 AND GtH 2 mRNA LEVELS AT DIFFERENT STAGES OF GONADAL DEVELOPMENT IN RAINBOW TROUT, *Oncorhynchus mykiss*

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To study the variations of α and ß GtH 1 and GtH 2 gene expresssion during male and female rainbow trout gonad development, chum salmon α and an ß GtH 1 and 2 probes were used. The α subunit cDNA probe used was identical to the cDNA encoding the α subunit common to both GtH 1 and 2. Total pituitary RNA preparations were performed and the validation of the use of these probes for studying the variations in GtH mRNAs was made by Northern blot analysis. The quantitative determination of GtH mRNAs employed slot blot hybridization. In males and females, GtH 1 predominates in early stages of gonadal development (spematogonia A and previtellogenesis), ß GtH 2 being weakly expressed. Both GtH 1ß and GtH 2ß are expressed during prespermiation, spermiation and in the periovulatory period with a predominance of ßGtH 2. In animals of both sex, α GtH variations follow ß GtH 2 variations.

OR-6

EFFECT OF TESTOSTERONE ON GROWTH HORMONE AND MATURATIONAL GONADOTROPIN SUBUNIT GENE EXPRESSION IN THE CULTURED GOLDFISH PITUITARY, *IN VITRO.*

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Testosterone is a natural steroid in goldfish and its concentration range from 2 ng/ml (in sexually regressed) to 25 ng/ml in fish with fully mature gonad. In this study we investigated the effect of testosterone on maturational gonadotropin (GtH-II) subunits and growth hormone (GH) gene expression in the cultured goldfish pituitary fragments *in vitro.* Previous studies demonstrated that treatment with testosterone, *in vivo,* results in a biphasic change in GtH-II-α and GtH-II-β mRNA levels; at low concentrations (0.2 - 2.0 µg/fish), testosterone injection stimulated GtH-II subunit mRNA levels, while at high concentrations (20 µg/fish) it inhibited GtH-II mRNA levels. The present results demonstrate that testosterone effect is direct at the level of pituitary. Incubation of goldfish pituitary fragments with testosterone (2-400 ng/ml) resulted in a biphasic effect on GtH-II subunit mRNA levels. The results demonstrate a seasonally-dependent shift in dose-related biphasic response to testosterone in the goldfish pituitary. In the same experiments we also quantified GH mRNA in the cultured goldfish pituitary fragments. Treatment with testosterone also stimulated GH mRNA level in a dose-related fashion. However, testosterone effect on GH mRNA production was not biphasic, indicating a different steroid-dependent mechanism of GTH-II and GH gene expression in the goldfish pituitary.

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OR-7

BLOCKS ALONG THE HYPOTHALAMO-HYPOPHYSEAL-GONADAL AXIS IN IMMATURE BLACK CARP, *MYLOPHARYNGODON PICEUS.*

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The objective of the present work was to localize sites along the hypopthalamic-hypophyseal-gonadal axis which are blocked in immature, 2 y old black carp (0.75-2 kg bw). Circulating cGTH in fish injected with sGnRHa + metoclopramide (10 μg/kg + 20 mg/kg), and estradiol in fish injected with carp pituitary extract (350 μg/kg cGTH) did not differ from the controls throughout the experiment (4-24 h post injection). cGTH release from culture of dispersed pituitary cells did not change after exposure to sGnRH (1pM - 1μM), however, a 300% increase occurred in response to activation of PKC by TPA (12.5 nM). This would indicate the presence of a functional transduction system for GTH release in the immature fish and a block at the GnRH receptor level. Estradiol release from incubated rudimentary gonads was stimulated by dbcAMP (0.3- 3 mM) but not by cGTH (0.1-4 μg/ml). This would indicate that the steroidogenic enzymes are present in the gonads and that the block is at the GTH receptor level. Exposure of cultured pituitary cells to testosterone (T) for 2 days facilitated cGTH release in response to sGnRH indicating that T may increase GnRH receptors in the pituitary cells. Injection of T microcapsules into 2+ y old black carp and *in vitro* challenge experiments of pituitary cells (see presentation by Gur et al.) support this conclusion.

OR-8

FUNCTIONAL INTERACTIONS BETWEEN NEUROENDOCRINE SYSTEMS REGULATING GTH-II RELEASE

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The neuroendocrine regulation of GTH-II release is a complex and interactive process. Environmental cues are transduced by poorly understood sensory neurons that in turn modulate hypophysiotropic systems controlling pituitary GTH-II synthesis and secretion. The preoptic-hypothalamic gonadotropin-releasing hormone (GnRH) and dopamine producing neurons are respectively the well described and principal stimulatory and inhibitory systems. Considerable progress has been made in identifying other possible stimulatory neuropeptides and neurohormones (15+), although for the most part their sites and mechanisms of action are poorly understood. Recent evidence indicates that amino acid neurotransmitters are important for GTH-II release. In goldfish, γ-aminobutyric acid (GABA) has clear stimulatory effects on GTH-II release. This results from both increased GnRH release and decreased hypothalamic dopaminergic activity, but not by direct action on the gonadotroph cell. Thus, the GABAergic cells may be considered as a modulatory neuronal system. Gonadal steroids produced in response to GTH-II release have important feedback effects on the GnRH, dopamine and GABA systems. Variations in the activity of these neurons are believed to underly the seasonal changes in pituitary and gonadal function in teleosts. A reductionist model is presented for the neuroendocrine regulation of GTH-II release. This model is composed of a stimulatory, inhibitory and modulatory system. The goal of this is to provide a framework for the functional classification of known neurohormones and to provide a basis for the discovery of new neuroendocrine regulators of reproduction.

OR-9

SOME INSIGHTS INTO SEX STEROID FEEDBACK MECHANISMS IN THE TROUT

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The functional significance of steroid feedback mechanisms is to inform the brain on the endocrine status of the animal and thus to synchronize all levels of the brain-pituitary-gonad axis. However, the precise mechanisms and neuronal pathways mediating these effects are largely unknown in fish. In trout, it is known that E2 (17ß-estradiol) levels are high during vitellogenesis and decrease when GTH2 plasma levels start increasing prior to final oocyte maturation and ovulation. Implantation of immature female trout with E2 resulted in increased pituitary GTH2 content without affecting plasma GTH2 levels, suggesting the existence of an inhibitory factor preventing GTH2 release from the pituitary. Recently, we obtained evidence that, similar to the situation in cyprinids, dopamine (DA) inhibits GTH2 secretion in trout, but that this DA inhibition occurs only when E2 levels are elevated. These data strongly suggest interactions between E2 and tyrosine hydroxylase (TH), the rate-limiting synthetic enzyme of catecholamines. To identify the neuronal substrate mediating the feedback actions of E2, the distribution of estrogen receptor (ER)-positive cells was compared to that of GnRH- and TH-positive neurons using a triple immunohistochemical detection on the same section. It was found that GnRH neurons do not express ER, whereas ER receptors colocalize with TH in a subset of preoptic TH neurons known for being dopaminergic and for projecting to the pituitary. Taken together, these data strongly suggest that, in trout, hypophysiotrophic DA neurons of the preoptic area inhibit GTH2 secretion during vitellogenesis and are target cells for E2. The drop in E2 levels would be part of the signals triggering the removal of the dopaminergic inhibition necessary for synchronizing the preovulatory surge of GTH2 with the maturation stage of the oocytes.

OR-10

THREE FORMS OF GONADOTROPIN-RELEASING HORMONE IN GILTHEAD SEA-BREAM AND STRIPED BASS: PHYSIOLOGICAL AND MOLECULAR STUDIES.

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Brains of gilthead seabream (*Sparus aurata*) and striped bass (*Morone saxatilis*) contain three forms of gonadotropin-releasing hormone (GnRH), salmon (s) GnRH, chicken (c) GnRH-II and seabream (sb) GnRH. Exogenous administration of all three forms induces both synthesis and release of gonadotropins. However, sbGnRH is 500-1000 times more abundant than sGnRH in the pituitary of fish undergoing advanced gametogenesis, while cGnRH-II is not present in the pituitary, indicating the relevance of sbGnRH to the control of reproduction. The full length cDNAs encoding the three GnRH precursors were cloned and characterized from cDNA libraries constructed from brains of sexually mature seabream and striped bass. The sGnRH precursor polypeptides show 65-90% homology with sGnRH precursors of other teleosts and the cGnRH-II precursor has 65-95% homology with cGnRH-II precursors of other fish. However, homology within the precursors of the three GnRH forms is restricted to the GnRH decapeptide and the cleavage site, whereas the GnRH-associated peptide region is the most variable. Using the sequence information, we designed specific RNA and DNA probes to localize, via *in situ* hybridization, each of the three GnRH forms in the brain and to monitor physiological changes in their mRNA levels. Based on the nucleotide and amino acid sequence information, and on anatomical considerations, we propose that sbGnRH is derived from the mammalian GnRH found in primitive fish and that sGnRH represents a separate evolutionary line.

OR-11

COMBINED AUTORADIOGRAPHIC AND IMMUNOHISTOCHEMICAL LOCALIZATION OF PITUITARY GnRH RECEPTORS IN MALE AFRICAN CATFISH.

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In the African catfish, *Clarias gariepinus*, two forms of gonadotropin-releasing hormone (GnRH) are present in the brain and the pituitary: catfish GnRH ([His^5,Asn^8]GnRH, cfGnRH) and chicken GnRH-II ([His^5,Trp^7,Tyr^8]GnRH, cGnRH-II). Both GnRHs stimulate the gonadotropin (GTH) release, but cGnRH-II is 100- to 1,000-fold more active than cfGnRH. The difference in GTH release activity matches the GnRHs' relative receptor affinities. However, this difference may be compensated for by an 1,000-fold excess of cfGnRH over cGnRH-II in the pituitary. In cyprinid fish GnRHs also stimulate growth hormone (GH) release. Accordingly, GnRH receptors were found on goldfish gonadotropes and somatotropes. The present study was conducted to investigate the possible involvement of cfGnRH and cGnRH-II on the GH release in the African catfish. GnRH receptors were localized on dispersed pituitary cells in a primary culture. Iodinated salmon GnRH analogue ([D-Arg^6,Trp^7,Leu^8,Pro^9-NEt]GnRH, sGnRHa) was used to radiolabel GnRH receptors and immunohistochemistry was used to identify GTH or GH cells. Following autoradiography, silver grains were exclusively found above GTH cells, indicating the presence of receptors binding sGnRHa on gonadotropes. Radioinert cfGnRH or cGnRH-II reduced dose-dependently the amount of silver grains on gonadotropes. No silver grains were associated with somatotropes. The *in vitro* release of GH after incubations with cfGnRH and cGnRH-II is currently under investigation. The present results suggest that the native forms of GnRH in the African catfish do not have a direct action on GH cells.

OR-12

Detection and measurement of gonadotropin II (GTH II) secretion from individual trout pituitary cells using a reverse hemolytic plaque assay (RHPA).

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A RHPA was developed for detecting and measuring GTH II secretion from individual trout, Salvelinus fontinalis gonadotropes. Fish pituitaries were removed and dispersed with a trypsin/DNAse procedure. A modified protocol published by Smith et al. (1) was used for the RHPA. Dispersed trout pituitary cells were mixed with protein A-conjugated sheep red blood cells and placed on Cunningham chambers constructed on poly-L-lysine-coated microscope slides. The gonadotropes were stimulated with a LHRH analog, des-Gly^{10},[D-Ala^6]-LH-RH in the presence of a salmon GTH II specific antibody (2). Guinea pig complement was used to develop the plaques. The number of GTH II secreting cells (plaque forming cells expressed as a percentage of total cells) as well as the amount of GTH II secreted (plaque size) were examined as a function of the LHRH concentration used. Plaque forming cells were loaded with the calcium indicator fluorescent dye, fura-2AM to study the effects of LHRH on the cytoplasmic concentration of free calcium ions ($[Ca^{2+}]_i$). LHRH was found to induced rapid and transient rises in $[Ca^{2+}]_i$ of individual gonadotropes.

1.Smith PF, Luke EH, and Neil JD. Methods Enzymol 124:443-465 (1986)
2.Swanson P. et al. Biol Reprod 44:29-38 (1991)

OR-13

DOPAMINE ACTION ON CALCIUM CURRENTS IN IDENTIFIED GOLDFISH (*CARASSIUS AURATUS*) GONADOTROPIN CELLS

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In goldfish, gonadotropin (GTH) release is under the stimulatory and inhibitory control of GTH-releasing hormone (GnRH) and dopamine (DA), respectively. Activation of voltage-sensitive Ca^{2+} channels and the subsequent increase in intracellular Ca^{2+} is believed to be an important component in the mediation of GnRH-stimulated GTH release. Consequently, it is likely that DA reduces Ca^{2+} influx through voltage-sensitive Ca^{2+} channels to inhibit GnRH-stimulated GTH release. Calcium currents in identified GTH cells recorded under whole-cell patch-clamp recording conditions were similar to L-type voltage-sensitive Ca^{2+} currents and were sensitive to the dihydropyridine Ca^{2+} channel activator Bay K 8644 (1 μM) and antagonist nifedipine (1-10 μM). The general DA agonist apomorphine (1 and 0.1 μM), reduced Ca^{2+} current amplitude, but did not affect the current-voltage relationship. These results suggest that DA can act on voltage-sensitive Ca^{2+} currents to reduce Ca^{2+} influx. The importance of DA-induced inhibition of voltage-sensitive Ca^{2+} channels was further examined by monitoring hormone release responses from dispersed goldfish pituitary cells in static culture. The Ca^{2+} ionophore A23187 was used to increase intracellular calcium concentrations independent of voltage-sensitive Ca^{2+} channels. Application of A23187 (10 μM) significantly elevated basal GTH release, while apomorphine (1 μM) had no significant effect. In combination, apomorphine was unable to reduce A23187-stimulated GTH release, suggesting that the inhibitory action of apomorphine is not on Ca^{2+}-dependent cellular signaling mechanisms. Taken together, these results suggest that DA inhibition of Ca^{2+} -dependent GTH secretion is mainly on Ca^{2+} influx through voltage-sensitive Ca^{2+} channels rather than on other Ca^{2+}-dependent events downstream of Ca^{2+} channels.
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OR-14

MONOAMINES METABOLISM IN THE HYPOTHALAMUS OF THE JUVENILE TELEOST FISH, *Chaetodipterus faber* (PISCES:Ephippidae)

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Monoamines are involved in different behavioural and physiological process, both in lower and higher vertebrates. Immunocytochemical studies carried out in our laboratory have shown the presence of immunoreactive dopamine (DA) neurons in various hypothalamic nuclei of *Chaetodipterus faber*. In addition many reports indicate that DA, noradrenaline (NA) and serotonin (5HT) are involved in the control of fish gonadotropin secretion. In the present study, we present data on 5HT, DA and NA metabolism in the hypothalamus of juvenile *Ch. faber* under farm culture conditions, related to gonadal development. A specific and sensitive HPLC-ED method was used to measure the levels of 5HT, DA, NA, 5-hydroxyindoleacetic acid (5HIAA), 3,4-dihydroxiphenylacetic acid (DOPAC), homovanilic acid (HVA) and methoxihydroxiphenylglycol (MHPG). Animals were sacrificed at the following ages: 6 months, stage 0 (undifferentiated gonad); 9 months, stage 1 (developing gonad); and 12 months, stage 2 (puberty). In all groups, a high NA and 5HT concentrations were observed. On the contrary, DA concentration was about 10-fold lower than NA concentration. Levels of HVA were under the technique detection limits. A decrease was found in the DOPAC/DA ratio in animals at puberty. However, an increase was observed in the MHPG/NA ratio during the same period. The content of 5HT and 5HIAA showed a decrease at initiation of puberty, however, 5HIAA/5HT ratio did not change significantly throughout gonadal development.

OR-15

A SEROTONERGIC CONTROL OF PITUITARY - GONADAL ACTIVITY IN THE FEMALE CATFISH, *HETEROPNEUSTES FOSSILIS*, UNDER NORMAL AND LONG PHOTOPERIODS

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Exposure of catfish to a 30-day long-photperiod regime (16L : 8D; 18 ± 1 °C) in gonadal preparatory phase increased hypothalamic 5-HT content and MAO activity significantly and abolished their day-night patterns. But the treatment amplified the day- night pattern in 5-HT turnover. The long photoperiod treatment stimulated pituitary-gonadal activity with significant elevations in plasma levels of gonadotropin (GTH), estradiol - 17β (E_2) and testosterone (T), and gonado-somatic index (GSI). Administration of para-chlorophenylalanine (p-CPA, a tryptophan hydroxylase inhibitor, 10 mg/100 g BW; 10 i.p. injections for 30 days) in both normal and long photoperiod groups decreased the 5- HT content and turnover, and MAO activity and obliterated their day-night patterns. Concomitantly, the treatment also decreased the plasme levels of GTH, E_2 and T, and the GSI; the reductions being greater in the long photoperiod group. Administration of 5-hydroxytryptophan (5-HTP, a 5-HT precursor, 5 mg/100 g BW; 10 i.p. injections for 30 days) restored the p-CPA-induced suppression of the 5-HT content and turnover, MAO activity, plasma levels of GTH, E_2 and T, and the GSI. These results strongly suggest that 5-HT stimulates GTH secretion and is a mediator of long photoperiod-induced stimulation of the pituitary-gonadal axis.

OR-16

CRYOPRESERVATION OF AQUATIC GAMETES AND EMBRYOS: RECENT ADVANCES AND APPLICATIONS

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Despite four decades of research using over 50 fish species, yields of viable post-thaw fish spermatozoa are unpredictable, low and variable and cryopreservation of fish eggs and embryos unsuccessful. Our knowledge of the underlying causes of variability of post-thaw spermatozoa is rudimentary and this has minimised the uptake of cryopreservation technology. Recent research on the possible factors, notably collection techniques, prefreezing storage conditions and precooling membrane damage, cooling methods and rates and protocol evaluation are critically reviewed and new approaches for larger scale cryopreservation of fish spermatozoa suitable for commercial application discussed.

Although successful cryopreservation fish eggs and embryos still remain elusive there is now a greater critical mass of information on fish egg preservation. Some recent progress has been made in cryopreserving a number of invertebrate species. Recent studies have reported some measure of cryosuccess of rotifers, mussel, pacific oyster and *Artemia* embryos. Reasons for cryofailure of fish eggs and embryos are unclear. High volumes of osmotically inactive water and low surface:volume ratios are cited as major obstacle. Recent studies on fish embryos suggest that major differences in prefreezing cryoprotectant tolerance, embryonic stages and cold shock are significant contributors to egg death. Current evidence suggest that although permeability of embryonic membranes is low, depending on embryonic stage used, up to 50% of internal water is osmotically active and can be removed.

OR-17

A UNIFORM CRYOPRESERVATION METHOD FOR SEMEN OF SALMONID FISHES AND ITS ADAPTION FOR PRACTICAL APPLICATION

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Basing on computer assisted cell motility analysis, cell viability stainings and measurement of spermatozoan LDH liberation we present a cryopreservation method for semen of salmonid fishes. This is uniform for the species investigated until now *(Oncorhynchus mykiss, Salmo trutta f. fario, Salmo trutta f. lacustris, Salvelinus fontinalis, Coregonus sp.)* and fertilization rates similar to the control with untreated semen are obtained at an average sperm/egg ratio of 2.5 - 5 x 10^6 spermatozoa/egg. The influence on the fertilization rate with respect to various thawing techniques, dilution ratios, equilibration period in the extender and duration of semen storage before deep freezing is described under aspects of practical application. For routine utilization in fishery management a technique allowing the fertilization of egg batches up to 500 ml with deep frozen semen is presented.

OR-18

THE PLASTICITY OF SEX DETERMINING GENOTYPES IN CHANNEL CATFISH

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The model of sex determination in channel catfish is female homogamety (XX). Paradoxical feminization resulting from dietary androgen administration during early development provided the opportunity to evaluate the effects of sex chromosome set manipulation. Sex-reversed (XY) females were identified when matings with normal males resulted in a 3:1 male:female sex ratio. One third of these males produced only male progeny when mated with normal females, which identified the YY sex genotype. Mating YY males with XY females also produced all-male populations; these could be feminized by exogenous hormones. The sex genotype of these offspring was expected to be XY:YY. Females from these populations mated with normal males produced six families of all male offspring, documenting the viability and reproductive competence of females with YY sex genotype. Remaining families had 3:1 sex ratios, identifying female parents with XY sex genotype. Males from the same population mated with normal females produced three families of all-male progeny, which verified the YY sex genotype. Other families with normal sex ratios were sired by XY males. Sex chromosome set manipulation by hormone administration and selective breeding has produced females with XX, XY and YY sex genotypes and males with XY and YY sex genotypes. A male-determining gene located on the presumptive Y chromosome may be turned off by exogenous sex hormones in channel catfish.

OR-19

APPLICATION OF A NON-INVASIVE SEX TEST IN THE AQUACULTURE OF STRIPED BASS

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Striped bass (*Morone saxatilis*) became an important fish for commercial aquaculturists in the last decade. Choosing broodstock is made difficult in part because mature striped bass are not sexually dimorphic. We tested the utility of our non-invasive sex test for mature striped bass in a hatchery. The test is based on detection of vitellogenin in the surface mucus of female striped bass. Vitellogenin is the precursor to egg yolk proteins. During the annual, extended period of oocyte growth, female striped bass produce vitellogenin in the liver in high amounts and transport it to the ovary. For unknown reasons, some yolk protein appears in the surface mucus. Using vitellogenin-specific antiserum in ELISA and Western blotting, we previously showed that the test discriminated with perfect accuracy wild female striped bass from wild male striped bass. In this study on captive fish, we collected surface mucus at three monthly intervals during the winter prior to spawning. All males (12/12) and most females (23/24) were correctly identified. One false negative represents a <5% error rate in discriminating females. The test had an overall success rate of 97%. The non-invasive sex test for striped bass and probably for related species could aid the industry in broodstock selection.
[Supported by the U.S. Dept. of Commerce and Maryland Agricultural Experiment Station.]

OR-20

COMMERCIAL FIELD TRIALS DEMONSTRATE EFFICACY AND ECONOMIC BENEFIT OF A CONTROLLED RELEASE GnRHa IMPLANT.

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It has been well established that sustained administration of GnRH analogues to male and female fish broodstock via controlled release implants is highly effective at inducing final gonadal maturation, ovulation, and spermiation. In this paper, we present results of commercial field trials of GnRHa implants (ReproBoost™, AquaPharm Technologies Corp.) in over 20,000 broodstock including the major cultured salmonid and marine species. We show that in a commercial environment these implants can enable, synchronize, and advance spawning and increase milt production. Examples of the enhancement of salmon broodstock performance include: 3-fold increase in Coho milt production, synchronized spawning of 95% of treated Atlantic females within 12-14 days, and 7 week advancement of Coho spawning season. We have analyzed these results from a microeconomic perspective and demonstrated that single-treatment GnRHa implants can significantly reduce egg production costs, enable efficient egg production planning, and facilitate new species culture and genetic selection. The use of this type of product can have a macroeconomic impact on global aquaculture production. The rapid increase in the production and sophistication of the aquaculture industry creates opportunities for the commercialization of basic discoveries in fish physiology and justifies the consideration of commercial application in the planning of basic research.

OR-21

INDUCED SPAWNING IN TELEOST FISH AFTER ORAL ADMINISTRATION OF GnRH-A

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The methods used to induce spawning generally need repeated fish capture and invasive treatments which are time consuming, stressful and can induced other disorders such as skin damages and associated pathologies. In order to avoid these problems, working on 3 species differing by the organisation of their digestive tract, the rainbow trout, the common carp and the African catfish, we developed a formulation containing an intestinal absorption enhancers whose safety for the intestinal mucosa has been tested, and proteolytic enzyme inhibitors.This formulation increased GnRH-A (D Arg^6 sGnRH) intestinal uptake after oral administration. This results in the stimulation of GtH2 secretion and induced ovulation at doses similar (20 to 40 µg/b.w.) to those used in intraperitoneal treatments. Furthermore, a microencapsulation procedure has been developed. The force feeding delivery of the capsules induced ovulation in the 3 species at rates comparable to those obtained after I.P. The first results indicate that there is no competition of GnRH uptake by nutrients. From this work, it becomes possible to develop a special diet for non invasive induced-spawning treatments in fish.

OR-22

THE ROLE OF DIETARY PROTEIN IN VITELLOGENIN SYNTHESIS AND OOCYTE DEVELOPMENT, AND ITS EFFECTS ON REPRODUCTIVE PERFORMANCE AND EGG QUALITY IN GILTHEAD SEABREAM *SPARUS AURATA* BROODSTOCK

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The potential use of different meals of plant or animal origin in seabream broodstock diet was evaluated. Results showed that fish fed a squid meal based diet produced three times more viable eggs than fish fed on standard grow-out diet or wheat-gluten based diet. Supplementation of the wheat gluten based diet with essential amino acids (EAA) profile similar to that of seabream eggs, was associated with doubling fish fecundity and 15d larval survival as compared with gluten protein diet only.

Dietary EAA controls seabream fecundity and egg quality mainly via the synthesis and selective uptake of yolk constituents. An in vitro binding assay was developed and the presence of specific binding sites for vitellogenin (VG) in oocyte membrane preparations was studied. VG binding capacity of oocyte was significantly affected by dietary EAA composition. The lowest level of plasma VG and lowest amount of VG bound per mg oocyte membrane protein was found in fish fed wheat-gluten based diet. The supplementation of EAA to this diet significantly increased by over 35% the plasma VG level and the amount of VG bound.

OR-23

THE EFFECT OF SEASONAL ALTERATION IN THE LIPID COMPOSITION OF BROODSTOCK DIETS ON EGG QUALITY IN THE EUROPEAN SEA BASS (*Dicentrarchus labrax* L.)

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There is now good evidence that the lipid composition of broodstock diets has a direct affect on the subsequent egg quality. This presentation investigates the assumption that broodstock lipid requirements would vary seasonally, depending on the state of gonad maturation. Fish were fed two pelleted, lipid enriched diets (maize oil, diet 1 or high quality fish oil diet 2), during four different periods in the annual cycle. The first group was fed diet 2 for twelve months and the second only during vitellogensis (Sep-Feb) with diet 1 fed for the remaining six months. Both these showed improved egg quality and higher hatch rates when compared to the remaining two groups fed diet 2 during pre-vitellogensis (Feb-Sep) and spawning (Feb-Mar). Improved egg quality was manifest in higher total n-3 fatty acids including enhanced levels of both docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

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PI-1

ISOLATION AND MORPHOLOGICAL IDENTIFICATION OF GONADOTROPIC CELLS FROM THE MEDITERRANEAN YELLOWTAIL (Seriola dumerilii Risso 1810) PITUITARY

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Sexually immature Mediterranean yellowtails (2-year-old specimens) were obtained from the Instituto Español de Oceanografía in Mazarrón (Murcia, Spain). They were killed by decapitation and the pituitaries removed. Pituitary cells were dispersed using the trypsin/DNAasa treatment procedure described by Chang et al. (1990) and separated by a discontinuous Percoll density-gradient as described De Leeuw et al. (1984) (modified). After centrifugation, the cells were removed from the Percoll interfaces, washed twice and resuspended in M-199 medium culture containing 1% P/S, 10% FBS and 0.35% NaCl. Cell viability was determined by trypan blue dye exclusion. Identification of gonadotropic cells was performed by immunocytochemistry and electron microscopy. Our results demonstrated that the ultrastructure of these cells was well preserved after dispersion and isolation and that they showed ultrastructural features similar to those found in intact tissue.

PI-2

MODIFICATION OF 3'-RACE (RANDOM 3'-RACE): PARTIAL CLONING OF THE ESTROGEN RECEPTOR OF THE AFRICAN CATFISH

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Degenerate primers were used to amplify the cDNA region encoding the DNA-binding region (DBD) of several nuclear receptors of the African catfish (cf) pituitary. DNA sequence analysis of cloned polymerase chain reaction (PCR) products showed that at least 13 of them were derived from cDNA sequences encoding different DBDs, one of them most closely resembling the DBD of estrogen receptors (ERs).
Using 3'-RACE (rapid amplification of cDNA 3'-ends), several attempts to amplify the estimated 5 kb cDNA region between the cfER-DBD and the poly (A) tail failed. Therefore, we have modified the 3'-RACE technique, using oligo dT-adaptor-primed cDNA, to random 3'-RACE, using the so-called FoFEiR-Ada primer (see below) for initiation of cDNA synthesis.
Random 3'-RACE takes advantage of the limited presence of specific nucleotide sequences (theoretically once every 4^n bases) in an RNA template. The FoFEiR-Ada primer consists of (from 5' to 3') a known (adaptor; **Ada**) sequence, **ei**ght random nucleotides (**EiR**; in order to facilitate annealing) and **fo**ur fixed nucleotides (**FoF**). The four fixed nucleotides at the 3'-end of this primer are crucial, since they determine the incidence of initiation of cDNA synthesis, allowing the primer to anneal within 100 to 1,500 nucleotides with a probability of 0.324 to 0.997, respectively.
Next, PCR was performed on the FoFEiR-Ada-primed pituitary cDNA, using a primer based on the cDNA sequence encoding the cfER-DBD, and the adaptor primer. DNA sequence analysis of the PCR products (of 1.2 kb, 1.0 kb, 0.85 kb and 0.7 kb) generated 1.1 kb of new sequence information, located 3' of the region encoding the cfER-DBD, and extending into the region encoding the so-called ligand-binding domain of the cfER.

PI-3

CLONING OF TWO PROLACTIN COMPLEMENTARY DNA FROM GOLDFISH, *Carassius auratus.*

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Prolactin (PRL), growth hormone (GH) and somatolactin (SL) are three major pituitary polypeptide hormones whose genes are considered to have evolved from a common ancestral gene. As a first step towards understanding the differential regulation of these hormones in goldfish, we here report the identification of two complementary DNA (cDNA) sequences encoding for goldfish PRL. Because these goldfish PRL cDNAs have distinct nucleotide sequences in their 5' & 3' untranslated regions, they are probably encoded by two different genes. Unlike the two tilapia PRLs identified to be very different from each others, the two goldfish PRLs are highly homologous and might be derived from recent gene duplication after the emergence of Cypriniformes. Comparisons of these two goldfish PRLs with other published sequences of PRL will be presented.

[Supported by Hong Kong Research Grant Council Earmarked Grants to KLY (HKU391/94M) and KMC (CUHK17/93M)]

PI-4

DEVELOPMENT OF RNASE PROTECTION ASSAYS FOR QUANTIFICATION OF GONADOTROPIN (GTH I AND GTH II) SUBUNIT TRANSCRIPT LEVELS IN COHO SALMON (*ONCORHYNCHUS KISUTCH*).

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In salmonids, several studies have indicated that the levels of gonadotropins (GTH I and GTH II) in the pituitary and plasma vary during gametogenesis. However, the mechanisms involved in the regulation of synthesis and secretion of GTH I and GTH II are poorly understood. To examine the role of reproductive steroids and hypophysiotropins in regulating the synthesis of GTH I and GTH II subunits at the transcriptional level, we have developed RNase protection assays (RPAs) to quantify α and β subunit transcript levels in coho salmon. The cDNAs for coho salmon α-2, GTH I β and GTH II β subunits were cloned and sequenced. Northern blot analysis revealed that a single transcript of 1 Kb for each of these subunits was present in the pituitaries of vitellogenic and post-ovulatory coho salmon. For the RPAs, antisense RNA probes and sense RNA standards were prepared from a region of the cDNAs which spanned the signal peptide and a portion of the mature protein. Specificities of the antisense RNA probes were verified by cross-hybridization with sense RNA standards of other subunits. These RPAs will be used to examine the effects of reproductive steroids and gonadotropin-releasing hormone on steady state mRNA levels.

PI-5

ISOFORMS OF GTHs FROM TUNA (*Thunnus thynnus*) PITUITARIES ISOLATED BY CHROMATOFOCUSING AND ION EXCHANGE CHROMATOGRAPHY.

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The processing of tuna pituitary crude extracts by Concanavaline-A-Sepharose chromatography gave ConA-I (carbohydrate-poor) and ConA-II (carbohydrate-rich) fractions. Ten fractions were eluted from ConA-II at different pH in Chromatofocusing (pH 8 to 3), four of which (5.3-5, 5-4.7, 4.5-4.2 and the peak eluted after ClNa) stimulated *in vivo* and *in vitro* production of steroids in *Fundulus heteroclitus* gonads and were immunoreactives with anti carp GTH serum. The SDS-PAGE of these fractions showed similar anti carp GTH immunoreactive bands of 11, 15-17, 18-20, 30-32 and 35-40 Kd; approximately.Such bands seem to correspond with α subunit, GTHII ß subunit, GTH I ß subunit, GTH II and GTH I, respectively. Ion exchange chromatography from the chromatofocused fractions gave different peaks and SDS-PAGE of them showed proteins with the same molecular weigths as described before. The results obtained suggest that these fractions could represent *isoforms* of the GTHs with the same molecular weigth but with different isoelectric points and net charges. This *microheterogeneity* could be explained by variations in the glycosilated domains and other protein modifications.

PI-6

ISOLATION AND CHARACTERIZATION OF TWO DISTINCT GONADOTROPINS FROM MEDITERRANEAN YELLOWTAIL (Seriola dumerilii, Risso 1810) PITUITARY GLANDS

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Two gonadotropins, GTRH I and GTH II, of Mediterranean yellowtail were chemically characterized. They were extracted from the pituitary glands with 35% ethanol-10% ammonium acetate and separated by ion-exchange chromatography on a DE-52 column. Each GTH was purified by reversed-phase HPLC on Asahipack C4P-50 and gel filtration on Superdex 75.

Molecular weights were estimated to be 47 kDa for GTH I and 29 kDa for GTH II by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, whereas they were 49 kDa (GTH I) and 42 kDa (GTH II) by gel filtration. GTH II were completely dissociated, while GTH I was partially dissociated by treatment with 0.1% trifluoroacetic acid. The α subunit showed 56-84% identity with the α subunits of other vertebrates. The Iß and IIß showed the highest sequence identity with those of bonito and tuna gonadotropins.

PI-7

MOLECULAR CLONING OF THE cDNAs ENCODING THE Prl, GH, SL and POMC PRECURSORS OF THE AFRICAN CATFISH, *Clarias gariepinus*

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The pituitary of the African catfish consists of six major cell types: prolactin (Prl) cells, growth hormone (GH) cells, pro-opiomelanocortin (POMC) cells, gonadotropic hormone (GTH) cells, thyroid-stimulating hormone (TSH) cells and somatolactin (SL) cells. Since these cell types are hormone-producing cells, we argued that the mRNAs encoding these hormones are abundantly expressed. Therefore, cDNA copies of these transcripts should also be adundantly present in a cDNA library of this tissue. We tested if isolation of these cDNAs could be achieved by grouping via cross-hybridization.
To this end, we constructed an oligo dT-primed cDNA library of the African catfish pituitary in λZAP II. After mass *in vivo* excision, five hundred clones were selected *ad random* for further analysis. The cDNA insert of one of the 500 clones was used to select cDNAs in the remaining 499 clones, containing identical or related sequences. Next, the cDNA insert of the next cDNA clone was used for identical purposes, etc. etc. In this way, four groups of cDNAs were identified.
DNA sequence analysis of the longest cDNA insert of the four groups showed that we isolated the cDNAs encoding the precursors of Prl, GH, SL and POMC. Northern blot analysis and *in situ* hybridization experiments were performed in order to determine the length and the site of expression of each transcript, respectively.

PI-8

LONG-TERM TESTOSTERONE TREATMENT STIMULATES GTH II SYNTHESIS AND RELEASE IN THE PITUITARY OF THE BLACK CARP, *MYLOPHARYNGODON PICEUS*.

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As part of a project designed to advance puberty in the black carp, microcapsules containing testosterone (T) were injected into immature 2 y old fish (1.8-3.2 kg bw) at 0.8, 1.6 or 3.2 mg/kg. Circulating T levels gradually increased, and reached peaks corresponding to the dose on day 14, after which they declined but remained above those of the vehicle-injected fish even on day 28. cGTH levels did not differ from the controls throughout the experiment (about 12 ng/ml). Nevertheless, cGTH content and cGTH-IIß mRNA levels were higher in T-treated fish (according to T dose) than in the controls. *In vitro* experiments using primary culture of dispersed pituitary cells have shown that GTH release in response to sGnRH or cGnRH-II (0.01-1 μM) occurs only in cells from T-injected fish; cGnRH-II appeared to be the more potent form. Apparently, T given *in vivo* stimulates the synthesis of GTH by elevating steady-state levels of GTH IIß mRNA. T also affects GTH release in response to GnRH, probably by amplifying GnRH receptors on the gonadotrophs. The fact that GTH plasma levels did not increase in T-injected fish, despite higher GTH content in the pituitary cells, suggests a strong dopaminergic inhibition of its release on the intact pituitary which is absent in the culture of dispersed cells.

PI-9

CLONING OF TWO GROWTH HORMONE COMPLEMENTARY DNA FROM GOLDFISH, *CARASSIUS AURATUS*.

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As a first step towards the development of a sensitive ribonuclease protection assay for studying the molecular mechanism of growth hormone (GH) gene expression in pituitary cells of goldfish *Carassius auratus*, we report the isolation of two complementary DNA (cDNA) clones encoding goldfish GH from a cDNA library prepared from pituitary poly(A)$^+$RNA. The complete nucleotide sequences of these two GH cDNA clones have been determined and predicted that both of them encode a polypeptide of 210 amino acids (aa) including a putative signal peptide of 22 aa. One of the GH cDNAs encodes for a polypeptide (GH1) with five cysteine residues (similar to other carp GH) whereas another encodes a polypeptide (GH2) with four cysteine residues (similar to most teleostean GH). Because these two GH cDNAs have distinct nucleotide sequences at their coding and 3' untranslated regions, they are likely to be encoded by two different genes. Comparisons of these two goldfish GH cDNAs with other published sequences of GH will be presented.
[Supported by Croucher Foundation Grant to KLY and Hong Kong Research Grant Council Earmarked Grants to KLY and KMC]

PI-10

FUNDULUS HETEROCLITUS GONADOTROPIN. 4. CLONING AND SEQUENCING OF GONADOTROPIC HORMONES (GTH)

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To amplify GTH cDNA from a pituitary library of *Fundulus heteroclitus*, a series of synthetic oligonucleotides based on conserved regions of teleost and mammalian α-subunit glycoproteins were used in polymerase chain reactions (PCR). The PCR products of the expected size were then subcloned and sequenced. The GTH α-subunit was constructed from eighteen clones consisting of fragments of the 5' end, 3' end and internal portions of the gene. The sequence obtained from multiple independent clones appears to be homologous to those of other teleosts. A signal sequence of 30 amino acids was predicted by PSIGNAL (PC/GENE). This predicted signal sequence was in exact alignment with other teleost amino acid sequences. Subtracting the signal peptide, the mature protein of the GTH α-subunit was found to be 95 amino acids. An alignment comparison of the *F. heteroclitus* amino acid sequence with the *K. pellamis* and *T. obesus* amino acid sequence indicates 72% identity with exceptional conservation at the cysteine positions (10:10).
Accession Number: FHGTH-A:U12923

PI-11

DIRECT POSITIVE EFFECTS OF TESTOSTERONE ON GnRH-STIMULATED GONADOTROPIN RELEASE FROM DISPERSED GOLDFISH PITUITARY CELLS

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In goldfish, positive feedback actions of testosterone (T) on gonadotropin (GTH) release were previously demonstrated in perifusion studies of pituitary fragments obtained from T-implanted fish. In this study, possible direct actions of T treatment on native GnRH (salmon, sGnRH and chicken, cGnRH-II) -stimulated GTH release were investigated using dispersed goldfish pituitary cells. In preliminary experiments using dispersed pituitary cells from both sexually regressed male and female goldfish, overnight treatment with 100nM T increased the GTH response to subsequent challenges of 100nM sGnRH or 100nM cGnRH-II, by approx. 2-fold, either in the presence or absence of T. However, acute applications of T at the time of sGnRH or cGnRH-II pulse applications were without effects. In dose-dependence studies, overnight pretreatments with 10, 100 and 1000 nM T were equally effective in potentiating subsequent GTH responses to 100nM sGnRH and cGnRH-II. Similarly in studies using pituitary cells prepared from female goldfish that were either sexually regressed or undergoing gonadal recrudescence, continuous exposure to 1nM and 10nM T for 24 h prior to and during perifusion potentiated the GTH response to 100nM sGnRH. These data strongly suggest the presence of direct positive actions of T on GTH cells. Since overnight treatment with T did not significantly alter cellular GTH contents or basal GTH secretion, this direct positive effect of T may be exerted on the sensitivity of GTH cells to GnRH stimulation at the receptor or post-receptor level. (Supported by Univ. of Utrecht, NSERC Canada, and a NATO Collaborative Research Grant).

PI-12

CLONING OF THE GOLDFISH GROWTH HORMONE cDNA

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The goldfish growth hormone (gGH) cDNA was cloned using the polymerase chain reaction (PCR). Two primers, each 30 nucleotide long, were designed based on the conserved sequences at the 3' and 5' ends of the carp growth hormone cDNA and were used to amplify the gGH cDNA from a goldfish pituitary cDNA library. The PCR amplification resulted in synthesis of a DNA fragment of 567 nucleotide displaying more than 94% homology with common carp GH cDNA. The deduced 188 amino acid sequence of gGH was found to have greater than 93% homology with those of other cyprinid species including silver carp, grass carp, common carp and bighead carp. In addition, gGH was found to have 64% homology with salmon GH, and 32% homology with human GH (hGH). The gGH appears to be unique among cyprinid species studied, since it contain four cysteine (Cys) residues rather than five reported in other cyprinid species. However, GH molecules in non cyprinid species including salmon and tetrapods also contain four Cys residues. Thus, the present findings and the results of previous mutation analysis in common carp, supports the hypothesis that the extra Cys 123 may not contribute to the folding of the GH molecule in other cyprinid species containing five Cys residues.

(Funded by a Natural Sciences and Engineering Research Council of Canada Strategic grant).

PI-13

PURIFICATION OF STRIPED BASS (*MORONE SAXATILIS*) GONADOTROPIN II AND DEVELOPMENT OF AN ENZYME IMMUNOASSAY FOR ITS MEASUREMENT.

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A highly purified striped bass gonadotropin-II (stbGtH-II) and its α and β subunits (stbGtH-IIα and β) were prepared from pituitaries of sexually mature fish by gel filtration, ion exchange and high pressure liquid chromatography. The stbGtH-II was identified by its *in vitro* estradiol-17β stimulatory activity as well as by SDS-PAGE and N-terminal amino acid sequencing. Molecular weights of stbGtH-IIα and β were 18KDa and 22 KDa, respectively. A competitive enzyme-linked immunosorbent assay (ELISA) was developed for stbGtH-II, using antibodies against the stbGtH-IIβ and the intact stbGtH-II for the standard curve. The sensitivity of the assay was 156 ng/ml (15.6 pg/well) and the intra- and inter-assay variability (coefficient of variation at 50% binding) were 7.71% and 8.72%, respectively. Using this ELISA, changes in blood and pituitary GtH-II levels were monitored in striped bass at different reproductive stages and a surge of GtH-II secretion was monitored in mature females treated with an analog of gonadotropin-releasing hormone (GnRHa). Displacement curves obtained with serial dilutions of pituitaries and plasma of species closely related to striped bass, such as white bass (*Morone chrysops*), white perch (*Morone americana*) and seabass (*Dicentrarchus labrax*), were parallel to the standard curve. This indicates that the stbGtH-II ELISA can be used for measuring GtH-II levels in fish belonging to the family Moronidae. The availability of the stbGtH-II assay will enable the intensification of studies on reproductive physiology and spawning manipulations of striped bass and its relatives, fish which are important to the aquaculture industry.

PI-14

PREOVULATORY CHANGES IN GONADOTROPIN GENE EXPRESSION AND SECRETION IN THE GILTHEAD SEABREAM, *SPARUS AURATA*.

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The female *Sparus aurata* has a non synchronous ovarian development. Oocyte maturation, ovulation and spawning occur at 24 hour intervals over a period of three months. Therefore, seabream is an excellent model to study preovulatory hormonal profiles. Changes in oocyte morphology, gonadotropin gene expression and secretion were monitored at 4 hour intervals during the 24 hours preceding spawning. At 20 to 12 hours before spawning, oocytes did not show signs of final maturation. During that period plasma GtH-II and pituitary ßGtH-II mRNA levels were low. The process of final oocyte maturation (germinal vesicle migration, coalescence of yolk globules and germinal vesicle breakdown) was initiated eight hours before spawning. Ovulation occurs within the 4 hours preceding spawning. GtH-II gene expression and secretion increased with the initiation of final oocyte maturation and remained elevated until ovulation and spawning. Throughout the preovulatory daily cycle, GtH-II synthesis closely paralleled GtH-II secretion. Removal of males from a spawning group resulted in the arrest of spawning. Within a week, a high percentage of atretic oocytes were observed in the isolated females and no signs of oocyte maturation were found. levels of GtH-II in the plasma and of ßGtH-II mRNA in the pituitary of these females dropped dramatically.

PI-15

INTRACELLULAR MEDIATION OF THE GnRH EFFECT ON TRANSCRIPTION OF THE TILAPIA GtH IIß GENE

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GnRH increased GtH IIß mRNA levels both *in vivo* (sGnRHa 50 μg/kg, 24 hrs after injection) and *in vitro* (sGnRH 10 nM, after 12-36 hrs exposure). Components of the GnRH signal transduction cascade mediating GtH release were examined for possible effects on GtH mRNA and protein synthesis, using a primary culture of pituitary cells. RNA was extracted and hybridized on Northern and slot blots with tilapia GtH IIß cDNA, and mRNA levels were standardized with those of ß actin. The phorbol ester TPA (12.5 nM) increased GtH IIß mRNA 3-fold after 10-36 hrs exposure and the protein levels (GtH cell content + release) were elevated as from 24 hrs. Activation of adenylate cyclase by forskolin (10 μM), or inhibition of phosphodiesterase by IBMX (0.2 mM) both increased the GtH IIß mRNA levels 2-3 fold (after 24 hrs), the latter being more effective. Ionomycin (1 μM) increased the GtH IIß transcripts after 4-8 hrs, an effect which was shared by the same dose of the ionophore A23187, whereas a higher dose (10 μM) of A23187 had a marked detrimental effect on all mRNAs measured (GtH IIß, GH and ß actin). Exposure of cells to actinomycin D (8 μM) for 1.5-10 hrs reduced GtH IIß mRNA levels to nearly a quarter of initial levels; this was abated by preincubation of the cells with forskolin (10 μM for 24 hrs). Such an effect was not seen after preincubation with 12.5 nM TPA. These results suggest that both the PKA and PKC pathways are involved in mediation of the GnRH effect on GtH IIß synthesis in tilapia, cAMP possibly having a stabilizing effect on the mRNA.

PI-16

LOCALIZATION OF PITUITARY CELLS BY IMMUNOCYTOCHEMISTRY IN THE "ARGENTINE SILVER SIDE" *ODONTHESTES BONARIENSIS*.

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Odonthestes Bonariensis, (Atheriniformes) is a fish with economic importance in Argentina. We are interested in studing the reproductive biology of this species and in this context we analized the pituitary cell types using immunocitochemistry. Prolactin cells were revealed with two different antisera: anti-chum salmon and anti-carp PRL. ACTH producing cells were stained with anti-human ACTH. Both cell types were found mainly in the rostral pars distalis (RPD). Somatotrops were immunostained with anti-chum salmon and anti-carp GH in the proximal pars distalis (PPD). GTH cells stained with anti-croaker GTH and anti-coho GTH I and II were mainly localized in the central area of the PDP with a diffuse distribution in the other areas. Thyrotropic cells were identified with anti-human βTSH and scattered in small groups. Talking about the pars intermedia (PI), MSH and somatolactin producing cells were detected in this area. These cells were revealed with anti-human ACTH and anti-carp SL respectively. The distribution pattern did not differ to that reported in several other species. This work will provide a basis for future research on the hypothalamus-pituitary-gonadal axis.

PI-17

CHANGES IN SERUM AND PITUITARY LEVELS OF PROLACTIN AND GROWTH HORMONE WITH REPRODUCTION AND FASTING IN THE TILAPIA, OREOCHROMIS MOSSAMBICUS

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Female tilapia, (Oreochromis mossambicus), brood eggs and larvae in their buccal cavity for up to 3 weeks following spawning. Reduced feeding during this period may affect hormone levels. The objective of the present study was to characterize and compare changes in circulating and pituitary levels of 2 distinct forms of PRL ($tPRL_{177}$ and $tPRL_{188}$) and GH during brooding and 21 days of fasting in the tilapia. Serum and pituitary levels of $tPRL_{177}$ were elevated in brooding tilapia compared with tilapia late in vitellogenesis. Serum and pituitary levels of $tPRL_{177}$ were also elevated in vitellogenic female tilapia within 10 days of fasting and remained elevated at 21 days of fasting. Serum and pituitary levels of $tPRL_{188}$ did not appear to change significantly with brooding or fasting. Serum and pituitary levels of increased in brooding tilapia with the rise in serum levels occurring prior to the rise in pituitary GH levels. In contrast, pituitary levels of GH were elevated in female tilapia fasted for 21 days but serum levels were not altered. These data suggest that the changes in circulating and pituitary GH levels observed during the reproductive cycle of the female tilapia derive from changes in reproductive status while changes in $tPRL_{177}$ levels may be due to an important extent, on changes in nutritional status. Supported by NSF Grant DCB 91-04494 and NOAA/Sea Grant No. NA36RG0507/ R/AQ-37 to E. G. Grau.

PI-18

MOLECULAR CLONING OF cDNAs ENCODING TWO GONADOTROPIN β SUBUNITS (GTH Iβ AND IIβ) FROM GOLDFISH

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Two types of cDNAs (GTH Iβ and IIβ) encoding the β subunit of gonadotropin were cloned using the polymerase chain reaction for a cDNA library prepared from goldfish pituitary mRNA. The nucleotide sequence of GTH Iβ cDNA was 616 bp long, encoding 135 amino acids, and GTH IIβ cDNA was 546 bp long, encoding 140 amino acids. When compared to the amino acid sequence of known teleost GTH β subunits, goldfish GTH IIβ showed high homology (99%) to carp GTH IIβ with only one deffering amino acid. Goldfish GTH Iβ showed homology ranging from 40-49% to known teleost GTH Iβ, with well-conserved regions specific to teleost GTH Iβ. These results showed that there are at least two distinct forms of GTH in the goldfish pituitary. We also analyzed the levels of GTH Iβ and IIβ mRNA in reference to ovarian maturity (immature, maturing, and mature) by Northern blotting. Both GTH Iβ and IIβ mRNA could be detected at all stages. The level of GTH IIβ mRNA showed a tendency to increase with the progression of maturity.

PI-19

PLASMA SOMATOLACTIN CONCENTRATIONS IN ATLANTIC CROAKER DURING GONADAL RECRUDESCENCE

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The observation that plasma somatolactin (SL) levels are elevated in salmonids during gonadal recrudescence suggests that SL may be involved in teleost reproduction. A radioimmunoassay developed for measurement of somatolactin (SL) in red drum (*Sciaenops ocellatus*) was validated for measurement of SL in plasma of Atlantic croaker (*Micropogonias undulatus*) which belongs to the same family, Sciaenidae. Blood was collected from wild caught Atlantic croaker every two weeks during the period of gonadal recrudescence (August to November). There was an overall decline in plasma SL concentrations during gonadal development in both male and female croaker. Plasma SL concentrations were highest prior to gonadal recrudescence (females: SL=7.53±1.60 ng/ml, n=21; males: SL=6.7±1.90 ng/ml, n=11) and were significantly lower in fully recrudesced fish (females: SL=2.92±0.48 ng/ml, n=17; males: SL=3.22±0.84 ng/ml, n=6). The present results do not support the hypothesis that SL is involved in reproduction in sciaenid fishes.

PI-20

CYTOSOL SEX STEROIDS BINDING IN BRAIN AND ITS LEVELS IN BLOOD OF FEMALE STURGEON (ACIPENSER GUELDENSTAEDTI BR.) DURING ANADROMOUS MIGRATION

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In female sturgeon migrating into Volga one year before spawning (vitellogenesis) specific cytosol binding (SB) of androgens (A) and estrogens (E) in the forebrain, hypothalamus end pituitary were determined using ^{3}H testosterone and ^{3}H estrodiol. The results are presented in fmol/mg protein. SBE in hypothalamus were 122,4±10,6, SBA-77,3±8,3; in the forebrain SBE achived 136,9±29,0, SBA were 76,3±9,6;in the pituitary SBE were 50±2,9, SBA-28,0±2,2. Testosterone (T) concentration in the blood serum of these fishes were 14,8±1,3 ng/ml, estradiol 17B(E_2)- 1,3±0,2 ng/ml. Positive correlation for E_2 in blood and SBE in the forebrain (Rs+0,72), for SBE in the forebrain and hypothalamus (+73) were found. Positive correlation (+0,73) for SBA in the forebrain and hypothalamus were determined. Negative correlation for T in blood and SBA in the forebrain(-0,86)as well as SBA in hypothalamus (-0,77) were elucidated. Differences of SBA,SBE and blood sex steroid concentrations at anadromous migration and vitellogenesis are discussed.

PI-21

SEROTONIN METABOLISM IN THE BRAIN OF THE JUVENILE MARINE TELEOST, *Chaetodipterus faber* (PISCES:Ephippidae).

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In teleost fishes, immunohistochemical techniques have revealed widespread monoaminergic innervation of different areas of the nervous central system. In the present study, we have investigated the changes of 5HT and 5-hydroxyindoleacetic acid (5HIAA) in the brain of a marine teleost, *Chaetodipterus faber* and its was related to gonadal development. An specific and sensitive HPLC-ED method was used to measure the levels of 5HT and 5HIAA. Animals were sacrificed at the following ages: 6 months, stage 0 (undifferentiated gonad); 9 months, stage 1 (developing gonad); and 12 months, stage 2 (puberty). 5HT was not detected in the olfactory bulbs and the cerebellum, however, 5HIAA was present in low concentrations, suggesting a low metabolic activity in these regions. The higher 5HT concentrations were observed in the hypothalamus (455, 557 and 104 pg/mg tissue) in the stages 0, 1 and 2 respectively. In contrast, the optic tectum showed the lower concentrations (93,133 and 28 pg/mg tissue) in the same period. In the hypothalamus, telencephalon and optic tectum the content of 5HT and 5HIAA showed a decrease at puberty, however, 5HIAA/5HT ratio did not change significantly throughout gonadal development.

PI-22

MOLECULAR CHARACTERIZATION OF THE SEABREAM GONADOTROPIN-RELEASING HORMONE GENE ISOLATED FROM STRIPED BASS (*MORONE SAXATILIS*)

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Gonadotropin-releasing hormone (GnRH) plays a fundamental role in the control of reproduction for all vertebrates. Nine distinct forms of GnRH have been identified to date, seabream (sb) GnRH being the most recent one. Of the three forms of GnRH present in the brain, sbGnRH was demonstrated to be the most abundant form in the pituitary of sexually mature gilthead seabream (*Sparus aurata*) and striped bass (*Morone saxatilis*), indicative of its role as an endogenous gonadotropin releaser. In order to further understand the underlying mechanisms which regulate the synthesis of the reproductively relevant form of GnRH, the sbGnRH gene has been cloned from a striped bass genomic library. This is the first non-salmonid GnRH gene to be cloned from a teleost species. Overall, the sbGnRH gene is more similar to the mammalian GnRH and chicken GnRH-I than to other GnRH forms. The 5' upstream region corresponding to the promoter has been characterized and analyzed for unique regulatory elements. Using the promoter sequence, constructs can be designed to produce transgenic fish with desired phenotypes such as sterility, precocious puberty or controlled spawning.

PI-23

BRAIN SEX STEROID BINDING IN IMMATURE, PRE- AND POST-SPAWNING TROUT, SALMO TRUTTA L.

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Testosterone and estradiol cytosol specific binding (T-SB and E_2-SB) was determined (fmol/mg protein) by radioligand method in different areas of trout brain: bulbus olfactorius+corpora striata, hypothalamus, tectum opticum, cerebellum. Wild immature and mature fish were used for this study. High levels of T-SB and E_2-SB were characteristic for telencephalon. The values of brain sex steroid binding increased during the period of sexual maturation and decreased from the pre- to post-spawning period of trout annual reproductive cycle. The differences between males and females were revealed also. The data obtained are discussed in connection with the problem of feedback mechanisms involved in control of maturation.

PI-24

THE EFFECTS OF SUPERACTIVE Gn-RH ANALOG ON MATURATION AND SERUM SEX STEROIDS LEVELS IN WOLFFISH (ANARCHICHAS LUPUS L.)

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In the present study the levels of estradiol (E), testosteron (T) and progesteron (P) in wolffish blood serum were investigated during final stages of gonads maturation after GnRH-A treatment. Single or double GnRH-A injections (14-43 mg/kg b.w.) caused final oocyte maturation and ovulation in prespawning period of wolffish (GSI=12-18%). The lenght of this period (7-13 days) and the value of effective doses of GnRH-A were dependent on initial stages of oocyte maturity. The treatment of GnRH-A combined with dopamine receptor antagonists did not suggested significant role of gonadotropin-release inhibitory factor in control of wolffish reproduction. We observed the differences in blood serum levels of sex steroids (RIA) in males and females during maturation after GnRH-A treatment. In females E and T serum concentrations decreased from $0,61\pm0,15$ to $0,18\pm0,03$ ng/ml ($P<0,05$) and from $3,61\pm1,05$ to $1,17\pm0,10$ ng/ml ($P<0,05$), respectively. In males E level decreased from $0,30\pm 0,03$ to $0,12\pm0,02$ ng/ml ($P<0,001$), but T level increased from $1,33\pm0,18$ to $2,31\pm0,30$ ng/ml ($P<0,05$). No changes of P serum levels were observed in males as well in females during maturation after GnRH-A injections.

PI-25

SEX STEROID BINDING IN BRAIN CYTOSOL OF STELLATE STURGEON

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The characteristics of ^{3}H-testosterone (^{3}H-T) and ^{3}H-estradiol (^{3}H-E) binding in brain cytosol of mature males (M) and females (F) were studied. Competitive analysis has shown high specificity of radio ligand interaction with macromolecular component of cytosol. Only estradiol effectively displaced ^{3}H-E from binding sites. Testosterone had relative competitive ability (RCA) at the 5% level and the values of RCA for other steroids (progesterone, dexamethasone) were negligible (<0.1%). Approximately the same results have been obtained in the ^{3}H-T specificity binding study. Specific binding dates of saturation analysis treated by Scatchard plot revealed the single type of binding site to each sex steroid. The parameters of ^{3}H-T binding were similar for both M and F - Kd = 7.6 nM, Bmax = 240 fmol/mg protein (f/m) and Kd = 8.1 nM, Bmax = 250 f/m correspondingly. The specific binding of ^{3}H-E in M brain cytosol had higher affinity and lower capacity - Kd = 3.6 nM, Bmax = 190 f/m than in F - Kd = 5.5 nM, Bmax = 280 f/m. It is concluded that such hormone binding characteristics as high specificity, high affinity and limited capacity binding sites in brain cytosol of Stellate sturgeon fulfil the criteria required of a putative sex steroid receptors.

PI-26

NEUROENDOCRINE CONTROL OF GONADOTROPIN II RELEASE IN THE ATLANTIC CROAKER: INVOLVEMENT OF γ-AMINO BUTYRIC ACID

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It has previously been shown that serotonin stimulates gonadotropin II (GtH II) release in the Atlantic croaker (*Micropogonias undulatus*) whereas an inhibitory dopaminergic control mechanism demonstrated in several teleosts is absent in this species. In the present study, the possible involvement of γ-amino butyric acid (GABA) in the control of GtH II release was investigated. Intraperitoneal administration of GABA (100 μg/g body wt.) elicited a significant elevation in GtH II levels in regressed croaker similar to the observations in goldfish. However, the same dose of GABA slightly inhibited GtH II levels in croaker with fully developed gonads. Comparable results were obtained with Muscimol (1 μg/g), a $GABA_A$ agonist, whereas Baclofen (1 μg/g), a $GABA_B$ agonist failed to induce any significant alterations in plasma GtH II levels in either group. Pretreatment of croaker with Bicuculline (1 μg/g), a $GABA_A$ antagonist blocked the stimulatory effect of GABA on GtH II release in regressed fish. Neither GABA nor the GABAergic drugs could influence LHRH analog-induced GtH II release in the mature or regressed individuals. The results indicate that effects of GABA on GtH II release are dependent on the reproductive stage of the fish and are mediated by $GABA_A$ receptors. In addition, the inhibitory effect of GABA on GtH II release in Atlantic croaker with fully recrudesced gonads differs from the goldfish model in that GABA has no effect on GtH II release in gonadally mature goldfish.

PI-27

CARACTERIZATION OF MOLECULAR VARIANTS OF GnRH IN THE BRAIN OF PROTOGYNOUS "SWAMP EEL", *SYNBRANCHUS MARMORATUS*.

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Synbranchus marmoratus (Synbranchiformes), a protogynous hermaphrodite fish, lives in swamp areas from Argentina to Mexico. It developes initially as a female and then changes sex to became a functional male. The physiological basis of sex reversal in *S. marmoratus* has not been shown yet but in a related species *Monopterus albus*, the "ricefield eel" (Tao et al., 1994) it has been shown that GnRH may play a role in the induction of sex reversal. The aim of the present study was to identify molecular variants of GnRH in the brain of *S. marmoratus*, using HPLC and RIA. A first study was done with three different antisera cII678, PBL#45 and PBL#49. With these three systems we could detect two main immunoreactive peaks coeluting with synthetic cIIGnRH and sGnRH. The immunoreative peak in fractions coeluting with cIIGnRH was analyzed by serial dilutions with an homologous cIIGnRH RIA system and paralelism with respect to synthetic cIIGnRH was obtained. The same kind of analysis was done with the fractions coeluting with sGnRH using two different homologous RIA systems with s1668 and Aida sGnRH antisera. Paralelism with respect to synthetic sGnRH was obtained in both cases. In summary, HPLC analysis combined with RIA of brain extract of *S. marmoratus* has two different variants of GnRH: cIIGnRH and sGnRH.

PI-28

STRUCTURE-ACTIVITY RELATIONS OF GONADOTROPIN-RELEASING HORMONE IN GOLDFISH: ORIGINS OF SUPERACTIVE ANALOGS

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The relative ability of mammalian, salmon and chicken-II gonadotropin-releasing hormone (GnRH, sGnRH, cGnRH-II, respectively) analogs, containing D-amino acid substitutions at position 6, to release gonadotropin-II (GtH-II) from goldfish pituitary fragments in a perifusion system was quantitated. [D-Arg^6]-GnRH was more potent than sGnRH, itself significantly more potent than [Arg^6]-GnRH. [D-Ala^6]- and [D-Leu^6]-GnRH were as potent as sGnRH, whereas the corresponding L-amino acid containing analogs, [Ala^6]- and [Leu^6]-GnRH, were significantly less potent. [D-Arg^6]- and [D-Trp^6]-cGnRH-II had high potency relative to sGnRH. Although [D-Trp^6, Trp^7, Tyr^8]-GnRH had decreased potency, [D-Arg^6, Trp^7, Tyr^8]-GnRH and [D-Lys^6, Trp^7, Tyr^8]-GnRH had high potency relative to sGnRH. Although [His^5]-GnRH had a lower potency, [His^5, D-Trp^6]-GnRH had a higher potency than sGnRH. These and other findings indicate that substitution of selected D-amino acids in position-6 is essential for development of superactive analogs of GnRH in goldfish. Generally, [D-Arg^6]- or [D-Lys^6]-GnRH in combination with constituent hydrophobic amino acids of salmon GnRH and chicken GnRH-II provides analogs of highest potency in the goldfish.

PI-29

The Effect of Pinealectomy and Enucleation on Circulating Melatonin Levels in Atlantic Salmon Parr

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The pineal in salmonids is thought to use photoperiodic information to synchronise both daily and seasonal endogenous rhythms. However, it is not yet clear whether this is acheived through neural or hormonal pathways. The production of melatonin by the pineal has a distinct diel rhythm with elevated levels during the hours of darkness. As smoltification and maturation in Atlantic salmon is known to be under the influence of photoperiod a simple and effective method of pineal removal was developed for future long-term experiments. This technique allowed fish from 8g to several kg to be pinealectomised with mortality rates of less than 7%.

In one experiment, removal of the pineal from Atlantic salmon parr significantly reduced the nocturnal levels of circulating melatonin from a mean of 587 $\pm$ 29.6 pg/ml and 608 $\pm$ 19.3 pg/ml in control and sham-pinealectomised groups to 96 $\pm$ 20.7 pg/ml in pinealectomised fish. However, photophase levels for the three groups did not differ significantly having an overall mean of 66 $\pm$ 10.5 pg/ml. The fish were maintained for up to 18 months after the operation with no adverse effects. The results of bilateral enucleation experiments designed to establish the source of the remaining melatonin secretion will also be described. This work was supported by SERC and NERC awards to Mark Porter and Niall Bromage.

PI-30

GnRH-INDUCED CALCIUM FLUXES IN PRIMARY CULTURES OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) GONADOTROPES.

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Two forms of GnRH have been identified in African catfish (*Clarias gariepinus*) brain, chicken GnRH II (ChGnRH-II) and catfish GnRH (cfGnRH). Both GnRHs have been characterised regarding their places of synthesis in the brain, axonal projections, GTH II release activity and GnRH receptor binding. Both GnRHs induced a similar maximal GTH II release which was, however, attained at chGnRH-II doses 500- to 1000-fold lower than those of cfGnRH. Accordingly, 1000-fold higher concentrations of cfGnRH than of chGnRH-II were needed to displace salmon GnRH analogue from pituitary membrane preparations. To investigate if the potency difference is related to a different use of second messenger systems, we studied the GnRH-induced Ca^{2+} fluxes in enriched gonadotrope cell populations using a video-imaging system. Both chGnRH-II and cfGnRH gave rise to transient elevations of intracellular Ca^{2+} concentration. The amplitude of the Ca^{2+} flux depended on the dose of GnRH. Again a 1000-fold potency difference between chGnRH-II and cfGnRH was found. The minimum effective GnRH concentration regarding secretion of GTH II above basal levels and regarding increases in intracellular Ca^{2+} concentration were similar. We conclude that both GnRHs activate Ca^{2+} fluxes in catfish gonadotropes in the same fashion. Future experiments on GnRH-modulated IP_x and cAMP levels in gonadotropes will provide further information on intracellular signalling pathways which might help to understand differences in the GnRHs' GTH II release potency. As for now, the weak affinity of cfGnRH to pituitary receptors appears to be an important parameter.

PI-31

OBSERVATIONS OF THE BRAIN-PITUITARY AXIS IN IMMATURE BLACK CARP

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Mylopharyngodon piceus, the black carp (BC), is the subject of aquaculture studies in Israel. A prime goal of these studies is to induce precocious puberty in this late (5-6 yrs) maturing species. Our histological studies have determined that young (4-6 mos), immature fish have a well-developed olfactory system and a pituitary gland oriented in a dorsal ventral axis. Immunocytochemical studies utilized antisera to variant forms of GnRH [salmon (s), chicken II (cII), mammalian (m), and lamprey (l)], GTH I and II, and a variety of neuropeptides. Immunoreactive (ir)-sGnRH was localized in the olfactory lobe, in the short stalk between the lobe and the olfactory epithelium, in the nucleus preopticus and nucleus lateralis tuberis and in cells of the caudal pars distalis. Ir-mGnRH was seen in similar areas as sGnRH but there was no colocalization. cIIGnRH was seen in the olfactory lobe and lGnRH was limited to cells in the pars intermedia. Anti-salmon GTH I was localized in cells in the CPD: GTH II was not seen. IR-FMRF-amide, -galanin, and -neurotensin were also localized in specific regions of the brain and pituitary; ir-FMRF-amide had the greatest distribution and relative amount. These observations indicate that many of the components required for puberty are already present long before the event actually occurs. Experimental manipulation of these factors may allow for the induction of early puberty. (See Yaron et.al, this meeting). (Supported by BARD #IS-2149-92).

PI-32

CHARACTERIZATION OF AN ANDROGEN RECEPTOR IN THE BRAIN OF THE ATLANTIC CROAKER, *MICROPOGONIAS UNDULATUS*.

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A nuclear androgen receptor was characterized in the brain of the male Atlantic croaker, *Micropogonias undulatus*. Scatchard analyses demonstrated a single class of high-affinity, low-capacity binding sites for testosterone (T) in the nuclear fraction (K_D = 2.73 ± 0.35 nM and B_{max} = 0.61 ± 0.15 pmol/g brain, n=3) as well as in the cytosolic fraction (K_D = 4.13 ± 0.98 nM and B_{max} = 0.93 ± 0.16 pmol/g brain, n=3). Analyses of binding kinetics revealed that the assocation rate was rapid for both the nuclear fraction ($T_{1/2}$=14.4 min.) and the cytosolic fraction ($T_{1/2}$=28.8 min.). However, the dissociation rate was slower in both the nuclear and cytosolic fractions ($T_{1/2}$= 6 hours). Competition studies indicated that T had the highest affinity, while dihydrotestosterone and methyltestosterone bound with an order of magnitude less affinity and 11-ketotestosterone bound with two orders of magnitude less affinity. No displacement was detected with 5,000-fold excess 11β-hydroxytestosterone or androstenedione. Nuclear binding was also found in the brain of female *M. undulatus* with Scatchard analyses showing a single class of high-affinity, low-capacity binding sites for T, similar to those in the male (K_D = 4.4 ± 2.1 nM and B_{max} = 0.62 ± 0.16 pmol/g brain, n=2).

PI-33

CHROMATOGRAPHIC AND INMUNOLOGICAL EVIDENCE FOR A THIRD FORM OF GnRH IN ADDITION TO cIIGnRH AND sGnRH IN THE BRAIN OF *ODONTHESTES BONARIENSIS* (ATHERINIFORMES).

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In the present study, the structures of GnRH (Gonadotropin Releasing Hormone) molecular forms in the brain of *Odonthestes bonariensis* were studied using high perfomance liquid chromatography (HPLC) and radioinmunoassay with different antisera. A firt screening of GnRH inmunoreactivity in brain extracts was done with three antisera (cII 678, PBL45 and PBL49) and three GnRH immunoreactive peaks were revealed. The first one eluted in the same position as cIIGnRH. This peak yielded a serial dilution displacement curve parallel to that of cIIGnRH, using a cIIGnRH homologous assay with cII675 antiserum. The second peak did not coeluted with any of the synthetic GnRH variants assayed, and showed crossreactivity in the cII675 homologous assay but not in a sGnRH homologous assay with s1668 antiserum. The third peak eluted in the same position as synthetic salmon GnRH. This peak yielded a curve paralell to that of sGnRH in two homologous RIA system (sGnRH 1668 and sGnRH Aida antisera). A cochromatographic study was performed adding cII and sGnRH to brain extracts. A screening with PBL49 antiserum showed three immunoreactive peaks. When meassured with specific RIA systems for cII and sGnRH an increase in cII and sGnRH concentration was observed either in the first and third peaks with no raise in the second one. In summary, this study suggests that this fish has at least two different types of GnRH, cII, sGnRH and possibly a third variant of GnRH.

PI-34

DISTRIBUTION OF NEUROPEPTIDE Y-LIKE IMMUNOREACTIVITY IN THE FOREBRAIN AND RETINA OF THE KILLIFISH, *FUNDULUS HETEROCLITUS*

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Intense NPY-like immunoreactivity was encountered in the neurons of the nucleus olfactoretinalis, in the large population of neurons in the floor of telencephalon and in the nucleus entopeduncularis of *F. heteroclitus*. Isolated immunoreactive (ir) neurons were located in the nucleus preopticus, nucleus lateralis tuberis and the dorsomedial thalamic nucleus. NPY-containing fibers were almost ubiquitous. Whereas a network of beaded ir fibers coursed through the olfactory bulb, the telencephalon contained thick radiating fibers basally and terminal fields with varying densities in the dorsal and lateral regions. NPY-containing fibers were particularly rich in the preoptic area, around the suprachiasmatic nucleus, tuberal hypothalamus and paraventricular thalamic regions. CSF-contacting ir fibers were seen around the preoptic and the lateral recesses. In the pituitary gland, fibers of the neurohypophysis displayed intense NPY immunoreactivity. Ir fibers were also seen in the habenular ganglia, in the slender pineal stalk, and arborizing into the pineal organ. In the retina, immunoreactivity was displayed in the amacrine cells and in the inner plexiform layer. Whereas the general pattern suggests a broad range of functional attributes for NPY, a particular role for the peptide in neuroendocrine regulation and circadian rhythms is suggested by these observations.

PI-35

EVIDENCE FOR TISSUE-SPECIFIC AROMATASE TRANSCRIPTS IN GOLDFISH BRAIN AND OVARY

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Teleost fish, including the goldfish Carassius auratus, demonstrate exceptionally high levels of brain aromatase activity and mRNA when compared with mammals and other vertebrates. By contrast, enzyme activity in ovary is <10% of that in brain, and aromatase mRNA is undetectable using a goldfish brain-specific cDNA as hybridization probe. To obtain evidence of aromatase transcripts in ovarian RNA and, if present, to determine whether brain and ovarian transcritps are identical, RT-PCR was undertaken using primers complementary to a sequence encompassing the substrate binding domain of the neural aromatase cDNA. Isolated cDNAs were subjected to restriction analysis. PCR products obtained from both brain and ovarian RNA were of the predicted size (1 kb); however, comparison of the restriction map of the putative ovarian cDNA fragment with the brain aromatase cDNA sequence showed differences in the restriction enzyme site patterns. Although sequence analysis is in progress, results suggest there may be tissue-specific differences in the coding region of aromatase transcripts in goldfish brain and ovary, a finding that has not been reported previously for mammals.
Supported by NSF-DCB89-16809.

PI-36

THE NEUROENDOCRINE REGULATION OF GONADOTROPIN SECRETION IN THE BAGRID CATFISH, MYSTUS MACROPTERUS

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The effects of intraperitoneal injections of (1) a luteinizing hormone-releasing hormone analog; (2) the dopamine antagonist domperidone (DOM) and (3) a combination of these substances, on gonadotropin (GtH) secretion and on ovulation in the bagrid catfish, Mystus macropterus were investigated. The GtH concentrations of serum samples were measuring using the African catfish GtH radioimmunoassay described by Goos with minor modifications.
LHRH-A (D-Ala^6,Pro^9-NEt-LHRH)(0.1 ug/g b.w.) alone stimulated an increase in serum GtH levels significantly 6 h after injection, but was ineffective for the induction of ovulation. DOM (10 ug/g b.w.) alone was ineffective in increase serum GtH level in mature bagrid catfish within 24 h after injection. However, DOM caused a marked potentiation of the GtH-release and ovulation response to LHRH-A. These results suggested that dopamine does not affect the GtH release directly in sexually mature fish, but indirectly by blocking the effect of gonadotropin-releasing hormone on GtH secretion; and the dopamine antagonist domperidone, potentiates the GtH release and ovulatory response to LHRH-A,which is consistent with the concept that dopamine functions as a gonadotropin release inhibitory factor in the teleosts.

PI-37

CONCENTRATION OF GONADOTROPIN-RELEASING HORMONES IN BRAIN OF LARVAL AND METAMORPHOSING LAMPREYS OF TWO SPECIES WITH DIFFERENT ADULT LIFE HISTORIES

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Our objective was to compare brain concentrations of gonadotropin-releasing hormones (GnRH) in similar life cycle intervals of nonparasitic and parasitic species of lampreys where the time of sexual maturation differs. We used HPLC and an RIA for lamprey GnRH-I to measure the brain levels (ng/brain) of GnRH-I and GnRH-III in larvae, metamorphosing individuals (transformers), juveniles, and sexually mature adults of landlocked sea lamprey (*Petromyzon marinus*, a parasitic species) and the western brook lamprey (*Lampetra richardsoni*, a nonparasitic species). Body size variations permitted trends and intraspecific, but not interspecific, GnRH values to be compared. In *P. marinus*, GnRH-III was the predominate form in larvae (1.4:1) and throughout most of metamorphosis (eg. stage 6, 2.5:1) and GnRH-I concentration increased significantly ($P<0.05$) between early (2) and later (5 and 6) stages to become the predominate form in juveniles (1:3.0). GnRH-I dominated over -III (1:4.8) during the feeding phase in *P. marinus*. GnRH-III in larvae and transformers through stage 3 of *L. richardsoni* was generally two-fold higher than GnRH-I but by stage 4 there was no difference in their concentrations. Sexually mature *L. richardsoni* showed a 1:2.7 dominance of GnRHI but also an additional unknown form of GnRH. Differences in the concentration patterns of lamprey GnRHs in transforming brains of nonparasitic and parasitic lampreys is an important feature which ultimately dictates their varied adult life histories. Supported by grants from NSERC and NSF and a GLFC contract.

PI-38

EFFECTS OF CRYOPROTECTANT, SPERM DENSITY AND STRAW SIZE ON LONG-TERM CRYOPRESERVATION OF BLUE CATFISH, *Ictalurus furcatus*, SPERM

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Sperm from nine blue catfish, *Ictalurus furcatus*, were cryopreserved for 12 months. Cryopreserved sperm was then used to fertilize lots of 450 channel catfish, *Ictalurus punctatus*, eggs. Mean relative fertilization percentage for the frozen sperm was 32% (26-39%) of the fresh control. Sperm frozen with the combination of an intracellular cryoprotectant, methanol, and an extracellular cryoprotectant, skim milk, produced no fertilization. No difference was observed between fertilization percentage of sperm frozen in two straw sizes ($P>0.05$). The highest level of relative fertilization percentage (54%) was achieved with an insemination dose of 6.00×10^9 sperm per straw ($P<0.05$). There was no difference ($P>0.05$) in fertilization percentage between insemination doses of 3.75×10^8 sperm and 1.70×10^9 sperm per straw.

PI-39

INDUCTION OF SPAWNING USING NON-CONVENTIONAL GONADOTROPHINS.

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Gonadotrophins from crude extracts of Tilapia (Mixed Species) and amphibian (frog and toads) were used in induced breeding of the African catfish, Clarias gariepinus. Spawning success, percentage fertilization and hatchability were used in assessing effectiveness of the inducing agents. The results are compared with those obtained by using synthetic hormones.

PI-40

mRNA EXPRESSION AND ENZYMATIC ACTIVITY OF CATHEPSIN D RELATED WITH SEABREAM EGG QUALITY

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In seabream, egg quality appears to be related to yolk composition. It has been well established that the yolk protein components derive from hepatic vitellogenin; moreover, cathepsin D seems to be responsible for intraoocytic processing of this precursor molecule to form egg protein components. The aim of this work is to establish whether in the seabream *Sparus aurata* egg quality may be related to the expression of cathepsin D and its mRNA. Cathepsin D probe was obtained by DNA extraction from seabream testis. After examination and quantitation, the DNA was subjected to polymerase chain reaction (PCR) amplification in the presence of sense and antisense oligonucleotides with a program consisting of 30 cycles. The amplification product of 550 bases was eluted and then directly cloned in a pCR II vector using T-A cloning kit. Nucleotide sequencing was performed using M13 primers; the resulting DNA was analyzed on the EMBL database and showed a close sequence homology with rat, mouse and human cathepsin Ds, making it suitable for use as a probe. This study correlates cathepsin D mRNA expression with enzymatic activity in the seabream ovary during vitellogenesis as well as in both good and poor quality eggs.

PI-41

CONTROL OF REPRODUCTION IN GRASS CARP *Ctenopharingodon idella*.

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The effectiveness of a Chinese system for the fry Carp production was evaluated. 31 gravid females were induced to spawning through the hypophyzation method in the fishery El Peaje at San Luis Potosí, México.
The results obtained show a mean rate of spawning of 71%. The average number of eggs was 635,072 for female. The fecundation value rate was 84.33% and the hatch rate obtained was 68.79%. We have displayed this spawning and incubation chinese system is adequate for the control of grass carp reproduction at the fish farms of México.

PI-42

MILT QUALITY AND QUANTITY PRODUCED BY YELLOWTAIL FLOUNDER (*PLEURONECTES FERRUGINEUS*) FOLLOWING GnRH-ANALOGUE TREATMENT BY MICROSPHERES OR PELLET.

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The GnRH analogue (GnRH-a), D-Ala^6,Pro^9-NHEtLHRH, was implanted intramuscularly either by microsphere injection (30μg/kg) or in 100% cholesterol pellets at two dosage levels (0, 44 or 197μg/kg) in four different groups of males (n=29) during the spawning season. Samples of blood and milt were collected on 5 occasions until 88 days after treatment. Compared to pellet control fish, all groups of hormone treated fish showed an increase in milt volume, with a longer-term response in the group receiving the highest dosage pellet. Although milt volume was increased by GnRH-a treatment, spermatocrit decreased throughout the duration of the experiment in all groups of fish and this may be a seasonal phenomenon or it may be due to the GnRH-a treatments. Milt quality of individual males was compared by using egg fertilization trials at 4 and 12 days after treatment, and fertilized eggs were maintained until hatch. No significant difference in milt quality was found between treatments.
In conclusion, GnRH-a treatment of yellowtail flounder males results in a stimulation of milt volume without a negative affect on milt quality.

PI-43

CO_2 EFFECTS ON FLAGELLA OF NATIVE AND DEMEMBRANATED TURBOT SPERMATOZOA

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CO_2 inhibits the movement of flagella of intact as well as demembranated spermatozoa. When exposed to a gentle flux of CO_2, intact turbot spermatozoa swimming at the surface of a drop of sea water, immediatly stop; they rapidly resume a normal swim right after the CO_2 flux is arrested. The swim is also rapidly and reversibly stopped by addition of Sodium azide. The inhibitory effect is similar on spermatozoa devoid of membrane and ATP reactivated : this is observed when adding either NaH CO_3 or CO_2 in buffered conditions (pH 6.0 to 9.0), showing that inhibition is not due to a pH shift. Sodium azide at low concentrations leads to similar inhibition either with native or with reactivated movement. In all cases, the inhibition was observed during its process right after application of CO_2. The use of high speed video-stroboscopy and image analysis allows the measurement of the degree of curvature along the axoneme. The major inhibitory effect is on the bending amplitude, with minor change of frequency : in presence of CO_2, the last wave initiated close to the head continue along the axoneme, but the following wave is not initiated. In contrast, ATP induced sliding process of trypsinised (demembranated) axonemes is not affected either by CO_2 nor by related effector such as NaN_3. From this set of observations, it is concluded that CO_2 blocks a flagellar target which is responsible of the transformation of sliding into bending and not on the sliding process itself.

PI-44

OVARIAN MORPHOLOGICAL CHANGES AND PLASMATIC SEX STEROID PROFILES IN TWO CULTURED SALMON (*O.kisutch* and *S. salar*) BROODSTOCK POPULATIONS IN CHILE.

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Chile is, at present, the second world producer of cultured salmonids with 60,000 tons in 1993. Because of the recent development of this activity in the country, no equivalent research efforts have ocurred yet in biological aspects of salmon in culture conditions, included reproduction. In the present study, ovarian and hormonal changes in females of two salmon populations of two farms from south of Chile, (one coho salmon an another Atlantic salmon) were monitored. Samples of ovarian tissue and blood (25 individuals each) were took five times beetwen September and May in both species. Histological studies were carried out on 4 µm thick sections of ovaries fixed in ALFAC. The tissues were stained with hematoxylin-eosin, and Masson van Gisson trichromic. Radioinmunoassay (RIA) and high pressure liquid chromatography (HPLC) were performed for sex steroids determinations. Main ovarian morphofunctional changes and hormonal profiles are described for both species and comparatively discussed with northern hemisphere literature.

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PI-45

PLASMA STEROID HORMONES IN ADULT TRIPLOID TILAPIA (OREOCHROMIS NILOTICUS)

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Testosterone (T) and estradiol-17ß (E2) were determined by enzyme immunoassay in plasma of adult diploid and triploid tilapia (O. niloticus), kept in pond culture, over a 60 day period. Profiles and maximum concentrations of T were the same in diploid and triploid males. Diploid females showed cyclical hormone profiles for T and E2 corresponding to average breeding intervals of 15-20 days. Triploid females showing advanced stages of gonadal development exhibited comparable amounts of T and E2 though cycles were extended to 20-40 days. In triploid females with retarded ovaries no endocrine signs of maturation were found. Steroid hormone concentrations are connected with the attained stage of sexual maturity. Thus all triploid male and part of the triploid female tilapia may be considered endocrinologically fully competent.

PI-46

PRODUCTION OF CLONAL LINES OF ATLANTIC SALMON X BROWN TROUT HYBRIDS BY GYNOGENESIS

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Clonal full-sib progeny groups of Atlantic salmon female x brown trout male hybrids were produced by gynogenesis. Eggs were stripped from two mature Atlantic salmon x brown trout F_1 hybrids and activated with UV irradiated rainbow trout sperm. The eggs were incubated until the fry reached the initiation of feeding stage, at which time average survival for the progeny of the two females was 69% and 62%. Flow cytometric analysis indicated that the fish were diploid, and protein electrophoretic analysis for 4 diagnostic loci confirmed that the fish possessed alleles for both species. Isogenicity within the progeny groups and to the maternal parent was indicated by identical DNA fingerprint patterns. It appears that a large portion of the eggs in the developing gonads of females of this hybrid undergo a premeiotic chromosome doubling, or a disruption of the first meiotic division. This results in ovulation of eggs possessing a full complement of both Atlantic salmon and brown trout chromosomes identical to that of the maternal somatic cells. Lines of clonal hybrids could therefore be perpetuated by continued gynogenetic production.

PI-47

IMMERSION OF NILE TILAPIA IN 17α-METHYLTESTOSTERONE AND MESTANOLONE FOR THE PRODUCTION OF ALL-MALE POPULATIONS

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Although hormone therapy for sex control has become nearly routine in aquaculture, there is room for improvement in selecting the safest and most effective hormones, as well as in developing procedures to minimize human and environmental exposure. Immersion of tilapia (*Oreochromis niloticus*) fry in 17α-methyltestosterone or in mestanolone was compared with feeding of 17α-methyltestosterone for production of all male populations. Immersion doses were 100, and 500 µg/Liter of water in two 3 hr exposures at 10 and 13 days post fertilization. Feeding dose was 60 mg/kg of food for 20 days beginning at the onset of feeding. Fish were sampled within four months after fertilization for microscopic determination of sex ratios. Preliminary results indicate that mestanolone is effective as an immersion treatment for production all male populations of tilapia.

PI-48

FAILURE OF GYNOGENETICALLY-DERIVED MALE CHANNEL CATFISH TO PRODUCE ALL-MALE OFFSPRING

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Female homogamety (XX) in channel catfish was demonstrated when all-female offspring were produced by meiotic gynogenesis (UV-irradiated blue catfish sperm; 8000 PSI, 3 min duration, beginning 5 min post-fertilization). Hormonal manipulation and selective breeding has produced females with XX, XY or YY sex genotype. To further elucidate the mode of sex inheritance in channel catfish, females with XY sex genotype were used to develop gynogenetically-derived YY males. We evaluated the sex ratios of offspring from gynogenesis and from matings of YY males with normal females. Gynogenetic offspring from three XY females were 48, 50 and 53% males. At 18 months of age, 194 surviving offspring were individually tagged and stocked into a pond until they reached sexual maturity. Presumptive YY males were mated with normal females, and families were maintained separately until fish were large enough to determine sex. Of the 18 families produced, seven (39%) had all-male offspring, five (28%) had offspring with sex ratios of 62-98% males, and six (33%) had equal percentages of males and females. As sex ratios from matings of normal male and female channel catfish rarely deviate from the expected 50% males, aberrant sex ratios from gynogenetically-derived YY male channel catfish (< 100% males) was not expected on the basis of single factor sex determination in this species.

PI-49

ULTRASTRUCTURAL STUDY OF OSMOLALITY EFFECT ON SPERMATOZOA OF THREE MARINE TELEOSTS

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With particular emphasis on mitochondria that may provide endogenous energy for spermatozoan motility, the morphological changes of the spermatozoa of three marine teleosts, black porgy (*Acanthopagrus schlegelli*), black grouper (*Epinephelus malabaricus*), and Atlantic croaker (*Micropogonias undulatus*), were compared either after activation in artificial sea water or when immersed in various osmotic pressure media. The midpieces of these three teleosts spermatozoa are composed of mitochondria surrounding the flagellum. Each mitochondrion is enclosed by distinct outer and inner membranes. The inner membrane separates the organelle's volume into two phases: the matrix and the intermembrane space. The inner membrane displays numerous infolding cristae that vary in number and shape and extend into the matrix. Following activation with artificial sea water, spermatozoa became motile and both the size and number of mitochondria decrease and then totally disappear. The present study strongly suggests that an energy source(s), responsible for motility, is located within the mitochondria in the midpiece of these three marine teleost spermatozoa.

PI-50

THE EFFECTS OF SUSTAINED ADMINISTRATION OF TESTOSTERONE AND GnRHa ON GtH-II LEVELS AND GAMETOGENESIS IN IMMATURE STRIPED BASS, MORONE SAXATILIS

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The goal of this study was to develop a delivery system for the long-term administration of testosterone and to use this device alone or in combination with a GnRHa delivery system to study and manipulate the onset of puberty in striped bass. For this purpose, we developed a biodegradable microspheric preparation, using a copolymer of polylactic and polyglycolic acid, which releases testosterone in striped bass for at least 7 weeks. Groups of immature striped bass were treated with the testosterone delivery system (3 mg T/Kg BW), with a GnRHa delivery system (225 µg GnRHa/Kg BW) or with a combination of both. Fifty days after the initiation of the experiment, fish were sacrificed, plasma was analyzed for circulating testosterone and GnRHa levels, pituitaries for GtH-II levels, and gonads were examined for stage of development. Plasma levels of both testosterone and GnRHa were significantly higher in the treated fish than in the controls, but none of the hormonal treatments affected the developmental stage of the ovaries or testes. However, in the females, both the testosterone and the combined treatment increased pituitary GtH-II content significantly (10.7 and 14.5 µg/pit., respectively), while pituitary GtH-II content in the GnRHa treated fish (6.1 µg/pit.) did not differ significantly from the controls (4.7 µg/pit.). None of the treatments affected the incidence of precocious males, which were present in all groups. Nevertheless, sustained administration of GnRHa causes an increase in pituitary GtH-II content of both immature and precocious males.

PI-51

BREEDING AUSTRALIAN LUNGFISH IN CAPTIVITY

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In 1992, Macquarie University began developing a facility for lungfish research. It now comprises 2 large earthen ponds (15X50m each), 5 large holding tanks, a well-equipped laboratory in close proximity to the holding tanks and a soon-to-be-constructed aquarium room for rearing young lungfish. The ponds have a shallow (0.5m depth) end planted with *Vallissneria* and a deep (2.5m depth) end. To maintain physical conditions appropriate for keeping fish, floating islands of *Eichornia* and paddle wheel aerators have been introduced. The ponds were completed in September, 1992 and one of these was stocked progressively over the ensuing 12 months with 9 adult lungfish, obtained from several sources and representing fish originally derived from the Brisbane, Mary and Burnett Rivers.

In December 1993, the lungfish began exhibiting spawning behaviour and soon after eggs were collected and incubated in sterile pond water. They hatched early in January and, although many have been used experimentally, the remainder are growing well in small aquaria. The majority of the eggs were left in the pond and periodically sampled to compare growth rates with those in the aquaria. Unfortunately, the ponds became attractive to several fishing birds in mid-1994 and many of the young lungfish were lost before the ponds could be completely enclosed in bird-proof netting.

This year spawning began again in October so that it now appears likely that these lungfish will breed naturally in captivity on an annual basis, thus providing large numbers of larval, juvenile and later adult lungfish for ontogenetic and physiological studies.

PI-52

REPRODUCTIVE VARIATION IN HATCHERY-RELEASED SOCKEYE SALMON

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Anadromous sockeye salmon (Oncorhynchus nerka) were artificially produced from nonanadromous kokanee salmon in Lake Shikotsu on Hokkaido. Hatchery-released smolts migrated seaward and returned to the Bibi River after one or two years of ocean life. A few precocious males migrated seaward with maturing condition and returned to the river several months after occan life. Their variations of reproductive characters were compared with those of kokanee salmon. Adult females had higher fecundity, as they were larger in body size. Fecundity and fork length were observed to fit allometric formula within each population. There was no relationship between fork length and egg size in kokanee salmon. Although age-1.1 (one winter in fresh water and one winter in ocean) sockeye salmon were approximately 65% larger in fork length than age-3.0 kokanee salmon, there was no significant difference in the egg size between the two populations. In contrast, age-1.2 sockeye had about 12% larger eggs than kokanee and age-1.1 sockeye salmon. Hatchery-released sockeye salmon are excluded from breeding competition and parental care. Then their egg size may be strongly affected by genetic component, whereas their fecundity vary with body size caused by environmental component.

PI-53

INCREASED MILT PRODUCTION BY GONADOTROPIN RELEASING HORMONE ANALOG (GnRHa) -TREATED ATLANTIC SALMON AFTER INJECTION OF 17α-HYDROXYPROGESTERONE

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Both GnRHa and gonadotropins have been used successfully to stimulate milt production in a variety of teleosts. Evidence suggests that some of the effects of these hormones are mediated through gonadal progestogens, particularly 17α,20β -dihydroxy-4-pregnen-3-one (DHP). However, the high cost of DHP precludes its routine use in broodstock management and our past use of a commercial salmon GnRHa preparation containing domperidone (dom) on non-spermiating Atlantic salmon has given equivocal results. The effects of GnRHa+dom, 17α-hydroxyprogesterone (HP) and DHP, alone or in combination, on milt production by Atlantic salmon were investigated in three trials over two years of study. Non-spermiating males received an i.m. injection of GnRHa+dom, or vehicle on day 0. On day 6 and day 7, they were injected i.m. with HP, DHP (1 mg/kg) or vehicle. On day 8, animals were stripped and blood samples taken for steroid analysis. GnRHa+dom increased milt volume 2-4 fold, compared to controls, in all three trials. HP or DHP alone caused a 2-fold increase in milt volume. In all cases, GnRHa+dom in combination with HP, but not in combination with DHP, caused a 2-fold increase in milt production compared to GnRHa+dom alone. Increased milt production was related to a decline in 11-ketotestosterone levels. HP alone or in combination with GnRHa+dom resulted in greater increases in plasma DHP levels, compared with other treatments. These results support the involvement of progestogens in milt production and indicate that HP treatment is a cost-effective method of promoting spermiation in GnRHa-treated Atlantic salmon.

PI-54

INTERACTION OF CRYOPROTECTANTS WITH TROUT SPERM PLASMA MEMBRANE DURING FREEZE-THAWING : A BIOPHYSICAL STUDY.

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Besides the problem of ice crystal formation during freezing, plasma membrane (PM)destabilization is one of the major damages encountered during cell cryopreservation. As individual lipids reach their phase transition temperature (Tm), i.e., they move from the fluid-crystalline phase to enter a much more rigid gel phase, phase separation of membrane components occurs. This results in packing faults and irreversible protein aggregation in the PM. Membrane cholesterol as well as cryoprotectants are thought to broaden lipid Tm, and this may reduce damages during freeze-thawing. In this work, we present the lipid phase transition profile of trout sperm plasma membrane as assessed byFTIR. Surprisingly, when compared to mammalian sperm, trout sperm lipid transition occurred over a broad range of temperature. This should provide a great stability of the cell PM toward freezing, but it is not so as shown by propidium iodide experiments. The specific interaction of the diluent components with the lipid fraction of trout PM was analyzed. Stability of liposomes made with trout sperm PM phospholipids was tested in freeze-thawing experiments. DMSO induced a better liposome stability than glycerol. However, DMSO interacted with phosphate- or TES-buffers and this increased liposome leakage during thawing. All the sugars tested had the same high cryoprotective effect. We also showed that cholesterol can either stabilize or destabilize artificial membranes depending on their lipid composition and the cryoprotectant used. This study showed that incompatibilities between different molecules can reverse their cryoprotective effect and that each cell might have its own set of requirements for protection depending on its lipid composition.

PI-55

EVALUATION OF PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS FOR ESTIMATION OF SEMEN FITNESS FOR CRYOPRESERVATION AND FOR QUALITY DETERMINATION OF DEEP FROZEN SEMEN IN THE RAINBOW TROUT *(ONCORHYNCHUS MYKISS)*

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Semen of teleost fishes reveal wide fluctuations in its quality and fertilization capacity. From practical experience it is known that also the semen fitness for cryopreservation differs between various batches and furthermore up to now no assays are avaibale for quality determination of deep frozen semen. Using semen of rainbow trout *(Oncorhynchus mykiss)* as a model and using the cryopreservation method of Lahnsteiner *et al.* (1995 - Aquaculture and Fishery Management, in press), the present study investigates physiological (sperm motility) and biochemical (sperm metabolites and enzymes) parameters before and after cryopreservation as well as the seminal fluid composition under aspects of correlations with semen postthaw fertility. We demonstrate, that parameters of seminal fluid composition, sperm motility and sperm metabolism correlate with the postthaw fertility of semen. The suitability of these parameters for evaluation of semen fitness for cryopreservation and for quality control of deep frozen semen is discussed.

PI-56

OVA MANAGEMENT AND OPTIMIZATION OF ARTIFICIAL INSEMINATION IN THE EUROPEAN CATFISH (*SILURUS GLANIS* L.)

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Artificial spawning of European catfish was evaluated by quantity of ova and sperm, effect of females and males on hatchability, storage of oocytes *in vivo*, influence of NaCl concentration and ratio of eggs and activation solution (AS) on fertilization expressed by percentage of hatching. For oocytes ovulated and left in the ovaries, the percentage of total hatching significantly decreased from the initial 74.5 % immediately after ovulation to 54.2 % and 37.9 % after 4 hours and 6 hours storage, respectively ($P<0.001$). On the other hand, the percentage of abnormal hatched fry from such oocytes increased from initial 8.2 % immediately after ovulation to 17.9 % and 50.0 % after 4 hours and 6 hours storage, respectively ($P<0.001$). The highest fertilization expressed by hatching rate 89.8±3.8 % was found for AS of 17 mM NaCl and than significantly decreased ($P<0.01$) at 68 mM NaCl to the level of 70.3±6.4 %. The ratio of 1:2 (volume of AS in ml : grams of ova) increased the hatchability (68.6±6.4 %), rather than ratio of 1:1 in the control batch. Hatching significantly decreased ($P<0.05$) at the ratios 1:4 and 2:1 to levels of 45 and 41.9 %, respectively. ANOVA showed significant effect of females on hatchability. The effect of different quantity of spermatozoa per egg was not significant.

PI-57

REPRODUCTION OF DELTA SMELT (*Hypomesus transpacificus*) IN CAPTIVITY

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Delta smelt, *Hypomesus transpacificus* is a threatened species endemic to the Sacramento-San Joaquin estuary. In this small, short lived, and apparently semelparous fish, a successful reproduction must be accomplished each year by a large number of individuals in order to sustain the population. To obtain first experimental information about the reproductive cycle of this species, we have initiated rearing and spawning of delta smelt in captivity. Wild fish were collected from the Sacramento and San Joaquin Rivers and raised in recirculation systems with controlled temperature, photoperiod, and feeding. Captive populations were monitored for growth, gonadal development and gametogenesis. From August to May, fish grew in fork length from 47 to 70 mm, body weight from 0.8 to 2.6 g, and their gonadosomatic index increased from 0.1 to 1.9% (males) and 0.5 to 10.9% (females). Vitellogenesis and testicular meiosis were completed within 2-3 months. Spawning occurred naturally in April and May. Embryonic development to hatching continued 9-14 days at 14-16°C, and newly emerged pelagic larvae ranged 5.1-5.7 mm in total length. The yolk sac and oil globule were absorbed in 6 and 10 days after hatching, and larvae started exogenous feeding on rotifers at 4-5 days after hatching. However, the differentiation of fins and functional swimbladder did not occur until the age of 2-3 months, suggesting that delta smelt has delayed metamorphosis and a long larval stage adapted to life in the entrapment zone of a tidal estuary.

PI-58

PERMEABILITY OF ORNAMENTAL CARP FERTILIZED EGGS TO ^{3}H-DMSO AND ITS TOXICITY TO DEVELOPING EMBRYOS

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^{3}H-DMSO was found to permeate into fertilized eggs with embryos at post-gastrulation stages. Embryos reaching these stages were found to tolerate cold thermic shock, and most of them survived exposure to 4°C for 2 h. Of the total amount of cryoprotectant permeating into fertilized eggs that were incubated in 0.5 M ^{3}H DMSO, the yolk fraction contained 32% (~0.05 M DMSO) and 36% (~0.1 M DMSO) after 15 min or 2 h of incubation, respectively. The relative amount retained in the non-soluble membrane fraction decreased from 35% to 22% during the same periods of incubation. The remainder was found in the supernatant fluid obtained after washing the membrane fraction. The amount of ^{3}H-DMSO that permeated these fertilized eggs increased with the concentration of DMSO in the incubation media, duration and temperature of incubation. About 0.2 M of DMSO (10%) entered fertilized eggs after 2 or 4 h of incubation in 2 M DMSO. All the tested concentrations of DMSO (0.5-2.0 M) reduced the viability of fertilized eggs, and the effect was expressed after 1 h of incubation.

PI-59

PRODUCTION OF RECOMBINANT CARP GROWTH HORMONE MOLECULE

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The objective of this work is to produce recombinant carp growth hormone (rcGH) in *Brassica napus* (canola) seeds. The plant derived rcGH may be used to develop a food supplement that enhances fish growth rate and could be applied in fish farming. The rcGH was initially produced in *E. coli* in order to test its bioactivity and use it as a standard for further studies. The rcGH cDNA was amplified by polymerase chain reaction (PCR) using primers that generated appropriate restriction sites for cloning in the bacterial expression vector pGEX-2T. Bacterial rcGH was produced as a fusion to GST (glutathione S-transferase, a protein from the parasitic organism *Schistosoma japoniccum*) which is part of pGEX vectors. The expressed fusion protein was purified then subjected to thrombin cleavage to separate GST and rcGH. The rcGH was then identified by specific antiserum against carp GH, and tested for biological activity using the insulin-like growth factor I (IGF-I) assay in goldfish. The results indicated that thr rcGH stimulated IGF-I production in the goldfish ovary. The rcGH cDNA was subsequently fused to the 18 kDa *Arabidopsis* oleosin gene driven by 800 bp of its own promoter. Such constructs have been shown to correctly target oleosin protein fusions to the oil bodies in *B. napus* seeds, allowing their relatively simple purification. We are presently working on transforming *B. napus*, cv. Westar, with the oleosin-rcGH gene fusion.

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PI-60

STERIOD INDUCED SEX REVERSAL OF PADDLEFISH

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Phenotypic sex of juvenile fishes can be directed if sex hormones at proper dosages are effectively administered throughout a critical period of gonadal differentiation. The objective of this study was to evaluate the effect of 17-alpha methyltestosterone (MT) capsule(s) implanted in juvenile paddlefish. Nine-week old paddlefish weighing 30 to 40 g were implanted intraperitoneally with silicone elastomer capsule(s) in the following treatments: 1) empty capsule (control), 2) one 5 mg capsule, and 3) two 5 mg capsules. Ninety fish per treatment were implanted with capsule(s) and PIT tags; and then, thirty fish from each treatment were stocked into three 0.04-ha ponds. Blood samples were taken monthly and analyzed for plasma testosterone by radioimmunoassay. When fish were 70-weeks old, fish were sacrificed and gonadal tissue removed for sex ratio determination. Level of testosterone measured in the plasma responded quadratically to the amount of implanted MT. There was a significant increase in testosterone level from fish implanted with one and two capsule(s) compared to those with an empty capsule, but levels between one and two MT capsule(s) were similar. Sex ratios were the following: control treatment 48% females (F) and 52% males (M); one capsule treatment 7% F, 26% mixed sex, and 67% M; and two capsules treatment 0% F, 10% mixed sex, and 90% M.

PI-61

PREPARATION AND EVALUATION OF GnRHa-LOADED, POLYMERIC DELIVERY SYSTEMS FOR THE INDUCTION OF OVULATION AND SPERMIATION IN CULTURED FISH.

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We have developed a variety of polymer-based delivery systems containing gonadotropin-releasing hormone (GnRHa) for inducing final gonadal maturation in fish. Delivery systems are prepared in the form of biodegradable, injectable microspheres or solid-matrix, implantable disks. Sustained elevations in circulating GnRHa levels are achieved for periods of 2 to 10 weeks. The effectiveness of these delivery systems has been evaluated in a variety of commercially important fishes, both in laboratory and field conditions. They have been shown to be effective both in species that spawn once during their reproductive season (Salmon, trout, striped bass, white bass), as well as in multiple spawners (American shad, plaice and yellow-tail flounder). Treatment of females with these delivery systems induced 100% ovulation with good egg quality characteristics. Similar treatment of males induces a prolonged elevation of spermiation, and large amounts of expressible milt can be collected frequently for up to three weeks after treatment. Use of sustained-administration, delivery systems obviates the need for multiple GnRHa injections, thus limiting the handling of sensitive and valuable broodstock to a single time. Moreover, the GnRHa treatment does not have to be precisely timed according to the fish's stage of maturity. Since the delivery systems can maintain elevated GnRHa plasma levels for long periods, they proved to be effective in fish at various stages of gonadal development.

PI-62

EFFECT OF THE LIPID COMPOSITION OF THE DIET ON THE HORMONAL LEVELS AND SPAWNING PERFORMANCE OF SEA BASS (*DICENTRARCHUS LABRAX*).

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The influence of dietary lipids on the reproductive cycle of sea bass was studied in fish that were fed for one year with diets containing different compositions of lipids: 7%, 15% (7% pelleted diet enriched with 8% of PUFA, mainly 22:6 n-3) and 28% of lipids in the diet. The control group was fed with sliced fish (*Boops boops*). Animals were fed in these conditions for one year before the beginning of the sampling. During the spawning period, eggs were collected daily to determine egg quality parameters. The control group showed improved egg quality (≈50% egg viability) and higher hatching rate (≈30%) than all the experimental groups (3-13% viability and 1-4% hatching rate). The egg quality for groups 15% and 30% was similar, and slightly higher than group 7%. Blood samples were collected monthly to determine the annual cycles of GtH II, estradiol-17ß (E_2) and vitellogenin (VTG) in plasma. Similar profiles of E_2 and VTG were observed in all groups but interstingly, a marked bimodal profile of VTG (peaks before and after the spawning period) was observed in the groups with the lowest egg quality (7% and 15%). The annual profile of GtH II levels in plasma has been determined for the first time in this species, using a newly developed ELISA. In controls, a single peak of GtH II (≈4 ng/ml) was observed at pre-spawning stages, followed by a continous decrease, until basal levels (0.5-1 ng/ml) were reached after the spawning period. In the experimental groups, the quantity and profile of GtH II levels was not affected by the different diets.

PI-63

CHARACTERIZATION OF S0 REPRODUCTIVE CYCLE FOR COHO SALMON *(Oncorhynchus kisutch)* IN CHILE.

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The availability of warm freshwater in lakes and springs in south of Chile has permitted the generalized use of S0 smolts. This practice allows sexual maturation at two years of age.

We characterize, based on data of four different farms, the reproductive cycle and the main reproductive parameters of coho salmon in Chile.

Spawns occurs during May, smoltification during next January (8 months); fishes reaches sexual maturation during May of the next year (16 months more) growing in salt water.

After a total period of 24 months, mature females, about 4 kg, produce a mean of 3,802 eggs; relative fecundity was 986 eggs/kg and eggs diameter 7.11 mm. Survival at the eyed stage was 74.3%.

Favorable water temperatures allows essentially a fast growth and sexual maturation in two years with a very advantageous fecundity.

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PI-64

COOL STORAGE OF THE JAPANESE EEL (*Anguilla japonica*) SPERMATOZOA

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Appropriate ionic composition of the Japanese eel sperm diluent for cool storage was examined. Spermatozoa at 50 times dilution with artificial seminal plasma, consisting of 149.3 mM NaCl+15.2 mM KCl +1.3 mM $CaCl_2$+1.6 mM $MgCl_2$ buffered with 5 mM $NaHCO_3$-NaOH at pH 8.2, or with isotonic NaCl+KCl solution showed high percentage motility 3 hours after cool storage at 5 °C. However, those diluted with isotonic NaCl solution, mannitol solution, NaCl+$CaCl_2$ solution, or NaCl+$MgCl_2$ solution showed significantly lower motility compared to the above two solutions. Spermatozoa were then diluted with isotonic solutions of various potassium concentrations (0, 5, 15, 25, 35, 45, 75, and 164.5 mM) at 50 times dilution and stored at 3 °C for up to 28 days. Spermatozoa diluted with the 5 - 45 mM KCl solutions showed a sharp decrease of percentage motility at 24 hours, however, they showed a marked recovery of the motility by the 7th day. The severity of the decrease at 24 hours became smaller with an increase of potassium concentration in the isotonic diluent. Spermatozoa diluted with the 75 or 164.5 mM KCl solutions did not show the temporal decrease of motility at 24 hours, but their motility at 14 - 28 days were considerably lower than those in lower concentration KCl solutions. Spermatozoa diluted with the 15, 25, 35, and 45 mM KCl isotonic solutions showed high motility indices (percentage motility / initial percentage motility $\times$100) of 47.9 - 64.8% at 21 days, and of 29.7 - 35.1% at 28 days, respectively. These results indicate that potassium ion is an essential constituent of isotonic diluent for cool storage of the Japanese eel spermatozoa. The optimum KCl concentration for short term storage (up to 7 days) is 45-75 mM, and that for longer storage is 15-45 mM.

PI-65

EFFECT OF METYHLTESTOSTERONE CONCENTRATION ON SEX RATIO,GROWTH AND DEVELOPMENT OF NILE TILAPIA.

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Methyltestosterone (MT) has been proven to be an effective drug to produce male tilapia but questions remain regarding its minimum effective dose and impact on growth and development. Tilapia fry were fed feeds containing 0, 3.75, 7.5 , 15, 30, 60, 120, 240, 480, 600, or 1,200 mg MT/kg for 28 days. Growth, survival and FCR were determined at the end of treatment. Fish were then grown to a minimum size of 5 cm, the sex and GSI determined and any gross abnormalities noted.

Sex ratios of 97% and greater were obtained at MT rates of 15 to 60 mg/kg. The percentage males was reduced at higher rates averaging approximately 50% at 240 to 1200 mg/kg. Intersex fish, where the gonad was predominantly tesicular tissue,were most common at MT rates of 3.75 to 15 mg/kg. At MT levels of 120 mg/kg and greater the gonadal tissues of intersex fish were predominantly ovarian. Male GSI differed among treatments. No anabolic effect was evident. No gross abnormalities could be attributed to MT concentration.

PI-66

ALTERATION OF CARP SPERMATOZOA MOTILITY BY URINE CONTAMINATION DURING SAMPLING

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It was shown previously that some samples of carp semen obtained by gonadotropic stimulation exhibited a poor motility which could be improved after incubation in a 200 mM KCl buffered solution (Redondo-Müller et al., Mol. Rep. Dev., 1991, 29: 259-270). Current laboratory observations of a large number of milt samples indicate that spermatozoa (spz) are spontaneously motile and loose 40-50% of their initial ATP content within one hour storage in a test tube. In experimentally contaminated milt, motility can be induced by addition (<10% in volume) of urine or freshwater (buffered or not). Intact sperm without urine contamination have an ATP content in the range of 10-12 nmolx10^{-8}spz and an initial velocity of 130 $\mu m.s^{-1}$ after a 1/2000 dilution in an activating medium, while in the case of sperm contaminated with 6.8% of urine during one hour, the ATP content is 5-7 nmolx10^{-8}spz and most spz have a low initial velocity of 10-20 $\mu m.s^{-1}$. It appears that the low osmolality of urine is responsible for the degradation of carp spz quality by a previous activation in the collecting tube and a premature ATP exhaustion. The contamination during stripping can be avoided by pressing the abdomen before sampling and by the use of a catheter introduced into the urinary bladder to collect urine prior to sperm. During artificial fertilization, ova and spz must be mixed as soon as possible to obtain a maximum fertilization rate.

PI-67

INTERACTIONS OF FEEDING RATE, WEIGHT GAIN, REPRODUCTIVE CHARACTERISTICS AND SPAWNING OF MALE CHANNEL CATFISH.

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Brood stock management procedures for channel catfish *Ictalurus punctatus* are not well defined. To better understand the interaction of feeding practice and reproduction, male channel catfish were fed at 0.5% or 3% body weight during the warmer months and at half the rates during the cold months. The development of the secondary sexual characteristic, head confirmation, was monitored. Spawning success of males from both feed rates of known head confirmations were determined. Serum steroid levels, GSI and sperm counts of males from both feed rates of known head confirmations were determined.

The mean percent spawning for males fed at the low feed rate was 44% and was significantly greater than that of 23% for the high feed rate. Head confirmation was not different for males at either feed rate. Males which spawned had a greater head width to body length ratio than those that did not. Sperm count, GSI, and hormone concentrations did not differ between feeding rates and were not correlated to head confirmation.

PI-68

CRYOPRESERVATION OF YELLOWTAIL FLOUNDER (*PLEURONECTES FERRUGINEUS*) SEMEN

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Pooled fresh semen from male yellowtail flounder was frozen in diluents A (diluent 1 from Gallant et al. but without lecithin) and B (plaice ringer, Cobb et al.) with 10% DMSO or propylene glycol added to make extender. Semen was diluted 1:3 (semen:extender) and frozen in 0.25 mL straws which were suspended 5.5 cm above the surface of liquid nitrogen on a floating styrofoam rack. After the temperature of the extended semen reached -95°C the straws were plunged into liquid nitrogen. Fertility trials were conducted to compare the following treatments: fresh pooled semen, fresh pooled semen diluted 1:3 with each diluent and each extender, and semen frozen in each extender. The straws were thawed for 7 seconds in a 30°C water bath. Freezing yellowtail flounder semen using the techniques described resulted in fertilization rates, hatch rates and percentage crooked larvae of approximately 70%, 65% and 8%, respectively. All four extenders produced acceptable results for cryopreserving the semen but diluent A with DMSO may have been the best combination.

References: 1. Gallant RK et al. 1993; Theriogenology 40: 479-486. 2. Cobb JLS et al. 1973; J Fish Biol 5: 587-591.

PI-69

CRYOPRESERVATION OF SPERM FROM STRIPED TRUMPETER *LATRIS LINEATA*

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Spermiating striped trumpeter males may not be available for the fertilization of eggs, especially early in the spawning season which lasts only 8 weeks (August to October). A bank of frozen semen which may be thawed as required would assist hatchery management. This study examined the handling and cryopreservation of semen for the fertilization of eggs.

Freshly-collected semen diluted with an extender containing 2 M glycerol in 300 mOsm saline was frozen as pellets on dry ice before storage in liquid nitrogen. Sperm motility was higher for pellets thawed at 20°C in dry test tubes than in tubes containing 0.5 ml sea water (40.5±4.2% and 34.5±4.3%). There was no difference in recovery of sperm for pellets thawed at 10°, 20° or 30°C. For fresh sperm at 20°C, motility declined from 83.3% immediately after activation with diluent to less than 2% within 9 minutes. The motility after activation of undiluted fresh sperm stored at 4°C was maintained for two days and then declined markedly so that by the eighth day, sperm were mostly immotile. There was no effect of pellet volume (0.2, 0.4 and 0.6 ml) or the number of pellets thawed in a tube (1, 2 and 4) on post-thawing motility. A dilution rate of 1:5 (semen:diluent) was better than 1:2 or 1:11 (30.3±4.2%, 24.9±3.8% and 27.2±3.8% motile sperm).

Fresh and frozen-thawed sperm (600-1,000 million motile cells) were used for fertilization of eggs (aliquots of about 400) collected from females treated with cholesterol implants containing LHRHa. Fertilization rate of floating eggs was lower for frozen-thawed than for fresh sperm (71.2±17.7% and 82.7±5.3%) and hatch rate was also lower (37.1±13.0% and 45.0±8.5%).

PI-70

DELAYED OVARIAN DEVELOPMENT AND REDUCED FECUNDITY IN LARGEMOUTH BASS RAISED ON A PELLETED FEED CONTAINING HIGH LEVELS OF STEROIDS AND LOW LEVELS OF ARACHIDONIC ACID.

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Largemouth bass, *Micropterus salmoides*, were raised on forage (goldfish) or a pelleted feed which contained significantly higher levels of testosterone (T) and estradiol-17β (E2), and lower levels of the prostaglandin precursor arachidonic acid, than the forage goldfish. Although there was no diet-related difference in maximum gonadosomatic index (GSI) attained, pellet-fed bass reached peak GSI 1 month later than forage-fed bass. In pellet-fed bass, serum levels of T and E2 were significantly suppressed during the period of delayed ovarian growth. Ovaries of pellet-fed bass had significantly lower levels of arachidonic acid than did ovaries from forage-fed bass. In controlled raceway spawning trials, pellet-fed bass had significantly fewer spawns, and produced fewer eggs and fry per spawn, than did forage-fed bass. Egg viability, determined by fertilization and hatching rates, was not affected by diet. Observed delays in ovarian development and reduced fecundity of pellet-fed largemouth bass may be due to the negative feedback effects of dietary steroids and a reduced capacity to synthesize prostaglandins necessary for ovulation and spawning behavior.

PI-71

GYNOGENESIS IN ALBINO GRASS CARP, Ctenopharyngodon idella (Val.)

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Diploid gynogenesis was induced in albino grass carp (AGC), Ctenopharyngodon idella (Val.) eggs obtained from three (AGC) females. Two batches of 36,000 and one of 90,000 eggs were inseminated with UV-irradiated (800 J/m²) heterologous sperm of common carp (CC), Cyprinus carpio (L.) and heat- or pressure-shocked at 40.0±0.5°C or 7,500 psi for 2 minutes (0.2-0.3T_o; 4-6 min post-activation at 22°C). Survival rates of gynogenotes (79-95%) was indicated by diploid albino larvae and did not differ significantly from their respective diploid controls, AGCxCC and CCxCC (t-test<0.87; P=0.5). Although high survival (about 70%) was found in AGCxCC (AGC eggs fertilized with intact CC sperm) embryos, examined 10h post-fertilization, no embryos hatched.

Another set of experiments used four AGC females to examine the possibility of incorporating pigment genes of paternal origin. In these, sperm of tricolored (sanke) Japanese ornamental (koi) carp (KC), common carp (CC) and wild-type colored grass carp (WGC) were gamma-irradiated (Co^{60}) with three different dosages, 60, 75 and 90 Krad. Irradiated sperm was used to inseminate AGC eggs. Batches of 18,000 eggs, activated at 22-24°C prior to shock, were diploidized either with early heat- (42±1°C for 1 min) or pressure-shocks (8,000 psi for 2 min), four minutes (0.2-0.25T_o) post-activation. The crosses AGCxKC yielded some (3 ind.) pigmented (wild-type) fish plus some (10 ind.) albinos, or solely large number of albinos. We presume that the genes responsible for the black pigment (e.g. tyrosinase) from the KC, were transferred to the AGC on chromosome fragments and were expressed in the dark-colored individuals (this will be further examined by DNA-fingerprintings). In other crosses, AGCxCC and AGCxWGC, all offspring were albinos. Survival of albino fish in the latter combinations was about 10% (total of about 2,500 hatch-out larvae).

PI-72

CHARACTERIZATION OF EMULSION PREPARED WITH LIPOPHILIZED GELATIN AND ITS APPLICATION FOR INDUCING VITELLOGENESIS IN JAPANESE EEL

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A new water-in-oil-in water (W/O/W) type emulsion using lipophilized gelatin (LG) and cottonseed oil was developed for the administration of hormones. LG was prepared by attaching fatty acid anhydride to gelatin. LG with palmitic anhydride was found to be most effective for the preparaton of W/O/W emulsion. The best conditions for preparing a stable emulsion were determined as follows: the final concentration of LG and the volume ratio of oil to water were 2% and 2:1. Plasma profiles of salmon GtH II in young eel (BW 200g) showed gradual changes when administered LG emulsion containing salmon pituitary, while sGtH II levels were constantly low when treated with W/O emulsion prepared with Freund incomplete adjuvant (FIA). Plasma profiles of sGtH II showed acute changes when treated with pituitary saline solution. Immature Japanese eel (BW 566-1017g) received weekly intramuscular injections of LG emulsion, FIA emulsion or saline solution containing salmon pituitary. LG emulsion was found to be more effective than others in inducing vitellogenesis in eel.

PI-73

GONADAL SEX DIFFERENTIATION IN CHANNEL CATFISH

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Culture of monosex male catfish populations is desirable for aquacultural purposes, but control of sex in this species has proven difficult. This study was designed to describe the process of gonadal differentiation in channel catfish in order to optimize current methods of sex control. Mixed-sex and monosex populations were used in this investigation. The mixed-sex population was from a normal cross between an XX female and an XY male; the all-male population was from a cross between an XX female and a YY male; and the all-female population was obtained by steroid treatment of all-male progeny. Fish were serially sampled for histological inspection of the gonads. All fish had indifferent gonads 7 days after fertilization (first sampling date). At 19 days post-fertilization, the gonads of some individuals showed tissue outgrowths at the hilar and distal regions indicating the onset of somatic organization. The extension and subsequent fusion of these outgrowths to form a cavity could be seen by 22 days post-fertilization along with the first appearance of the zygotene stage of germ cell meiosis. By 48 days post-fertilization perinucleolar oocytes could be seen in these gonads. This pattern of gonadal differentiation was identical to that of known females. The gonads of the rest of the mixed-sex population and those of known males showed no evidence of somatic or germ cell differentiation up to 3 months of age. Therefore, sex differentiation occurs first in females with the onset of somatic organization followed by germ cell differentiation. Also, the critical period of female sex differentiation coincides with the time in which catfish are sensitive to feminization by steroid treatment.

PI-74

EFFECTS OF STRIPPING FREQUENCY ON THE SEASONAL SPERMIATION RESPONSE AND SPERM QUALITY IN MALE WINTER FLOUNDER, *PLEURONECTES AMERICANUS* (WALBAUM)

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The seasonal spermiation response was characterizied and sperm quality evaluated in male winter flounder held without females throughout the summer spawning season. Milt volume, spermatocrit, sperm production, motility and sperm fertility, when eggs were available, were monitored according to a weekly (1/wk) or biweekly (1/2wk) sampling protocol. Generally, males spermiated for many weeks with a tendency for spermatocrit to rise during the spawning season with fluctuations from 30-81%. The greatest motility was found at the beginning of the season in May and it tended to vary with changes in milt volume. Motility was correlated to estimates of sperm quality, evaluated by fertilization and hatching rates. Mortality (83 vs 33%) was increased while duration of spermiation (6-10 wk vs 10-16 wk) was decreased in males sampled biweekly. Total milt volume and sperm production also decreased by 66% and 72% respectively. Lower motility (23 vs 43%) and fertilization rate (37 vs 57%) were obtained from males sampled biweekly. These data suggest that the spermiation response of male flatfish undergoes seasonal changes and that a delay in sampling reduces sperm production and sperm quality.

PI-75

GONADAL DIFFERENTIATION OF BLACK CARP

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Black carp (Mylopharyngodon piceus) have potential for biological control of nuisance mollusks. Development of reproductively limited fish permits testing and/or utilization in an ecologically sensitive manner. Development of broodstock to produce monosex populations is a practical management system. The protocol involves a combination of steroid-induced sex reversal and breeding. A preliminary requisite is the characterization of gonadal differentiation so as to identify the appropriate sex-reversal regime. Gonadal development was studied in pond-grown black carp during early ontogeny. Fish examined were 9-26 months old, 70-500 mm (T.L.) and 5-1180 g. Gonads were cytologically undifferentiated throughout the first year of life at sizes smaller than 340-400 mm (390-600 g). Initial cytological differentiation of gonads occurred at age 12-18 months and in fish between 600 and 1000 g. Some slower growing fish 24-26 months-old and less than 285 mm still had undifferentiated gonads.

PI-76

INDUCED OVULATION OF CHINOOK SALMON USING A GNRHa IMPLANT: EFFECT ON SPAWNING, EGG VIABILITY AND HORMONE LEVELS.

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The purpose of this study was to compare the effectiveness of a gonadotropin releasing hormone (GnRHa) implant with an aqueous solution of a similar dose for accelerating and synchronizing ovulation in chinook salmon *Oncorhynchus tshawytscha*. Groups of 30 mature salmon (mean weight 7.6 Kg) received either 1) an implant containing 250 μg of GnRHa ([D-Ala^6,Pro^9 NEt]-LHRH; ReproBoost™ AquaPharm Tech. Corp.), 2) an injection containing the same amount of GnRHa in saline solution, or 3) an injection of 0.7% saline. Within 18 days post-treatment 93, 60 and 13% of the fish in each of the groups had spawned. By day 22 post-treatment 97% of the fish in group 1 (implant) had spawned, while the cumulative spawning of fish in the injected groups were 93% after 35 days and 87% after 37 days, respectively. Mean egg viability to hatch of 5 random egg samples from each group was not significantly different. During the course of the study blood samples were collected from 10 fish from each group for measurement of cortisol, estradiol-17ß, 17α20ß dihydroxyprogesterone, and GnRHa.

PI-77

POST-SMOLT MATURATION IN ATLANTIC SALMON FED DIFFERENT RATION LEVELS

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Atlantic salmon (*Salmo salar* L.) 1+ smolts were maintained in freshwater past the time of smolting (mid-May), and fed four different ration levels: 100% ration, 50% ration, 25% ration and 0% (starved). No signs of disease or aggression were observed, and no mortalities were recorded. In early July all groups were returned to excess feeding. In October all fish were killed and their maturational status determined. Growth rate and condition factor were significantly reduced on restricted ration levels. After transfer to excess feeding fish from restricted rations grew faster than previously full fed fish. K-factor did not differ significantly among the groups in early October. Mature males were found in all groups. From 22% to 43% of males matured as post-smolts, with no apparent trend among ration levels. Mature female post-smolts were found only in the 25% ration group, where 16% of females matured. The present results indicate that post-smolt maturation of male Atlantic salmon can occur independent of energy status in the early post-smolt phase. Further, the rare phenomenon of mature female post-smolts in groups on restricted ration suggest that initial stages of maturation in females may co-occur with the development of smolt characters in the spring.

PI-78

CRYOPRESERVATION OF RAINBOW TROUT (ONCORHYNCHUS MYKISS) SPERM IN STRAWS

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Fertilization of small batches of rainbow trout eggs with sperm cryopreserved by the pellet technique is an established technique (HOLTZ, 1993, Aquaculture 110).
In order to deal with larger batches of eggs, in this study semen was frozen in 4 ml-straws. Various diluents, concentrations, freezing and thawing rates were tested. Eventually staisfactory fertilization rates were achieved with batches of about 1800 eggs.
In comparison to pellets the large straws proved to be more efficient and much more convenient to handle when fertilizing all eggs stripped from one female at a time.

PI-79

CRYOPRESERVATION OF FISH SPERM: LABORATORY, HATCHERY AND FIELD STUDIES OF TWENTY SPECIES

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Cryopreservation of sperm offers benefits across a broad range of aquaculture and fisheries applications. These include use for genetic improvement of species that are cultured for food, such as channel catfish *(Ictalurus punctatus)* or tilapia *(Oreochromis niloticus)*, or selective breeding of ornamental fishes such as koi carp *(Cyprinus carpio)* in which a single fish can be worth thousands of dollars. Other applications include stock enhancement of species such as spotted sea trout *(Cynoscion nebulosus)* or Mekong giant catfish *(Pangasius gigas)* that support commercial or recreational fisheries, or conservation of genetic resources of endangered species, such as razorback sucker *(Xyrauchen texanus)* or bonytail chub *(Gila elegans)*. These applications can have divergent goals: in endangered species, cryopreservation could be used to maximize genetic diversity, while in culture species, it could be used to minimize genetic variation in selective breeding. Cryopreservation reduces the cost and risk of broodstock maintenance, simplifies production of interspecific hybrids, and can be accomplished in the laboratory or on the riverbank. During the past four years we have combined laboratory, hatchery and field studies to develop procedures for collection, storage, cryoprotection, freezing and thawing of sperm, and fertilization of eggs. Best results have been obtained with the use of 0.5-ml straws, penetrating cryoprotectants (e.g. methanol or DMSO), and rapid freezing (-45°C/min) and thawing (~22 °C/sec).

PI-80

EFFECT OF CATIONS, pH AND OSMOLALITY ON SPERM MOTILITY OF MALE WHITE CROAKER, *MICROPOGONIAS FURNIERI*

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Physico-chemical factors were analysed for the induction of sperm activation in male white croaker. Buffer Tris solution (0.02 M, pH = 8.9) with high molarities of NaCl (250 mM, 500 mM) induced maximum levels of activation in 100% of spermatozoa for 3 min. When lower concentrations of NaCl were used (175 mM to 31.2 mM) spermatozoa were activated after 50 seconds and motility did not reach the maximal levels. Hiperosmotic solutions containing a mixture of differents sea salts without NaCl (PO = 560 and 832) were able to activate spermatozoa, but the maximal motility was seen only when NaCl solutions (PO = 478 and 930) were used. Different concentrations of KCl, CaCl2 or MgCl2 had no effect on sperm activation neither in the presence of NaCl nor in it absence. Buffer Tris solutions at different pH (4.8 to 9.9) did not activate spermatozoa. When NaCl (250 mM) were added, pH 8.6 and pH 9.9 induced the maximal activation, while pH 6 and 5 were negative for sperm motility. These results suggest that in this estuarine fish hiperosmotic solutions induces the sperm motility like in marine fish. The high concentrations of Na+ associated with high pH are the best conditions to activate spermatozoa of white croaker.

PI-81

CRYOPRESERVATION, MOTILITY AND ULTRASTRUCTURAL CHANGES OF SPERM FROM THE OCEAN POUT (Macrozoarces americanus L.), AN INTERNALLY FERTILIZING MARINE TELEOST

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Male ocean pout, a marine teleost in Northwest Atlantic Ocean, produces mammalian-like sperm; sperm had a long midpiece and tail and were motile in male and female reproductive tracts but were instantly immobilized by seawater. In this study, sperm were extended in diluent containing various cryoprotectants and immediately frozen in liquid N_2 for storage. Three diluents and four cryoprotectants (dimethyl sulfoxide, dimethyl acetamide, glycerol and 1,2-propane diol) were tested and the results showed that cryoprotectant in extender was essential for protecting post-thaw sperm motility and semen extended in a diluent containing a similar electrolyte composition to that of seminal plasma as well as 20% dimethyl sulfoxide produced the highest post-thaw motility. Effects of freezing and thawing rates, dilution ratio and the presence or absence of seminal plasma proteins in diluent on sperm motility were also examined. Sperm motility dropped considerably after freeze and thaw due to changes in sperm midpiece, where the plasma membrane shrank leading to the exposure of mitochondria.

PI-82

SUSTAINED ADMINISTRATION OF GnRHa INCREASES SPERM VOLUME WITHOUT ALTERING SPERM COUNTS IN THE SEA BASS (*DICENTRARCHUS LABRAX*)

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Although gonadotropin-releasing hormone (GnRH) regulation of female reproduction has been widely investigated, the role of GnRH and its analogs in spermiogenesis is not well understood. This study examined the effects of different modes of GnRHa ([D-Ala^6,Pro^{9}NEt]-LHRH) administration on spermiogenesis in the sea bass, *Dicentrarchus labrax*. Groups of male sea bass received either a GnRHa injection in saline (inj; 25 µg/Kg BW), or one of three different types of GnRHa sustained release polymeric devices: a faster releasing implant (EVAc; 100 µg/fish), a slower releasing implant (EVSL; 100 µg/fish) or biodegradable microspheres (mcs; 50 µg/Kg). Total expressible sperm was collected and assessed for volume, motility and counts (sperm/ml) at varying intervals for 35 days. Untreated control males produced an average of 2.8 ± 0.5 ml of milt for 21 days, with milt production ending by day 28. All GnRHa treatments induced a significant increase in milt volume, peaking by day 2 (inj: 7.3 ± 1.3 ml; EVAc: 8.9 ± 1.5 ml; EVSL: 10.1 ± 2.0) or day 7 (mcs: 8.8 ± 1.8 ml) post-treatment. However, while sperm volumes in the injected males returned to control values by day 14, in all groups treated with sustained GnRHa delivery systems, sperm volumes remained significantly elevated for 28 days (EVAc group) or 35 days (EVSL and mcs groups). Sperm motility was consistently good (70-80% vigorously active) to excellent (90-100%) in all groups when sperm volume was above 1.0 ml. There were no significant differences in sperm counts (averaging $5.5x10^9 \pm 1.2x10^8$) between the different groups. These data show that sustained administration of GnRHa significantly increases and prolongs spermiogenesis in the sea bass without altering sperm quality or concentration.

PI-83

SEX PHEROMONE-ENHANCEMENT OF FERTILITY IN MALE CYPRINIDS: APPLICATION IN GOLDFISH AND CARP

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In cultured cyprinids, milt from untreated stock males is often of poor quality or low volume; culturists routinely deal with this problem by treating stock males with exogenous hormones. However, such treatment may be costly, and requires that the fish be caught, handled and injected individually. Recent studies suggest that sex pheromones provide a non-invasive alternative to hormone-induced spermiation. In both common carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*), water-borne 17α,20ß-dihydroxy-4-pregnen-3-one (17,20ß-P) increases strippable milt volume and spermatocrit, and in goldfish increases sperm motility. Here we show that exposure to 17,20ß-P increases fertilization rates during spawning in goldfish, and present the results of studies that investigate the frequency with which milt may be stripped from common carp using repeated stimulation by 17,20ß-P. Finally, we present data from electro-olfactogram recordings suggesting that similar sex pheromones may be exploited to stimulate spermiation in other commercially-important carp species. These data indicate that hormonal pheromones may be used to increase both the quantity and quality of milt in cultured cyprinids without resorting to direct hormonal intervention.

PI-84

THE EFFECT OF PHOTOPERIOD ON VITELLEGENIN SYNTHESIS AND OOCYTE ENDOCYTOSIS IN RAINBOW TROUT (*Oncorhynchus mikiss*)

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Two groups of a precocious winter strain of rainbow trout were taken from the same population at the peak of first spawning. Six weeks later, the two groups were submitted to different photoperiod conditions, one natural and one artificial. Whereas the natural photoperiod induced spawning in early winter, 12 months after first spawning, the artificial photoperiod shortened the sexual cycle, and spawning occured in late summer, at only 9 months.
The increase in oocyte diameter, reflecting vitellogenin accumulation, was faster in the artificial photoperiod group only during the slow development phase. Equivalent diameters to the natural group were achieved at spawning.
Six females of each group, with mean oocyte diameters of 3.5mm at the beginning of the rapid development phase, were taken from each group, and yolk endocytosis was compared by studying the binding of vitellegenin to its specific receptors. The resulting Scatchard plots show the association coefficient (Ka) for the shortened cycle group to be more than twice as small as for the natural group. On the contrary, the total binding, expressed in femtomoles of vitellogenin per mm2 of oocyte surface, is over twice that of the natural group.

PI-85

EFFECTS OF AROMATASE INHIBITORS ON SEXUAL MATURATION IN THREE-SPINED STICKLEBACK, *GASTEROSTEUS ACULEATUS*.

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Aromatization of androgens to estrogens is very active in the teleost brain, it has been implicated to be important for feedback mechanisms on the hypothalamus-pituitary-gonadal axis. The aim of the present study was to find out if it is involved in the photoperiodic control of breeding. Non-breeding males were caught in December and implanted with Silastic capsules containing two aromatase inhibitors 1,4,6-androstatriene-3,17-dione or CGS16949 A, 4-benzonitrile monohydrochloride or empty capsules. The fish were exposed to Light:Dark (LD) 8:16h or 16:8h at 18° C. After six weeks the fish were dissected. The kidney of the male stickleback hypertrophies in the breeding season under androgen stimulation and produces a "glue" which is used in the building of the nest. Kidney hypertrophy was used as criterion for maturity. Control fish matured in LD 16:8 but not in LD 8:16, whereas fish treated with both aromatase inhibitors matured also in LD 8:16. The results suggest that aromatization is involved in the suppression of maturation under short photoperiod. One possibility for its involvement could be by influencing feedback mechanisms at the hypothalamus and/or pituitary level.

PI-86

DOES CORTISOL INFLUENCE EGG QUALITY IN THE RAINBOW TROUT, *ONCORHYNCHUS MYKISS*?

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Stress has been shown to have deleterious effects on ovarian function in many animals. In the rainbow trout stress influences both oocyte development and the viability of the eggs produced. A major component of the stress response is elevation of plasma cortisol levels. Cortisol has adverse effects on many physiological processes, including reproduction, although the specific actions of cortisol on the reproductive system have yet to be fully determined. Although egg quality ('quality' defined as survival to hatching) is reduced in stressed fish, it is not known to what extent both levels of cortisol in females prior to ovulation and the concentration in ovulated eggs influence egg survival. Female rainbow trout, 6 weeks before ovulation received one of three treatments; Group 1 - controls, Group 2 - vehicle implant only, Group 3 - cortisol implants. The eggs produced were fertilised with a single pool of milt and one half dosed with 500ug/l cortisol for 2 hours. Cortisol levels in the plasma and the eggs were measured by RIA. Implantation of fish with cortisol raised both plasma levels and concentrations of cortisol in the eggs. Dosing of ovulated eggs with cortisol caused a 30 fold increase in egg cortisol. Survival to hatching data indicated that cortisol does not directly affect egg quality.

PI-87

THE EFFECT OF SPAWNING TEMPERATURE ON EGG VIABILITY IN THE ATLANTIC HALIBUT, (HIPPOGLOSSUS HIPPOGLOSSUS).

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Water temperature is known to be an important environmental parameter influencing reproduction in fish. The Atlantic halibut inhabits deep waters characterised by constant physical environmental conditions including low, relatively stable temperature. The aim of this work was to determine whether temperature control through water chilling is a necessary feature of halibut broodstock management in Scotland, at the southern extreme of its distribution. The effect of controlled versus ambient temperature on the performance of a halibut broodstock was assessed over two spawning seasons in terms of egg morphology and viability. Results showed that as the season progressed, the quality of eggs from females held at ambient temperature deteriorated as the temperature increased during the spring. In addition, the proportion of eggs exhibiting normal cleavage patterns and the hatching rates both decreased with time. No such changes were observed in the eggs from females held at a controlled temperature of 6°C. It was concluded that a constant water temperature of 6°C is necessary for a consistent production of viable halibut eggs in Scotland. Work funded by Trouw Aquaculture.

PI-88

LINKS BETWEEN NUTRITION AND REPRODUCTION IN FISH

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Evidence already amassed shows that some fish will not enter a reproductive cycle if nutritionally compromised during a critical period. This implies that the fish are responding to intrinsic signals which reflect either negative nutritional status and a consequent halting or non-start of reproduction, or a positive nutritional state enabling gametogenesis to either start or continue. Analysis of plasma samples from winter flounder of different nutritional status close to the critical period shows at least two highly significant differences including a potential direct excitatory signal to the hypothalamic-hypophyseal-gonadal (HHG) axis. Preliminary localization studies within the HHG axis have shown highly specific responses, not at the hypothalamic level, as originally expected, but within the pituitary.

PI-89

EFFECT OF STRESS ON THE REPRODUCTIVE PHYSIOLOGY OF RAINBOW TROUT, *Onchorhynchus mykiss*..

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Despite the need for high quality gametes in aquaculture, the environment under which the broodstock are maintained before reproduction is often stressful, but the impact of stress on broodstock and progeny is not well known. We designed experiments where stress was administered over the period of early vitellogenesis, late vitellogenesis-final maturation, and during both periods. Fish that experienced stress during final maturation, and those that were under stress during the whole experiment spawned earlier than the control group. In contrast, fish stressed during the period of early vitellogenesis spawned at the same time as the controls. No significant differences were found in percent of fertilization for the four groups studied (p-value = 0.53). Preliminary results suggest significant diffences among stress and control groups in sac fry weight. Future analyses will include fecundity, egg size, cortisol levels in eggs and embryos, survival, and growth.

PI-90

Density-dependent inhibition of spawning in the substrate spawning cichlid *Tilapia tholloni* (Sauvage).

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Spawning periodicity in the multiple spawning tilapias has been reported to be dependant upon various factors commonly including latitude, temperature, photoperiod, salinity and food ration. Whilst it is commonly accepted that stocking density and sex ratio of tilapiine broodfish under farming conditions may further exert particular influences on subsequent breeding intensity, comparatively little is known of the quantitative endocrine and ovarian physiology underlying such observations. The findings of the present study involving *T. tholloni* suggest that whilst under even 'semi-crowded' holding conditions, levels of the circulating sex steroids testosterone and 17ß-oestradiol appear suppressed and exhibit little change during the period of confinement, levels of both steroids rise swiftly following transfer to individually partitioned aquaria and are maintained at much higher levels than those of a control group remaining under crowded conditions. Moreover, spawning was frequently found to occur within several days of transfer to individual conditions. Samples of ovarian tissue from both control and experimental groups of fish experiencing an experimental regime involving alternate 30 day periods of confinement and individuality were taken by serial ovarian biopsy and processed for histological comparison. Quantitative dynamics of ovarian histology in both groups of fish were assessed and compared using stereological analysis.

PI-91

Off-season maturation and factors influencing gamete production in yellow perch (Perca flavescens). K. Dabrowski[1], R.E. Ciereszko[1,4], A. Ciereszko[1,5], G.P. Toth[3], S.A. Christ[3] and J. Ottobre[2]. [1]School of Natural Resources, [2]Department of Animal Sciences, Ohio State University, Columbus, OH, U.S.A., [3]U.S. Environmental Protection Agency, Cincinnati, OH, U.S.A., [4]Agriculture University, Olsztyn, Poland, [5]Polish Academy of Sciences, Olsztyn, Poland.

Two-year old yellow perch (25.2 ± 5.4 g, males; 36.8 ± 3.8 g, females) were transferred in October from natural conditions into four different photothermal regimes. Two groups were maintained on a long-day cycle (14 h) until January (LL=long light) and then day light decreased. The two other groups experienced decreasing photoperiod until January down to 10h light (SL=short light), after which photoperiod gradually increased. One of each "photoperiod" manipulated subgroups was exposed to normal thermal conditions of decreasing water temperature from 20 to 10°C in January (No - normal), whereas the other was exposed to a prolonged period of summer temperature (20°C) and the decrease begun in January (Ma - manipulated). Four respective treatments are marked as LL-No, LL-Ma, SL-No and SL-Ma. We observed significant differences in the females gonadosomatic indices (GSI, % body weight) in February. SL-No and LL-No fish advanced in gonad development (GSI 18.3 ± 0.9 and 16.4 ± 0.5%, respectively) while SL-Ma and LL-Ma fish experienced arrested gonad growth (GSI 8.6 ± 1.8 and 4.6 ± 0.6%). GSI in LL-Ma males decreased of 7.9 ± 0.7% in October to 0.16 ± 0.03% in January, whereas males from all other groups had GSI higher than 2.06% and continued to spermiate. Preliminary results of a computer assisted sperm motion analysis (CASA) in November revealed that peak motility for group SL-No (18.7%) was lower than that for groups SL-Ma and LL-No, 25.9% and 38.1%, respectively, after activation in 50 mM NaCl, 10 mM HEPES, pH 8.0. The kinetics of activation over the first 17 s also differed for males from group SL-No. This research does not necessarily reflect USEPA policy.

PI-92

THE EFFECTS OF PHOTOPERIOD AND TEMPERATURE ON SERUM GTH I, GTH II AND THE TIMING OF MATURATION IN THE FEMALE RAINBOW TROUT.

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Four groups of 2 year old, post-spawned, female rainbow trout were exposed to ambient (56°N) or constant long and then short (18L:6D February 1st-May 10th, then 6L:18D) photoperiod together with constant (8.5±1°C) or seasonally-fluctuating temperatures (0-21°C). The two groups exposed to the stimulatory long-short photoperiod showed significant 4 month advances in spawning compared to the groups on ambient photoperiod. Egg viability was comparable for all four groups. Exposure to the constant water temperature resulted in a small 3-4 week advance in spawning compared to groups exposed to the seasonally fluctuating water temperature. For the advanced groups serum levels of GTH I fell rapidly during February-March and then increased to peak in May; levels fell in the period preceding and during spawning but rose immediately afterwards. Such large changes in GTH I levels were not seen in the groups kept on ambient photoperiod although small fluctuations did occur. Serum GTH II was only detectable close to and during spawning for all four groups. Serum testosterone, 17ß-oestradiol and calcium (as an indicator of vitellogenin levels) also showed peaks associated with the times of vitellogenesis and spawning for each group.
(Funded by grants from Scot Trout and BP Nutrition (UK) Ltd. to NB.)

PI-93

GROWTH, GONADAL DEVELOPMENT AND SPAWNING TIME OF ATLANTIC COD (*GADUS MORHUA*) REARED UNDER DIFFERENT PHOTOPERIODS

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Atlantic cod (*Gadus morhua*) were reared for 36 months under different photoperiod regimes. Cod reared under natural photoperiod spawned in the period between January and April at an age of two years. Oocytes of females reared under continuous light were arrested in the cortical alveoli stage. When transferred to a natural photoperiod, females ovulated within 4-5 months.

Two year old cod that spawned three months delayed (May-July) spawned again at their normal spawning time (as three year olds) when reared under a natural photoperiod. A few of the females reared under continuous light ovulated at an age of three years.

Sexual maturation reduced feed intake and growth of the cod. At an age of 26 months the weight of the cod reared under natural photoperiod and continuous light were 1.5 and 2.5 kg respectively.

PI-94

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DEPRESSION OF BLOOD LEVELS OF REPRODUCTIVE STEROID GLUCURONIDES IN MALE WINTER FLOUNDER EXPOSED TO SMALL QUANTITIES OF HIBERNIA CRUDE, CRANKCASE OIL, OILY DRILLING MUD AND HARBOUR BOTTOM SEDIMENTS IN THE FEW MONTHS PRIOR TO SPAWNING.

Crude oil was added at 5 levels (5-500 ml) to 45 kg of clean sand. Oiled sand was washed for one week before introducing flounder and flowing seawater was maintained.

Plasma levels of the principal male sex steroid, 11-ketotestosterone glucuronide, were greatly decreased following 4-month exposure to the oil fractions. For example, reduction was 72% using Hibernia crude and 40% with crankcase oil. Testosterone glucuronide was also reduced. It has been shown by others that androgen glucuronides can function as pheromones in fish so their reduction may affect spawning behaviour. Flounder exposed to St. John's harbour sediments suspended in sand gave a significant reduction in plasma glucuronide levels of 11-ketotestosterone and testosterone. It is of interest that the sensitivity of the response to steroids occurred at levels below those which produced a change in mixed function oxidase. There was a reduction of 38% in UDP-glucuronyl transferase enzyme in testes of fish exposed to harbour sediments. The possible mechanisms will be discussed.

PI-95

EFFECTS OF ENDOCRINE-DISRUPTING CHEMICALS ON MARINE FLATFISH REPRODUCTION: AN APPROACH TO ENVIRONMENTAL RISK ASSESSMENT

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In field studies conducted on the Northwest and Northeast coasts of the United States, we have investigated the impact of chemical contaminants on reproductive function in several Pleuronectid species. Important research goals include: 1) identification of mechanisms through which contaminants disrupt reproductive processes in marine fish; 2) development of reproductive biomarkers that can be measured in field studies to serve as early indicators of reproductive dysfunction; and 3) utilization of field data to evaluate potential impacts of reproductive dysfunction on fish abundance. We have found that exposure to and uptake of chemical contaminants such as aromatic and chlorinated hydrocarbons (AHs and CHs) is associated with several types of reproductive dysfunction in female fish, including inhibited ovarian development, altered endocrine function, and reduced spawning success, and are now examining the effects of endocrine-disrupting chemicals, especially environmental estrogens, on reproductive function is male fish. From these field data we are developing dose-response models to aid in identification of threshold sediment or tissue contaminant levels where signs of reproductive pathology initially occur, and have incorporated the data into population models to predict impacts on fish abundance. Preliminary results with English sole suggest that contaminant-related declines in reproductive output may have the potential to reduce the growth rate of English sole subpopulations in urban areas. This integrated multidisciplinary approach has enabled us to examine effects of endocrine-disrupting chemicals on marine fish not only at the biochemical or individual level, but at the population level as well, and to make progress toward developing methodologies for assessing the environmental risk of endocrine-disrupting chemicals.

PI-96

THE HORMONE MIMIC ß-SITOSTEROL ALTERS REPRODUCTIVE STATUS IN GOLDFISH.

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Recent evidence suggests that hormone mimics in the environment can alter the reproductive status of both wildlife and humans. Since ß-sitosterol (ß-sit) is a primary plant sterol in bleached kraft mill effluent (BKME) from pulp and paper mills, it might contribute to some of the reproductive changes observed in wild fish exposed to BKME. Male goldfish were exposed in the laboratory for 12 days to environmentally-relevant ß-sit concentrations (75, 300, 600 and 1200μg/L). ß-Sit at all doses decreased plasma levels of testosterone (T) and 11-ketotestosterone. This effect appears to be mediated at the gonad level, as *in vitro* T and pregnenolone (preg) production were decreased in testes from treated fish, while plasma GtH II levels were not significantly changed by treatment. ß-Sit might reduce the activity of the sidechain cleavage enzyme P_{450}, and/or steroid biosynthesis due to cholesterol availability, as treated fish have reduced gonadal cholesterol levels and *in vitro* 25-OH cholesterol addition to testicular pieces causes steroid production equal to/greater than control fish. In addition, ß-sit might be functioning as an estrogen mimic, as it it binds to goldfish hepatic estrogen receptors and increases plasma vitellogenin levels. This study demonstrates that ß-sit is potentially responsible for some of the reproductive dysfunctions in fish exposed to BKME.

PI-97

TRANSPORT AND OVARIAN ACCUMULATION OF o,p'-DDT IN THE ATLANTIC CROAKER (*MICROPOGONIAS UNDULATUS*) DURING GONADAL RECRUDESCENCE

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To ascertain the role of plasma lipoproteins, including vitellogenin, in the transport of xenobiotics, Atlantic croaker (*Micropogonias undulatus*) were exposed through the diet to o,p'-DDT at concentrations of 1.8, 10.8 and 54.6 μg/100g fish/ day for 14-77 days during gonadal recrudescence. Tissue samples were taken from the fish after 2, 3, and 11 weeks of exposure, and o,p'-DDT was extracted from the tissues by sonication in acetonitrile and analyzed by gas chromatography. Analysis of the ovarian tissue collected 2 and 3 weeks from the start of exposure revealed that the o,p'-DDT concentration increases as the gonadosomatic index (GSI) increases (r^2=0.85), with accumulation ranging from less than 1% to as much as 8% of the total dosage. Interestingly, o,p'-DDT did not accumulate in the testes during the same exposure period. Gel filtration of the female plasma showed that most of the o,p'-DDT elutes in the VLDL (very low density lipoprotein) fraction with a small amount in the vitellogenin fraction. Density ultracentrifugation of the plasma from dosed female fish revealed that 35% of the o,p'-DDT is carried in the VLDL fraction, while only 6% is carried by the vitellogenin fraction. Furthermore, uptake of o,p'-DDT by the ovary is six times greater when injected as an o,p'-DDT/VLDL complex rather than as o,p'-DDT alone. Our results show that VLDL is an important route of transport for the accumulation of xenobiotics in the ovaries of exposed fish.

OR-24

TEMPERATURE MANIPULATION OF SEX DIFFERENTIATION IN FISH

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Environmental factors define the reproductive strategies and the output of fish reproductive activity by setting the timing and the amount of energy available for reproduction. In a number of species, environmental factors also influence the relative contribution of each sex for the reproductive processes by temporally or permanently inducing functional sex-change (environmental sex determination, ESD). This paper reviews the effects of the thermal environment on the primary sex differentiation of fish, with special regard to the evidences of thermolabile sex determination (TSD, a form of ESD). TSD is common among Atherinids, and temperature effects on gonadogenesis in this group range from functional sex change to virtual castration. Within this group, exposure to extreme (sub-lethal) temperatures seems to allow sex manipulation even in species with otherwise strictly genetic sex determination (GSD). In other taxa, including several economically important species, there are instances in which phenotypical (gonadal) sex appears at least partly modulated by environmental temperature. This suggests that the sensitivity of sex determination mechanisms to environmental modulation in fishes may be more widespread than presently believed. Thermal manipulation of sex holds promising prospects for the control of fish sex in aquaculture.

OR-25

TEMPERATURE SEX DETERMINATION IN TWO TILAPIAS SPECIES, *OREOCHROMIS NILOTICUS* AND THE RED TILAPIA (RED FLORIDA STRAIN): EFFECT OF HIGH OR LOW TEMPERATURES.

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Very few studies have evaluated the role of external factors on sex determination in gonochoric fish species. In tilapia, our recent results clearly demonstrate that high temperature has a strong effect on *Oreochromis niloticus* sex-ratio. In reptiles and amphibians, 3 different patterns of temperature sex determination (TSD) have been described depending on the sex produced at high and low temperature. In order to define this pattern in tilapia, the effects of low and high temperatures on *Oreochromis niloticus* sex-ratio have been compared in the present study using classic or genetically female progenies. High temperatures (30-36°C) significantly increased the proportion of males (extreme percentages = 69-91% males) but low temperatures (19-20°C) do not affect the sex-ratio. As tilapia, according to genetic studies, can be divided in male (MH) or female heterogametic (FH) species, we also investigate TSD in the red tilapia from the Red Florida strain (considered as a gene pool of 2 MH and 2 FH species). In this red tilapia, 36°C treatments also significantly increased the male percentage in most progenies (extreme percentages = 60-97%). These results suggest that TSD could exist in several tilapia species, with strong genotype-temperature interactions. The pattern of this TSD resembles that of the amphibian *Pleurodeles waltl*.

OR-26

SALMON GnRH GENE EXPRESSION FOLLOWING PHOTOPERIOD MANIPULATION IN PRECOCIOUS MALE MASU SALMON

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The relationship between testicular maturation and salmon gonadotropin-releasing hormone (sGnRH) mRNA expression was investigated in underyearling precocious male masu salmon, *Oncorhynchus masou*. Testicular maturation could be manipulated experimentally by changing the length of the light-dark photoperiod; maturation was accelerated in the short photoperiod group (8L16D) and delayed in the long photoperiod group (16L8D). sGnRH synthetic activity in the preoptic area and the ventral telencephalon, i.e., the number of neurons expressing sGnRH mRNA and total silver grains in these loci in individual fish, increased with advancing testicular maturation. They were maximal in the short photoperiod group in August, and in the long photoperiod group in September, when spermiation was occurring. In contrast, marked changes in sGnRH synthetic activity in relation to testicular maturation were not observed in the terminal nerve ganglion and olfactory bulbs. sGnRH neurons in the preoptic area and the ventral telencephalon were clearly influenced by photoperiod, and are thus likely involved in the control of gonadal maturation probably via gondotropin secretion.

OR-27

HOW DO PHOTOPERIOD, THE PINEAL GLAND, MELATONIN, AND CIRCANNUAL RHYTHMS INTERACT TO CO-ORDINATE SEASONAL REPRODUCTION IN SALMONIDS?

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Although it is now beyond doubt that photoperiod coordinates the annual cycle of reproduction in certain salmonids the mechanisms underlying many aspects of the photoperiodic control of reproduction remain unresolved. This paper describes recent experiments designed to test the hypothesis that reproduction is ultimately controlled by an endogenous circannual clock, and that the photoperiodic entrainment of this clock is mediated by the pineal gland, either via a neural connection to the brain, or via release of melatonin. As in mammals in which melatonin has been shown to mediate the effects of photoperiod on the timing of reproduction, salmonids exhibit diel changes in circulating melatonin under both natural and artificial photoperiods. Melatonin levels are low during the day and elevated for the duration of the night, thus providing an accurate representation of the prevailing daylength. Results from experiments where fish were exposed to 'solstice-hold' and phase-shifted simulated seasonal photocycles show that, irrespective of time of year or temperature, the duration of the night-time increase in melatonin is determined by photoperiod and cannot be attributed to endogenous circannual variations in the production of this hormone. Despite melatonin rhythms providing such an accurate portrayal of the prevailing daylength it is not clear whether they are used to time daily and seasonal events in salmonids. The potential of the pineal to transmit photoperiodic information, by either neural or endocrine pathways, and to entrain the circannual clock controlling reproduction, will be discussed in relation to experiments in which fish were maintained under natural or artificial photoperiods and either pinealectomized or administered constant-release melatonin implants at different times of the year. (This work was supported by the NERC)

OR-28

PHOTOPERIOD CONTROLS THE TIMING OF REPRODUCTION IN ATLANTIC COD

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The Atlantic cod, *Gadus morhua*, is one of the commercially most important coldwater marine teleosts. For an efficient cultivation, a stable, year-round production of gametes is necessary. The objective of this study was to investigate the impact of photoperiod manipulation on reproductive development in female Atlantic cod. Immature Atlantic cod were divided into 4 groups and exposed to compressed annual photoperiod cycles of 6 (group 1) and 9 (group 2) months, respectively, extended 18 month annual cycle (group 3) and a 12-month annual cycle (group 4, control group). In all groups, these cycles were followed by a simulated natural photoperiod, which was thus phase-shifted 3 or 6 months in relation to the normal photoperiod. After completion of the experimental treatment, spawning was advanced by 6 months in group 1, by 2 months in group 2 and delayed by 6 months in group 3. Peak plasma levels of estradiol-17β and testosterone occurred immediately prior to or during spawning. In conclusion, the results show that photoperiod controls the timing of reproduction in the Atlantic cod, evidenced by plasma sex steroid levels and spawning activity. Photoperiod manipulation is a powerful tool for controlling reproductive development in this species.

OR-29

EFFECTS OF WATER TEMPERATURE IN SPRING ON SEXUAL MATURATION IN MALE ATLANTIC SALMON (*SALMO SALAR* L.) PARR.

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To date, experimental studies on the correlation between spring growth and the probability of sexual maturation in male Atlantic salmon (*Salmo salar* L.) parr have focused on the effect of food restriction. The present study reports on the effect of the rate of temperature increase in spring on the proportion of mature male parr the following autumn. Experimental groups of one-year-old Atlantic salmon parr, fed at excess, were subjected to different temperature regimes from mid May until late June, a period during which the ambient water temperature normally increases from ca 5°C to 16°C. In temperature regimes with a temperature of ca. 11°C and 16°C in late June the proportions of mature males were 12% and 47 % in 1993, and 5% and 37% in 1994, respectively. These effects on maturation rates are much larger than effects that have been reported in studies on the effect of food restriction. If maturation rates are plotted against corresponding growth rates it stands out clearly that maturation rate declines more rapidly with decreasing growth rate if temperature is controlled than if food ration is manipulated. Water temperature in spring might be a more reliable predictor of summer growth than spring growth rate *per se*.

OR-30

WHAT IS THE SIGNIFICANCE OF SUBTLE CHANGES IN THE REPRODUCTIVE PERFORMANCE IN RESPONSE TO ENVIRONMENTAL DISTURBANCES?

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Assessment of the reproductive performance of fish is increasingly being used to evaluate the impacts of environmental disturbances. Reproductive physiologists are being called upon to provide quick answers to questions related to the identity and actions of compounds in complex mixtures and the effectiveness of chemical process changes and remedial treatment regimes. Consequently, there is a real need to establish whether effects on the molecular and cellular events which mediate reproductive processes are predictive of significant whole animal and population level effects. Numerous in vitro (receptor binding, steroid production, vitellogenin production) and short term whole animal tests (plasma hormone levels; functional testing of the HPG axis) are available to assess responses of fish to xenobiotic chemicals and altered environmental conditions (e.g. thermal regimes). However, little is known of whether these responses translate to effects at the whole organism level and often less is known of population consequences. At this time, there are no surrogates for long term experiments although physiological testing of wild fish populations is providing a means of focusing attention to key components of the reproductive axis sensitive to environmental disturbance.

OR-31

A LONG TERM STUDY OF THE EFFECTS OF POLLUTED SEDIMENTS ON THE ANNUAL REPRODUCTIVE CYCLE OF THE FEMALE FLOUNDER, *Platichthys flesus*.

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The euryhaline flatfish *Platichthys flesus* inhabits coastal/estuarine waters and therefore lends itself for monitoring organic chemical pollutants. Compounds such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls and pesticides are easily incorporated and often metabolized by enzyme systems which are involved in steroid metabolism. Disturbanced reproduction may therefore occur because these compounds interfere with the endocrine system.

Fish were kept for at least three years in mesocosm systems which contained polluted sediment from the Rotterdam harbour and than sampled twice a year in May and November. The gonadal morphology and steroidogenesis was studied. Moreover the plasma levels of estradiol and vitellogenin was determined.

The November animals all contain ovaries with vitellogenic oocytes, comparable with those captured in the wild. In May, however, all the control animals were in the previtellogenic phase while the ovaries of the "polluted" animals contain not only previtellogenic oocytes large numbers of vitellogenic oocytes. Although the ovarian capacity to synthesize estrogens is more or less the same in both groups, the levels of estradiol and vitellogenin were elevated in "polluted" females. In "polluted" males, however, no trace of estradiol or vitellogenin could be detected. This indicates that a direct estrogenic effect of the polutants can be excluded. On the other hand compounds in the polluted environment may influence estradiol metabolism resulting in an early induction of vitellogenesis.

OR-32

THE REPRODUCTIVE ENDOCRINOLOGY OF ELASMOBRANCH OVIPARITY AND VIVIPARITY

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Although systematic and evolutionary relationships of chondrichthyan fishes remain unsettled and controversial, it is probable that they have been in existence for well over 400 million years. The fossil record suggests that the modern radiation of elasmobranchs began as early as the late Carboniferous, about 300 million years before present (mybp). Elasmobranchs as a group reached an evolutionarily stable condition in balance with the environment many millions of years ago, and some extant species have existed for up to 200 million years. Dating of reproductive adaptations may therefore be extended back in time to suggest that elasmobranchs experimented with a variety of reproductive adaptations antedating similar parallel developments in more recently evolved vertebrate groups. Using both oviparous and viviparous reproductive modes, elasmobranchs have adopted the strategy of giving birth to relatively few young at one time, each representing the investment of a great deal of maternal energy. The oviparous species foreshadow the situation common in oviparous reptiles and universal in birds. Conversely, viviparous species range from internal incubators, in which large yolked eggs are retained, to species in which the complexity of placentation and egg yolk reduction approach the eutherian condition. In elasmobranchs, histiotrophic nutrition attains greater importance and complexity than in other vertebrates. However, much remains to be discovered about the control of reproductive tract development, growth, differentiation, and function to protect minimal populations of these extraordinary animals.

OR-33

NEUROENDOCRINE CONTROL OF REPRODUCTION IN LAMPREYS

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This paper summarizes the recent studies in my laboratory on the structure and function of lamprey gonadotropin-releasing hormone (GnRH) in sea lampreys, *Petromyzon marinus*. A key neuroendocrine function of the hypothalamus is the release of GnRH which in turn acts on the pituitary regulating the pituitary-gonadal axis for all vertebrates. Lampreys are the first vertebrates to clearly demonstrate roles for multiple GnRH molecules as neurohormones involved in reproductive activity. Both lamprey GnRH-I and -III have been shown to induce steroidogenesis and spermiation and/or ovulation in adult sea lampreys. In lampreys undergoing metamorphosis, there was a demonstrated increase of brain lamprey GnRH-I and -III which coincided with the acceleration of gonadal maturation. In immunocytochemistry studies, both lamprey GnRH-I and -III immunoreaction were found in the cell bodies in the rostral hypothalamus and preoptic area in larval and adult sea lamprey. We have suggested that in the larval stage, the majority of irGnRH is lamprey GnRH-III indicating that GnRH-III perhaps is the more active form during reproductive maturation. In the lamprey, the neurohypophysis and adenohypophysis are separated by avascular connective tissue. From our most recent experiments, we provide further evidence that GnRH can diffuse from the neurohypophysis to the adenohypophysis and thus regulate its secretory activity. Lamprey GnRH-I and -III are the only two members of the GnRH family to have substitutions in the sixth position suggesting a different conformational structure. We suggest from structure-activity and receptor studies that lamprey GnRH receptor requirements for GnRH are different in the lamprey from those of all other vertebrates. (Supported by NSF IBN-8904919,-9022834,-9407767 and the Great Lakes Fisheries Commission)

OR-34

DIURNAL RHYTHM IN TESTICULAR ACTIVITY IN THE SECONDARY MALE OF A PROTOGYNOUS WRASSE, *Pseudolabrus japonicus*

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Under the captive condition (one male and two females in a 200 *l* tank), the secondary male of a protogynous wrasse *Pseudolabrus japonicus* spawned daily over one month between 6:00 and 9:00 from October to November. Sperm may be produced and released daily or males may store sperm for future release. In other word, males may exhibit a diurnal rhythm of spermatogenesis and spermiation. To answer this question, we performed the time course study on germ cell composition in the testes of the secondary males captured in the field in a day during the spawning season. Results obtained indicated that spermatogonial proliferation and meiosis occurred bertween 0:00 and 15:00 and spermiation (release of spermatozoa into the lobular lumen from the cysts) occurred between 18:00 and 6:00. The serum level of 11-KT showed a profile corresponding to that of B-type spermatogonium and spermatocyte number. Thus, this study demonstrates that the secondary male of *P. japonicus* has a diurnal rhythm in (1) spermatogenesis and spermiation, and (2) 11-KT production.

OR-35

A COMPARATIVE STUDY OF THE VITELLOGENESIS DYNAMIC AND REPRODUCTIVE ECOLOGY IN SINGLE AND MULTISPAWNER CYPRINIDS

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Dynamics of vitellogenesis correlated with oocyte growth and seasonal variations in hepatic activity has been compared in a single-spawner (the roach *Rutilus rutilus*) and three multi-spawner cyprinids (the white bream *Blicca bjoerkna*, the bleak *Alburnus alburnus* and the gudgeon *Gobio gobio*) collected in the river Meuse basin (Belgium). Just before the spawning season, the vitellogenin levels (expressed by alkali labile phosphoprotein phosphorus) and the hepatic activity (hepatosomatic index, abundance and status of mitochondria and rough endoplasmic reticulum) decreased markedly in roach. Different patterns of gonadosomatic index (GSI), oocyte growth, vitellogenin and estradiol levels, and hepatic activity have been observed in the three multi-spawner cyprinids. Compared to the rapid decline of GSI in the roach population, the GSI of the multispawners decreased progressively during the spawning season. However, the different parameters involved in the vitellogenesis process and oocyte growth indicated that the reproductive strategies among the multispawner species were quite different. In the white bream gonadal development was mainly oriented to the first batch of ova, the ovaries being fully mature and vitellogenesis markedly reduced at the onset of the spawning period whereas in the bleak and in the gudgeon vitellogenic activity remained high throughout the spawning season. These results are discussed in term of reproductive ecology of the mono and multi-spawner fish.

OR-36

PLASTICITY OF SEXUAL EXPRESSION IN AN AFRICAN CICHLID

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While sex change appears to be rare in freshwater teleosts, other forms of labile sexual development have been documented (reviewed in Francis 1992). Moreover, there is no *a priori* reason to expect labile sexual development, up to and including sex change to be confined to marine species. We attempted to determine whether the cichlid, *Haplochromis burtoni*, a highly polygynous species which, according to the size-advantage model, should be a protogynous hermaphrodite, would change sex under the appropriate circumstances. We first investigated whether, as in the new world cichlid, *Cichlasoma citrinellum*, primary sex could be influenced by social factors. From the day they became free-swimming, individuals were reared either in isolation, pairs of groups for 20 weeks, at which age they could be reliably sexed. The proportion of females was significantly greater in isolated and group-reared fish than in fish reared in pairs. Among fish reared in pairs, there were more heterosex pairs than would be expected by chance. We then placed young females with broods together in tanks without males. Repeated trials failed to induce sex change. However, in each case, one or two individuals would immediately adopt male coloration, including the black eyebar. These fish would also exhibit "pseudomale" behavior, defending a territory and courting other females, even inducing these females to spawn and begin mouth-brooding.

OR-37

HOMING MECHANISMS IN SALMON: ROLES OF VISION AND OLFACTION

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Salmon have an amazing ability to migrate thousands of kilometers from ocean to natal rivers for spawning after an oceanic life for a few years. Many behavioral and electrophysiological studies have confirmed that salmon return from the coast to the natal branch of rivers using their olfactory cue. Tracking of salmon in ocean is, however, rather difficult, so that behavioral experiments on the open water orientation have not been carried out. Moreover, few attempts have been made to investigate biochemical aspects of any particular molecules in the olfactory system during the salmon homing migration.

Kokanee salmon (*Oncorhynchus nerka*; non-anadromous sockeye salmon) in Lake Toya offers an excellent model system for studying the orientation mechanisms in open water, because matured fish return to their natal spawning area with a high accuracy. We telemetrically tracked kokanee salmon in Lake Toya, and found that matured fish released at a long distance from the natal area returned directly to the natal area. Interference of the magnetic cue did not affect their straight homing, whereas blockage of the visual cue caused them move randomly, suggesting that the fish return directly mainly using their visual cue. In addition, we used two molecular markers, a salmonid olfactory system-specific protein (N24) and salmon type gonadotropin-releasing hormone (sGnRH), and examined biochemical and cytophysiological changes in the olfactory system during salmonid homing migrations. Our results indicate that N24 and sGnRH may be importantly related to the olfactory homing mechanisms in salmonids.

OR-38

PLASMA SEX STEROIDS IN FEMALE NEW ZEALAND FRESHWATER EELS (*Anguilla spp.*) BEFORE AND AT THE ONSET OF THE NATURAL SPAWNING MIGRATION.

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Unlike *Anguilla anguilla* and *A. japonica*, ovarian development in one of the two New Zealand eel species, the longfinned eel, *A. dieffenbachii* (LF), has been shown to be early to mid-vitellogenic at the onset of the spawning migration. We therefore collected eels before and at the onset of the spawning migration in order to investigate changes in plasma sex steroid profiles with stage of gonadal development. For comparative purposes, specimens from another New Zealand eel species, the shortfinned eel, *A. australis* (SF), were collected at the same time. In non-migratory ("yellow") eels of both species, only previtellogenic oocytes and oogonia were found, whereas ovaries of migratory ("silver") eels also contained oocytes in the oil droplet stage (SF) and at early to midvitellogenic stages (LF). Plasma estradiol-17β (E_2) and testosterone (T) were very low in non-migratory LF (< 0.25 ng/ml), but had increased in migratory LF (2.52 ± 0.26 ng/ml and 0.88 ± 0.10 ng/ml, respectively). In non-migratory SF, plasma E_2 (0.82 ± 0.22) and T (0.46 ± 0.07) levels were higher than in non-migratory LF, but lower than in migratory SF (1.30 ± 0.14; and 2.46 ± 0.17, respectively). Levels of 17α,20β-dihydroxy-4-pregnen-3-one were low in all groups (< 0.25 ng/ml). In both LF and SF, plasma levels of 11-ketotestosterone (11-KT) were low in non-migratory fish (< 0.5 ng/ml), but increased dramatically in migratory fish, averaging 4.66 ± 0.28 ng/ml in LF and 21.43 ± 1.81 ng/ml in SF. These results indicate that: 1) at the onset of the spawning migration ovarian steroid production is greatly increased; 2) steroidogenic activity appears to differ between eel species, and cannot entirely be explained by the different stage of gonadal development; and 3) the presence of large quantities of 11-KT in migratory females suggests a role for this steroid in female anguillids.

OR-39

REPRODUCTIVE MECHANISMS ASSOCIATED WITH INTERNAL FERTILIZATION OF EGGS IN A BENTHIC MARINE TELEOST, THE OCEAN POUT *MACROZOARCES americanus*

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Both the ocean pout and the wolffish, *Anarhichus lumpus* L., have drawn the interest of mariculturists because the two species are cold tolerant and they have good growth rates at low temperature. Because these fish do not spawn spontaneously in captivity and since successful mariculture is dependent upon predictable seed supplies, recent studies have focused on the reproductive biology of these benthic teleosts.

Our biological studies of captive ocean pout show that oviparous females have a low fecundity (approximately 500 eggs/kg) and they produce a single batch of large diameter eggs (7-9 mm) each reproductive season. The relatively few eggs annually produced by females are fertilized inside the body cavity and after spawning females wrap themselves around the egg mass fanning and coating the eggs with mucus which may be important for embryo survival during a long (~3 month) egg incubation period.

Evidence favouring internal fertilization of eggs in the ocean pout includes observations of copulatory behaviour between males and females during the spawning season, the spawning of fertilized eggs by inseminated females in the absence of males, instantaneous immobilization of sperm by seawater and some characteristics of ocean pout sperm morphology suggesting a long life-span (several days duration) reminiscent of mammalian sperm.

OR-40

HORMONES AS SEX PHEROMONES IN FISH

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Fish are typical vertebrates in using hormones as endogenous signals that synchronize gonadal development with reproductive physiology and behavior. However, studies over the past fifteen years clearly demonstrate that fish also use released hormones and hormone metabolites as exogenous signals (*hormonal pheromones*) that synchronize the reproductive activities of conspecifics. Early studies on *Gobius jozo*, zebrafish, African catfish, and goldfish laid the groundwork for the concept of hormonal pheromones in fish by demonstrating synthesis, release, detection, and biological response to a variety of steroids and prostaglandins. More recently, we have been employing electro-olfactogram (EOG) recording to screen a variety of freshwater species for olfactory responsiveness to a large number of putative hormonal pheromones, on the assumption that olfactory response is indicative of pheromonal function. Although in the preliminary stages, these studies indicate hormonal pheromones are widespread among three major orders (Cypriniformes, Characiformes, and Siluriformes) and present in at least two families of Perciformes (Gobiidae, Cichlidae). In addition to prostaglandins, which are commonly detected by Cypriniformes and Characiformes, detected compounds include free, glucuronidated, and sulphated forms of a variety of C21, C19, and C18 steroids. Surprisingly, we find no evidence that hormonal pheromones function as species-specific isolating mechanisms; the similarity in compounds detected increases with decreasing phylogenetic distance to the extent that hormonal pheromones might serve as useful taxonomic characters. This apparent similarity in hormonal pheromone systems among related species suggests that, contrary to expectations, knowledge of hormonal pheromone systems in a few key species can be transferred directly to fundamental and applied research in a great number of species.

OR-41

A PHEROMONE IN FEMALE RAINBOW TROUT URINE.

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Earlier studies have shown that female trout urine contains a priming pheromone which stimulates blood levels of sex steroids and milt production in males.
Following physical isolation steroid hormone levels in spermiating males drop rapidly over the course of 24 hours. Levels can then be raised again in ± 70% of the males by exposing them to female urine.
In this study, we have looked in detail at the time course of hormone levels in both types of males. Males were exposed to 1 ml of female urine and blood samples were taken at: 0, 5, 10, 20, 30, 45, 60, 90, 120, 180 and 300 minutes, and were assayed for 17α,20β-dihydroxy-4-pregnen-3-one, testosterone, 11-ketotestosterone, cortisol and gonadotropin. Levels of sex steroids peaked, in responding males, at 180 minutes.
Our attempts to identify the female pheromone(s) have so far proved negative. We have investigated the possible role of estradiol in the production of the pheromone(s). However, estradiol injection into spermiating males did not induce the production of urine with priming properties; and five estrogen conjugates, when added to the water, failed to simulate the priming effect of female urine. Furthermore, urine from pre-spermiating males was found to have some pheromonal activity. Following on findings in Atlantic salmon, nine prostaglandins were also tested but found to be inactive.

OR-42

ORIGINS AND FUNCTIONS OF F PROSTAGLANDINS AS HORMONES AND PHEROMONES IN THE GOLDFISH.

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Although F prostaglandins (PGFs) have been implicated as both the hormone responsible for female sexual behavior and the pheromone which triggers male sexual behavior in cypriniform fish, the origins, identities, and fates of PGFs have yet to be elucidated. Using immunoassay and mass spectrometry we have recently found that circulating levels of prostaglandin F2α ($PGF_{2\alpha}$) increase 50-fold coincident with ovulation in the goldfish. Also, when the oviducts of non-ovulated females were filled with an 'egg substitute', these fish became sexually active after several hours at which time their circulating $PGF_{2\alpha}$ also rose: the oviduct appears to be the source of this behavioral hormone. Tracing the fate of circulating $PGF_{2\alpha}$ using a radio-label, we discovered that this compound is rapidly cleared to the water as 4 metabolites which appear to be unique to the goldfish and released via two routes (Appelt & Sorensen, this symposium). Interestingly, only one of these metabolites, 15-keto-$PGF_{2\alpha}$, has extreme olfactory activity in mature males as measured by electrophysiological recording (EOG), and mass spectrometry has demonstrated that it is released by ovulated goldfish at a rate exceeding 500 ng per hour. The other PGF metabolites are conjugated and lack pheromonal activity. In conclusion, the production and release of PGFs appears to represent a specialized mechanism responsible for stimulating both female and male behavior in the presence of ovulated eggs.
(Supported by National Science Foundation and MN Agricultural Experiment Station)

OR-43

F-SERIES PROSTAGLANDINS HAVE A PRIMING PHEROMONAL EFFECT ON MATURE MALE ATLANTIC SALMON PARR.

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Ovulated female Atlantic salmon urine contains at least one priming pheromone which enhances plasma GtH II, androgens, and 17α,20β-P concentrations and expressible milt in spermiating mature male parr. Recent data indicate that steroids and steroid conjugates are probably not responsible for this priming effect. Although potent odorants, waterborne testosterone and 17α,20β-P sulphate have no effect on the male parr plasma hormonal profiles or on expressible milt. Waterborne prostaglandins ($PGF_{1\alpha}$ and $PGF_{1\alpha}$), however, are potent stimulators of male reproductive physiology and mimic the priming effect of female urine. The responsiveness of spermiating male parr to prostaglandins was muted in October but increased markedly in November/December. This, along with previous observations that spermiating male parr are attracted to testosterone in October but not in November, suggests that testosterone may act as an attractant for males to bring them to the salmon spawning grounds and that ovulated females release at least one, probably a PGF, priming pheromone in their urine which enhances male spawning readiness.

OR-44

ALTERNATIVE LIFE HISTORY STRATEGIES AND DIMORPHIC MALES IN AN ACOUSTIC COMMUNICATION SYSTEM. A. H. Bass. Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853

Studies of the influence of life history strategies on mechanistic traits provide the opportunity to identify potential linkages between ecology, behavior, development and evolution. It is within this context that we have been studying the reproductive biology of a marine teleost fish, the plainfin midshipman (*Porichthys notatus*). Midshipman have two male reproductive morphs: Nest-building *Type I males* generate long duration, quasi-sinusoidal-like, advertisement calls ("hums") to attract females to nests and trains of short duration agonistic calls ("grunts") in defense of their egg clutch and nest against potential intruder males. "Sneak-spawning" *Type II males* do not build nests, guard eggs or acoustically court females; like females, they infrequently generate isolated grunts in non-spawning contexts. Other studies indicate distinct, non-overlapping developmental trajectories for Type I and II males with no evidence for sex or role reversal; Type II males become sexually mature and reproductively active about one year earlier than Type I males. Thus, Type I males, compared to Type IIs, have an extended juvenile stage during which they are investing in body growth (e.g. on average Type I males are 40% larger in body size) and "building" a vocal motor system that will function in mate attraction. In contrast, Type II males are investing in earlier reproduction. Sex- and morph-specific vocal behaviors are paralleled by a divergence in vocal traits ranging from the size and number of muscle fibers to the rhythmic firing properties of neurons. Gonadotrophin releasing hormone (GnRH)-containing neurons are considered to initiate a cascade of events leading to the initial maturation and adult maintenance of the pituitary-gonadal axis. A temporal shift in the onset of sexual maturation, triggered by the brain's GnRH neurons, is considered a key event determining precocious maturation of Type II males and delayed maturation of Type I males; immunocytochemical and molecular biological methods are being used to determine changes in GnRH mRNA expression. Research support from NSF.

OR-45

BEHAVIOURAL AND ENDOCRINE STUDY OF *OREOCHROMIS AUREUS*, WITH SPECIAL REFERENCE TO SEX-REVERSED MALES.

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The behaviour of tilapia *O. aureus* was studied under controlled conditions in an attempt to improve knowledge on cultured cichlid fish. Relationships between relative social status and hormones [Testosterone (T), Estradiol-17ß (E2), 11-Ketotestosterone (11-KT), T3 and T4] were investigated on 87 fishes kept in aquaria (temperature : 26°C; photoperiod : 12L/12D). Dominant females exhibited a T level which was higher than in subordinates. Moreover, a significant linear correlation was observed in females between T plasma levels and frequency of aggressive patterns (lateral display, tail beating, biting, jagen, mouth fighting). On the other hand, dominant males exhibited a significantly higher level of 11-KT, by comparison with subordinate and isolated ones as well as with individuals maintained in high density conditions which inhibit aggressivity. Comparisons were made also between 18 females and 18 "17α–ethynylestradiol sex-reversed males" (pseudofemales). Pseudofemales showed more aggressive behaviour and were more dominant than normal fish in face to face experiments or in groups of 4, 6, 8 & 10 fish. When both females and pseudofemales were kept in an aquarium containing a territorial male, pseudofemales never spawned. The similarity between the body and gonad morphology and the gonadosomatic index of females and pseudofemales supports the idea that neuroanatomical and neurophysiological differences could exist between brains of normal and sex-reversed fish.

OR-46

BEHAVIOR, BRAINS, AND BIOPHYSICS: STEROIDAL MODULATION OF COMMUNICATION SIGNALS IN ELECTRIC FISH.

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Exploring the actions of hormones on neural circuits underlying reproductive behaviors is difficult because these circuits are often distributed across a number of brain areas each of which may be synaptically complex. The Electric Organ Discharge (EOD) of electric fish, which is used for social communication, is an ideal behavior for this analysis. EODs are species-specific, sexually-dimorphic, stereotyped signals that convey information on the sex, reproductive status, and motivational state. The neurons and effectors which generate the signal are simple, accessible for biophysical examination, and modified by sex steroids. I will present two examples: in the genus *Sternopygus* males make longer EOD pulses than females and the EOD pulse is broadened by androgen treatement. We have identified a voltage-dependent Na^+ current in the electric organ which shuts off rapidly in females, slowly in males, and whose voltage-dependent kinetics are altered by androgen treatment. We have made similar studies on *Apteronotus leptorhynchus* in which the EOD frequency of females is higher than males and in which EOD frequency is lowered by estradiol 17-ß (E_2) and raised by 11 ketotestosterone. We have found that treatment with E_2 lowers and 11 KT raises the endogenous firing frequency of electromotor neurons.

OR-47

ANDROGEN-INDUCED CHANGES IN ELECTROCOMMUNICATORY BEHAVIOR ARE CORRELATED WITH CHANGES IN SUBSTANCE P-LIKE IMMUNOREACTIVITY (SPI-ir) IN THE BRAIN OF THE WEAKLY ELECTRIC FISH, *Apteronotus leptorhynchus*

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A. leptorhynchus, modulates its electric organ discharge to produce intraspecific communication signals called "chirps". Although males and females are known to produce chirps during aggressive and reproductive displays, they show clear sex differences in their propensity to chirp; males readily chirp in response to artificial electrosensory stimuli, whereas females generally do not. This species also shows sex differences in SPI-ir in the brain; SPI-ir is present in a number of forebrain regions in males but not in females. One such region is the prepacemaker nucleus (PPn), the command center for chirping behavior. We have recently demonstrated that female chirping behavior is androgen sensitive; androgens increase both the number and structure of chirps produced by females. In the present study, we quantified androgen-induced changes in chirping behavior in females and simultaneously examined whether androgens alter the sexually dimorphic pattern of SPI-ir in the PPn. Androgen-implanted females produced significantly more chirps with longer durations and more dramatic frequency and amplitude modulations compared to controls. Many of these chirps are similar to those reported to be produced during spawning. Moreover, these changes are correlated with an increased expression of SPI-ir in the PPn, in a direction similar to the normal male pattern. However, alterations in SPI-ir were not restricted to the PPn, but also occurred in regions that regulate pituitary function and reproductive behavior. The results suggest that androgens modulate female chirping activity and cause both specific and wide spread changes in SPI-ir that may relate to a functional system that synchronizes chirping behavior with discrete reproductive events such as ovulation and spawning.

PII-1

DIFFERENTIAL MODES OF HORMONAL DISRUPTION IN FISH EXPOSED TO VARIOUS ORGANIC CONTAMINANTS

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Studies conducted on white sucker exposed to bleached kraft pulp mill effluent identified a number of reproductive alterations associated with reduced circulating levels of the reproductive steroid hormones. Depressed steroid levels were not related to an increased rate of peripheral metabolism but to a reduction in the steroid biosynthetic capacity of the ovarian follicular cells. In this study, we determined whether fish exposed to different classes of organic compounds showed similar reproductive lesions. Studies conducted on the white sucker at a non-chlorinated pulp and paper mill identified comparable disruptions within the steroid biosynthetic pathway resulting in reduced circulating levels. By comparison, brown bullhead collected from a site heavily contaminated with polycyclic aromatic hydrocarbons from steel mill effluents had depressed steroid levels during gonadal recrudesence, but no consistent pattern in terms of steroid production by ovarian follicles incubated *in vitro*. Follow-up studies at a pulp mill location demonstrated species differences in responsiveness as female brown bullheads showed no effects whereas white sucker demonstrated reductions in both circulating and *in vitro* production levels.

PII-2

CHRONIC STRESS ACTIVATES OPIOID-ADRENAL SYSTEM IN GILTHEAD SEABREAM *Sparus auruta.*

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Relationship between POMC-derived peptides and stress has been found in fish, as well as in other vertebrate species; moreover, the activation of the hypothalamus-pituitary-adrenal axis is involved in the adaptive response. In fact, increasing cortisol plasma levels together with plasma changes of POMC-derived peptides have been demonstrated in trout. Using chronic (one month) stress confinement, both pituitary and plasma acetyl salmon endorphin (acetyl sEP), and plasma cortisol have been assessed in seabream. In this stress paradigm, plasma acetyl sEP titers significantly increased together with concomitant decrease of pituitary acetyl sEP content. The acetyl sEP changes parallel those of plasma cortisol found in the same animals. The stress-induced activation of the opioid-adrenal system is reversed by concomitant treatment with antaxone, the long-acting opiate receptor antagonist.

PII-3

SLOW RELEASE MELATONIN IMPLANTS ELEVATE DAYTIME PLASMA MELATONIN BUT DO NOT AFFECT THE REPRODUCTIVE SEASONALITY OF THE FEMALE RAINBOW TROUT, *Oncorhynchus mykiss*.

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Melatonin may be implicated in the transduction of the photoperiodic information to the reproductive axis in salmonids. In our investigations, however, no evidence was found for such a link in female rainbow trout. Intraperitoneal and intramuscular implants of microspheres containing melatonin were shown to elevate and change the diurnal profile of plasma melatonin for several weeks post treatment. In the initial trial, groups (n = 14) were implanted with melatonin or placebos at different times in their reproductive cycle over one year under natural photoperiod. In a second trial, treatment and control groups (n = 35) were implanted continuously every six weeks with melatonin or placebo respectively. The treatment and negative control group received natural photoperiod and the positive control group was subjected to continuous darkness. Melatonin exerted no significant effect on spawning time, annual vitellogenin profiles or final gonad size although an effect was found as expected in the positive control group. These results pose the question as to what is the role, if any, of melatonin in reproductive seasonality in salmonids.

PII-4

EFFECT OF LIGHT ON MELATONIN SECRETION *IN VITRO* FROM THE PINEAL OF THE HAMMERHEAD SHARK, *SPHYRNA LEWINI*.

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The influence of light on melatonin secretion was investigated in the pineal of the hammerhead shark, *Sphyrna lewini*, maintained in organ culture. Individual pineals were incubated under (1) normal 12L:12D (lights on 08:00 hr) and (2) reversed 12D:12L (lights on 20:00 hr) cycles for 6 days, (3) continuous light (LL) for 2 days followed by normal 12L:12D (lights on 08:00 hr) or (4) reversed 12D:12L (lights on 20:00) cycles for 4 days, (5) continuous dark (DD) for 4 days, and (6) DD for 2 days followed by normal 12L:12D (lights on 08:00 hr) or (7) reversed 12D:12L (lights on 20:00 hr) cycles for 2 days at 23°C. In addition, the effect of culture medium osmolality on melatonin release was tested in pineals cultured in low (283 mOsm kg^{-1}) or high osmolality (1000 mOsm kg^{-1}) media. Melatonin secretion was high during the dark and low during the light under normal ($P<0.0027$) and reversed ($P<0.001$) photoperiodic regimes. Moreover, melatonin release was inhibited under LL. Under DD, melatonin release was constantly elevated and no circadian rhythmicity in secretion was found ($P<0.93$). Melatonin secretion from pineals cultured in the high osmolality medium was surprisingly below the detectable limit of the RIA. This is the first report demonstrating that light affects pineal melatonin release *in vitro* in an elasmobranch. Thus, the shark pineal like that of other lower vertebrates examined thus far acts as a photoendocrine transducer, suggesting that the elasmobranch pineal may play a role in regulating seasonal aspects of shark reproduction.

PII-5

EVIDENCE THAT INHIBITION OF REPRODUCTION BY STRESS IS NOT MEDIATED BY THE ACTION OF CORTISOL ON OVARIAN STEROIDOGENESIS.

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Stress inhibits reproduction in most teleost fish studied, and this effect is reported to be mediated by the effects of cortisol, and in salmonids at least, the effect is suggested to be directed at ovarian steroidogenesis. In this study, we incubated ovarian follicles of goldfish, common carp and the sparid *Pagrus auratus* with GtH and steroid precursors in the presence and absence of physiological doses of cortisol. Follicles of all three species produced T and E_2 in response to treatment with steroid precursors or GtH, but co-incubation with cortisol had no inhibitory effect on the capacity of the follicles to synthesize E_2 in any of the species examined. Cortisol also had no effect on basal secretion of T or E_2. In a number of cases, cortisol treatment had a small stimulatory effect on steroid production. The experiments suggest that either the observed effects of stress on fish are not mediated by cortisol, or that they arise higher in the endocrine cascade that at the level of ovarian steroidogenesis.

PII-6

PRELIMINARY OBSERVATIONS ON THE EFFECTS OF MELATONIN IMPLANTS AND PINEALECTOMY ON THE TIMING OF REPRODUCTION IN RAINBOW TROUT

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It is well established that the annual reproductive cycle of the rainbow trout is ultimately controlled by an endogenous circannual rhythm which is entrained by the seasonal changes in daylength. However, it is not known how information on photoperiod is transmitted to the reproductive axis. In several mammals photoperiodic information is converted by the pineal gland into a daily rhythm of melatonin secretion; pinealectomy renders the animals reproductive axis unresponsive to changes in daylength and, because it is the duration of the nocturnal increase in melatonin that encodes photoperiodic information, constant-release melatonin implants can be used to mimic the effects of a 'short-day' on the timing of reproduction. To determine whether the pineal gland of the rainbow trout is also involved in the transmission of photoperiodic information female rainbow trout were maintained under either a constant long day (LD 18:6) from late winter, or a constant long day from late winter followed by a constant short day (LD 6:18) several months later. As expected, fish exposed to both long and short days spawned in advance of those which only experienced long days. Fish which were exposed to long days followed by short days, but were pinealectomized just before the reduction in daylength, did not exhibit the same advance in spawning time as sham-operated controls. However, spawning was also not advanced in fish which were subjected only to long days, but received melatonin implants at the same time as control fish were exposed to short days. These preliminary experiments, conducted as a prelude to a major study, suggest that the pineal gland is involved in conveying photoperiodic information to the reproductive axis of the rainbow trout, but they are unable to distinguish between neural and hormonal mechanisms.

PII-7

EFFECTS OF PHOTOPERIOD AND TEMPERATURE ON REPRODUCTIVE ACTIVITY OF THE MUMMICHOG *FUNDULUS HETEROCLITUS* DURING VARIOUS SEASONS

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One strain of the mummichog ('Arasaki' strain; originated from Chesapeake Bay, introduced to the NRIFS Japan in 1985) has been reared in NRIFS showing distinct and constant annual reproductive cycle. This fish has also showed regular daily spawning cycle during the spawning season, without lunar or semilunar rhythm. To clarify the mechanism regulating the annual reproductive cycle of this species, the fish was kept under various photoperiodic and temperature regimes during different periods of the year. Gonadal development towards the spawning season (spring and summer) was accelerated by the elevation of water temperature during early spring. Termination of the spawning season was mainly induced by the short daylength in early autumn, although high water temperature was also responsible in the underyearlings. This fish became photorefractory after the spawning season, and did not progress in gonadal recrudescence even under the long daylength and moderate temperature conditions during autumn. It was concluded that both photoperiod and temperature is important for the regulation of the annual reproductive cycle of this species, although their respective importance greatly varies among seasons. Interactions between environmental factors and presumed circanual rhythm are also discussed.

PII-8

THE EFFECTS OF STRESS ON SPAWNING PERFORMANCE AND LARVAL DEVELOPMENT IN ATLANTIC COD, *GADUS MOHURA.*

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Four different groups of mature male and female cod were held together without feed in 3m fibreglass tank at a stocking density of 5 kg/m^3 and allowed to spawn spontaneously during the spawning season from March to June. Two tanks of fish were undisturbed controls and fish in the remaining two tanks were exposed to stress by netting individuals for a 1/2 hr period 3x/wk. Daily volumes of floating eggs, obtained from the egg collectors, and records of egg batch fertilization rates and egg survival (24 hr and survival to hatch) were recorded. Twice during the spawning period and once after, blood samples were rapidly collected from 10 restrained individuals in one control and one stress-treated tank of fish to determine circulating levels of cortisol. Although plasma cortisol levels were elevated in stressed groups, no differences in total egg production, egg fertilization and egg survival rates were detected between control and stress-treated fish. The most obvious effect of stress occurred on larval development where higher frequencies of deformed larvae ("twirlers") were produced by stressed broodstock compared with the larvae from control broodstock.

PII-9

SEASONAL SHIFTS IN DAILY CYCLES OF FREE T4 AND T3 AND ROLE OF SEX STEROIDS IN BINDING ABILITY OF T4 AND T3 TO THEIR THYROID BINDING PROTEINS IN CLARIAS BATRACHUS

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Thyroid hormones upon entering circulation binds with transport proteins and is metabolically inactive. It is their free forms only which enter the target cells and bring characteristic changes. Hence, analysis of free T4 and T3 will reflect better physiological status of thyroid hormones rather than total T4 and T3. Therefore, in the present study, plasma free T4 and T3 were measured at 4 hr interval over 24 hr daily cycle during different reproductive phases in the test fish. Results indicate significant seasonal changes in their daily cycles. Interestingly they show two peaks; one in photophase while other in scotophase of 24 hr daily cycle in all reproductive phases. There were remarkable shifting in their peak timings and magnitudes over the annual reproductive cycle. The data are analyzed in relation to circulating levels of total T4, T3 and sex steroids. It appears from the in vitro study further that 17B-estradiol does not effect binding ability of T4 and T3 to their binding proteins while 17a,20B-dihydroxy-4-pregnen-3-one decreases their ability to bind.

PII-10

EFFECTS OF CONTINUOUS LIGHT ON GROWTH AND SEXUAL MATURATION IN SEA WATER REARED ATLANTIC SALMON

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The effects of exposure of Atlantic salmon (*Salmo salar* L.) post-smolts to continuous additional light (LL) from January until May on growth and grilsing were investigated by conducting an experiment on a commercial fish farm in Western Norway (Stolt Sea Farm AS). Salmon post-smolt (n = 30,000) were distributed among two conventional 2250m^3 sea cages, and two 800m^3 tanks with pumped sea-water in November. One tank (LL-TANK) and one sea cage (LL-PEN) were exposed to LL from January to May, whereas the fish in the other two groups received only natural light (NL-TANK and LL-PEN). The fish were fed *ad libitum* during the hours of natural day light. In the period immediately following onset of additional light the appetite of the LL-TANK and LL-PEN fish decreased substantially compared with the groups under natural light. Subsequently, appetite increased in the LL groups, and remained higher than in the NL groups during March-June. When the fish held in tanks were harvested in August, average body weight was found to be 2.9 kg in the NL-TANK group and 3.7 kg in the LL-TANK group. The fish from the sea cages were harvested in September, and mean weights were 3.3 and 3.9 kg in the NL-PEN and LL-PEN groups, respectively. The proportions of grilse (fish maturing after 1.5 years in sea-water) were 21 and 26% in the NL-TANK and NL-PEN groups, but only 11 and 9% in the LL-TANK and LL-PEN groups, respectively. The results suggest that an abrupt change from short to long photoperiod in January both increases growth rate and reduces the proportion of maturing salmon.

PII-13

POPULATION DENSITY EFFECTS GONADAL STEROIDS LEVELS IN BOTH TERRITORIAL AND NON-TERRITORIAL MALE DAMSELFISH, *CHROMIS DISPILUS*

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Our previous work on the temperate damselfish *Chromis dispilus* has shown that territory-holding males from areas of high population density have higher levels of gonadal steroids than fish from lower population density during periods of spawning activity. This is correlated with higher spawning frequency and frequency of territorial interaction at high density. Most males do not occupy territories, but hold station in the water column above. In this study we investigated whether or not levels of steroids in these non-territorial fish also show density effects. Blood samples collected from non-territorial fish showed that plasma levels of testosterone and 11-ketotestosterone, but not 17α,20β-dihydroxy-4-pregnen-3-one, were higher during the spawning period in non-territorial males from areas of high population density than in fish from areas of lower population density. This study provides further evidence that population density has a strong influence on endocrine status in male damselfish.

PII-14

AN EVALUATION OF ROCKFISH (*Sebastes spp.*) AS MODELS FOR THE STUDY OF REPRODUCTION AND DEVELOPMENT IN VIVIPAROUS MARINE FISH

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Two species of Pacific rockfish have been evaluated as models for the study of reproductive seasonality, larval development (*S. rastrelliger,* grass rockfish) and growth (*S. auriculatus,* brown rockfish) in viviparous marine fish. Captured grass rockfish exhibited testicular recrudescence during September and all specimens were in full spermatogenic condition by December. Progressive regression of the testis occurred during March and April. In females, ovaries in various stages of vitellogenesis were observed in December. Fertilization and gestation were recorded between January and March and by April all female fish were in a post-partum condition. Larvae cultured from grass rockfish spawned in captivity grew linearly from birth to day 40 ($y=0.133x+4.534$, $r=0.965$). Cultured brown rockfish showed a progressive acceleration in incremental growth rate from the young fingerling stage (0.23 g/day at 7 cm S.L.) until they reached a maximum growth rate (0.38 g/day at 12 cm S.L.) which was sustained for the remainder of juvenile development. Under subsistence conditions, brown rockfish are estimated to grow to sexual maturity and marketable size (400 g) in 3-3.5 years. In the aggregate, these data establish well-defined parameters of timed development and environmentally induced changes in reproductive status in viviparous marine fish.

PII-15

SEASONAL CHANGES IN WEAKFISH SONIC MUSCLE FIBER MORPHOLOGY AND METABOLIC SUBSTRATE CONCENTRATIONS

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Male weakfish, Cynoscion regalis, bear bilateral, sound producing muscles along the lateral walls of the abdomen. These sonic muscles undergo a seasonal tripling in mass during the spring spawning period, and this increase in mass is correlated with rising androgen titers. Spontaneous sound production by these muscles is noted only during this period. Changes in sonic muscle fiber structure and the availability of metabolic substrates within the muscle were examined during the spring and summer of 1992 in the Delaware Bay, along the eastern shore of the United States. Immediately after capture, a single sonic muscle was removed and frozen in liquid nitrogen for analysis of muscle lipid, glycogen and protein concentrations. The other sonic muscle was removed and preserved in formalin for examination of muscle fiber cross-sectional area (CSA). The CSA of the myofibrillar, or contractile tissue (mCSA) and that of the mitochondria-rich sarcoplasm surrounding the myofibrils (sCSA) both increased significantly concurrent with increasing sonic muscle mass, then decreased to minimal values in the late summer, as sonic muscle mass decreased. The sCSA was significantly greater than the mCSA until late in the summer, when the sarcoplasm surrounding the myofibrils disappeared. Muscle protein concentration increased significantly as CSA and sonic muscle mass increased. Sonic muscle lipids and glycogen decreased significantly and abruptly during the period of maximal sound production in the field, and changed very little thereafter.

PII-16

CONTROL OF REPRODUCTORY CYCLE IN FEMALE *TRICHOGASTER*

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Research over the last years has made it possible to describe hormone control of the reproductory cycle of the Blue gourami, *Trichogaster trichopterus* (Pallas 1770), as a model for male-dependent, multi-spawning, asynchronic species of fish. This model involvs the three main stages of oogenesis (oogenia, vitellogenesis and maturation) and subdivisions of each stage. In the second stage of oogenia, after the development of receptors, gonadotropin (GtH) begins to affect oogenesis. Vitellogenesis is controlled by GtH and 17β-estradiol (E_2) in the first instance. Later, when a high percentage of oocytes are in vitellgenesis, the testosterone level increases, E_2 decreases, and GtH-II is synthesized in the pituitary. Male sexual activity and pheromones now lead to the secretion of GtH-II to the blood plasma and converts 17α -hydroxyprogesterone to 17α, 20β-dihydroxy-4-pregnen-3-one, which controls maturation. This stage can again be divided into five sub-divisions. All three stages can be identified and controlled in the laboratory.

PII-17

THE REPRODUCTIVE CYCLE OF THE COMMON SNOOK, CENTROPOMUS UNDECIMALIS

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Reproductive cycles have been elucidated in the common snook, Centropomus undecimalis. Snook are multiple spawners in inshore waters primarily around passes with deep channels which are near grassbeds where the shoreline is bordered by mangroves. They are reproductively regressed from November through March. Gonadal recrudescence starts in April. Spawning lasts from May through September when gonadal regression occurs. Plots of the gonadosomatic index (GSI) with water temperature and daylength show that they all coincide throughout the year. There is an inverse relationship between the reproductive season and mesenteric fat bodies. Fat bodies are largest during the winter, when the fish is regressed and are smallest during the summer, reproductive months.

Measurements of the GSI, maximum oocyte diameter, and oocyte histology indicate that snook spawn in the evening. Nuclear migration commences between 1100 to 1300 hours. Female snook with oocytes having undergone final maturation can be caught by 1500 hours. Preovulatory and ovulated females have been caught between 1700 and 1900 hours. Spawning is believed to occur into the late evening. A circadian spawning cycle is superimposed upon the annual spawning cycle.

PII-18

ONE FISH, TWO FISH,...GIRL FISH, GUY FISH? SOCIAL ENVIRONMENT AND GONADAL STEROIDOGENESIS IN TILAPIA

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Endocrine correlates of social environment with sexual status have indicated the importance of (circulating) hormones in reproductive behavior and physiology of teleost fish. Tilapine species exhibit relatively complex social interactions, especially during reproduction, and may serve as useful models to understand psychoendocrine phenomena. Reproductively mature male and female *Oreochromis niloticus* were maintained under three social regimes: 1) isolated individuals, 2) same-sex groups and 3) mixed-sex pairs. Gonads were excised, minced and incubated with ^{3}H-androgen precursors for up to 3 h. Organic-soluble steroidal metabolites were identified by TLC and related criteria. Less than 5 % of the total metabolites were present as aqueous-soluble compounds. Generally, the precursor androstenedione was converted into 6-12 organic-soluble metabolites. Testes produced mainly an unidentified metabolite (not 11β-hydroxy/keto- testosterone or androstenetrione), 5β-reduced androgens and a minor amount of testosterone. The production of this compound was most prominent in testes from single males. Ovaries produced predominantly testosterone, 5β-reduced androgens and estradiol in females paired with males. Sex-specific differences in gonadal steroid metabolism were more apparent than differences due to social environment. Consequently, we hypothesize that steroid biosynthesis in extra-gonadal sites may contribute significantly to the overall changes in behavior and physiology associated with social environment.

PII-19

THE WHITE PERCH, *MORONE AMERICANA* : A LABORATORY MODEL FOR REPRODUCTION OF TEMPERATE BASSES

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The reproductive cycle of white perch was characterized in detail and effectively controlled. Circulating levels of estradiol-17β (E_2), testosterone (T), 11-ketotestosterone (11-KT) and vitellogenin (Vg) were correlated with specific morphological and histological stages of gonad maturation. Increased levels of E_2, T and Vg accompanied oocyte growth. Progressive maturation of the testes coincided with increased T and 11-KT levels. Oocyte size-frequency distribution and histochemical analyses indicated that recruitment into vitellogenesis began in November and spawning extended from late-March to mid-May. In April, ovaries contained oocytes at all stages of development (multiple-clutch, group-synchronous maturation). Combined in vivo and in vitro experiments implicated 17α,20β-dihydroxy-4-pregnen-3-one (DHP) and 17α,20β, 21-trihydroxy-4-pregnen-3-one (20β-S) in control of final oocyte maturation in this species. Perch were routinely induced to mature out-of-season by manipulating temperature and photoperiod. They were reliably induced to spawn in the laboratory with implanted GnRH analogue and injected hCG. Our results demonstrate the utility of white perch as a laboratory model for reproductive physiology in temperate basses.
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PII-20

SEASONAL CHANGES IN GONADAL HISTOLOGY AND SEX STEROID HORMONE LEVELS IN THE PROTOGYNOUS HERMAPHRODITE, *EPINEPHELUS MORIO*

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Seasonal changes in gonadal histology and circulating sex steroid hormones (E_2, 11-KT, and T) were examined in a field population of the protogynous, sex changing red grouper, *Epinephelus morio*, collected in the eastern Gulf of Mexico to elucidate the roles of these hormones during the annual reproductive cycle and with sex change. During the spawning period (March-May) females were found with both ripe and ripening oocytes which indicated that this species may release several batches of oocytes over the spawning period. E_2 levels were highest in females with gonads containing both cortical alveoli and vitellogenic oocytes during the breeding season. On the other hand, males were still ripe as late as August although plasma levels of androgens (11-KT and T) had declined. Androgen levels were highest in March and decreased dramatically thereafter, reaching a low plateau for the rest of the months studied (May to November). A small proportion of red grouper with either previtellogenic or cortical alveoli stage oocytes were undergoing sex change both before and after the peak in reproductive activity. All three steroid hormones (E_2, 11-KT, and T) were detected in transitional fish but plasma levels were low. Proliferation of male tissue was not confined to a specific area of the gonad but was found in "pockets" within the ovarian lumen. The following sequence of an increase in gonial cells along the periphery of the lamellae, an increase in interstitial tissue, degradation of female elements, and formation of a sperm duct was concurrent with spermatocyte proliferation in these sex changing individuals.

PII-21

STURGEONS OF CASPIAN LAKE: BIOLOGY, ECOLOGY WAYS OF REPRODUCTION AND QUANTITY MAINTENANCE.

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The sturgeons are unique objects for fishering and investigations. Five species and several subspecies of sturgeons inhabit Caspian Lake and their total catch constitutes 90% of world catch. The rivers are their main spawning places. Nevertheless, due to blockade of migration ways from fattening areas to spawning places in basins of Kura and Volga Rivers, and to general aggravation of ecological situation in Caspian basin, the stable tendency toward their total number decrease is observed. Nowaday the sturgeon reproduction is realized by artificial way. The biotechnology of sturgeon reproduction and breeding till 3-4 monthed age is mainly elaborated, though the problem of increase of artificial reproduction efficiency is still remained that might be solved for account of elevation of adaptational capabilities of juveniles releasing from fish-breeding factories into natural conditions. For this purpose the investigations of sensitivity of larvas and juveniles to changes of main abiotic (salinity, temperature, oxigen content) and anthropogenic (oil pollution, detergents, pesticides, etc.) milieu factors were conducted. The data are envisaged and recommendations for the increase of reproduction efficiency of sturgeons reserves in Caspian Lake are offered in report.

PII-22

PLASMA LEVELS OF GONADOTROPIN AND STEROID HORMONES IN THE INDIAN MAJOR CARP, LABEO ROHITA DURING SEXUAL MATURATION

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Plasma gonadotropins (FSH and LH), steroids (progesterone, testosterone, oestriol, cortisol), and thyroid hormone (T3 and T4) in immature, maturing and mature males and females of Labeo rohita were determined in relation to season and to the development and timing of sexual maturation. Assay of FSH, LH and steroids by RIA in monthly pooled plasma samples revealed that gonadotropins and sex steroids levels were undetectable until the age 18 months in females and 10 months in males. Highest levels of FSH (1.95 mIU/ml) and LH (1.70 mIU/ml) were found in females during the prespawning (April & May) and spawning periods (July & August) respectively. In males, the highest levels of FSH (1.80 mIU/ml) and LH (1.70 mIU/ml) were observed during the late preparatory to the spawning period (March to August) in their second year of maturation. Progesterone and oestriol in females and testosterone in males, and cortisol, T3, T4 in both sexes also showed an increase during the pre-to the spawning period.

PII-23

CHANGES IN PLASMA ESTRADIOL AND TESTOSTERONE CONCENTRATIONS DURING A BROODING CYCLE OF FEMALE MOUTHBROODING TILAPIA, OREOCHROMIS MOSSAMBICUS, AND MALE MOUTHBROODING TILAPIA, SAROTHERODON MELANOTHERON

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A variety of parental care is observed in teleost fish, although not much is known about endocrine controls of parental behavior. We have examined plasma concentrations of estradiol (E_2) and testosterone (T) during a brooding cycle of 2 species of mouthbrooding tilapia, female mouthbrooders, Oreochromis mossambicus, and male mouthbrooders, Sarotherodon melanotheron. In O. mossambicus, E_2 concentrations were 6.7 ± 1.6 and 6.1 ± 1.4 ng/ml at early and late fry-brooding stages, respectively, and decreased to 2.5 ± 1.0 ng/ml after separation from the fry. T concentrations did not appear to change at these sampling times (17.8 ± 2.2, 32.6 ± 17.5, 15.3 ± 7.2 ng/ml). In male S. melanotheron, E_2 concentrations were 0.4 ± 0.1 ng/ml at early brooding stages, increased to 0.7 ± 0.1 and 0.8 ± 0.1 ng/ml at a late brooding stage and within a day of releasing fry, respectively, and decreased to 0.5 ± 0.2 ng/ml after the release. T concentrations were 5.7 ± 1.8 and 5.7 ± 1.9 ng/ml at early brooding stages, increased to 17.9 ± 5.8 and 69.2 ± 36.2 ng/ml at a late brooding stage and within a day of releasing fry, respectively, and decreased to 13.1± 5.1 ng/ml after release. These results suggest a possible involvement of sex steroids in mouthbrooding behavior of tilapia.
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PII-24

REPRODUCTIVE CYCLE AND EMBRYONIC GROWTH DURING GESTATION OF THE VIVIPAROUS TELEOST, *ZOARCES ELONGATUS*

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Within the Zoarcidae, three species of the genus *Zoarces* have evolved viviparity. Heretofore, almost all the information concerning such zoarcid viviparity has been centred on studies of the European species, *Z. viviparus*. We have thus initiated several investigations to elucidate the mechanisms in the maintenance of gestation, including endocrine control, in *Z. elongatus* which inhabits the Pacific coastal regions of northern Japan. The annual reproductive cycle of *Z. elongatus* can be divided into the following five periods; 1) early vitellogenesis (Dec to Apr): pre-vitellogenic oocytes in which begins gradual yolk accumulation when females become pregnant , 2) late vitellogenesis (May to Aug): when yolk accumulation proceeds rapidly after parturition, 3) early gestation (Sep to Oct): ovulated eggs (4.4 mm in diameter) fertilize and develop, 4) mid-gestation (Oct to Nov): hatched larvae absorb their yolk-sac, 5) late gestation (Dec to Apr): embryos continue to grow further until parturition. Serum levels of vitellogenin and estradiol-17β in females were high during the late vitellogenesis, showing good correlation with the advance of oocyte development, and declined rapidly during the early gestation period. During gestation, dry weight increased from 7 mg in ripe eggs in September to 65 mg (57 mm in total length) in the embryos in February. This demonstrates that *Z. elongatus* belongs to a matrotrophic type of viviparity which depends on maternal nutrients during gestation. Thus, it is considered that the embryonic growth of this species maintains its abundant yolk during early gestation, thereafter depending solely on additional maternal nutrition.

PII-25

VARIABILITY IN EGG QUALITY AND PRODUCTION IN A BATCH-SPAWNING FLOUNDER, PLEURONECTES FERRUGINEUS .

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The yellowtail flounder (Pleuronectes ferrugineus) follows a batch-spawning reproductive strategy also demonstrated in related flatfish, Scophthalmus maximus and Limanda limanda. A daily stripping protocol was used to study the variability in egg quality among ovulated batches and among different captive females. Fertilization rates and hatching rates varied greatly among batches within females and between females. Since no strict seasonal pattern for egg quality is evident within a female, it is not clear when a female's best batches are produced. Individuals can be regular in ovulation frequency or inconsistent with spawning interruptions which are associated with poor quality eggs. An inter-ovulatory period of 1 day accounted for 43 % of the pooled cases. An additional 39% of the cases were distributed among inter-ovulatory periods ranging from 2 to 4 days. Duration of spawning had a mean value of 44 days, an extreme value of 95 days was observed. The average egg production was greater than 500 000 eggs/female (avg pre-spawn female weight was 700 g), however, an individual of average weight may produce more than one million eggs. Mean fertilization and hatching rates observed for the eleven spawning females were 38% and 62%, respectively . The mean larval yield amounted to 27% of the total egg production spawned.

PII-26

SEASONAL CHANGES IN PLASMA LEVELS OF SEXUAL HORMONES IN THE TROPICAL FRESHWATER TELEOST, *Pygocentrus notatus* (TELEOSTEI: Characidae)

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In previous studies we have shown the changes that occurs in gonadotropin releasing hormone and catecholamines content in the brain of *Pygocentrus notatus* during the reproductive cycle. This fish, is a seasonal breeder which shows a pattern of gonadal maturation closely related to changes in the environmental conditions of the Venezuelan plains. In the present study, annual variations in the plasma levels of 17ß-Estradiol (17β-E) and Testosterone (T) in the male and female *P. notatus* have been determined. Adults *P. notatus* were collected from lagoons and ponds of the Guarico River. There was significant differences in plasmatic concentration of sexual steroids according to the sex or the physiological condition of the fish. In females, plasmatic 17β-E concentrations changed significantly during the year, lower levels were found in regressed, pre-spawning and spawning fish. Highest 17β-E levels were recorded during early recrudescence and recrudescence. Testosterone was detectable in plasma at all sample times and highest concentrations were found in females sampled in May. In males, T was low in fish that were spent, regressed, or in early recrudescence and elevated in recrudescence. A three-fold increase in the T levels in males compared to females levels was observed.

PII-27

Allozymes variation in two morphological types of the swamp eel. Synbranchus marmoratus, Bloch.

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Swamp eels, Synbranchus marmoratus, from Corrientes Province (Argentina) exhibit morphological differences in head shape and two well defined types can be distinguished. With the aim of learning whether these phenotypes correspond to different populations, isoenzymatic analyses were started to search for genetic markers to be correlated with such differences. Homogenates were obtained from three differentes tissues: liver, skeletal muscle and kidney. Electrophoretic assays of the esterase (EST), alcohol dehydrogenase (ADH), lactate dehydrogenase (LDH) and glutamic-oxaloacetic transaminase (GOT) systems were carried out. The activity for this enzymes, comprising 10 loci (Est-I, Est-II ,Est-III, Adh-I, Adh-II, Ldh-I, Got-I, Got-II, Got-III and Got-IV). The morphological types can be identified by both allelic frequencies and the number of alleles in some loci. From these preliminary results it arises that some systems could be genetic markers associated to the phenotypic differences in S. marmoratus. The continued exploration of the genetic structure of this species could provide very valuable information, in this sense.

PII-28

A MULTIDISCIPLINARY APPROACH OF THE REPRODUCTIVE ECOLOGY OF SINGLE AND MULTIPLE SPAWNER CYPRINID FISH IN RIVER

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Dynamics and regulation of oogenesis in single and multiple spawner fish have been investigated by a multidisciplinary approach including the profiles of gonadosomatic and hepatosomatic index in relation with the annual rhythm of oocyte growth, the seasonal profiles of steroid hormones (estradiol-17β, testosterone and 17,20β-dihydroxy-4-pregnen-3-one) and vitellogenin levels correlated with the most advanced oocyte stage. The hepatic activity including the hepatic uptakes (glycogen, phospholipids, glycerids) and the ultrastructure of the hepatocytes (abundance and status of mitochondria and rough endoplasmic reticulum) has been also studied to complete the informations about these two spawning strategies. In unique spawner, total fecundity (for one season of reproduction) has been calculated by counting the mature oocytes (limited to one size class). In multiple spawner, the fecundity by spawning act has been determined considering the largest oocytes during the reproductive season. The roach *Rutilus rutilus* (as a model of single spawner) and the white bream *Blicca bjoerkna*, the bleak *Alburnus alburnus* and the gudgeon *Gobio gobio* (as models of the multiple spawners) have been collected monthly outside the spawning season and weekly during it in the river Meuse basin (Belgium) by electrofishing, gill nets and fish pass control from 1992 to 1994. Different patterns of these parameters have been observed between single and multiple spawners but also among multiple spawners. In some multiple spawner species, recruitement and oocyte growth were markedly reduced during the reproductive seson (successive spawnings occurring from a predefined oocyte stock) whereas in some others vitellogenic activity remained high during the whole reproductive period.

PII-29

REPRODUCTIVE BIOLOGY OF WILD C. GARIEPINUS
FROM TWO SITES IN SOUTH WESTERN NIGERIA

[redacted] SALAMI AND A. BORODE

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This paper reports the cyclic changes in the gonads of the feral catfish, Clarias gariepinus, from the River Ogbese and Igbokoda Creek, South Western Nigeria. The effect of environmental factors in inducing this cyclic changes in gonads, reproductive behaviour, feeding and fecundity are discussed as it affects a tropical climate.

PII-30

REPRODUCTIVE BIOLOGY IN TEXAS CICHLID *Cichlasoma cyanoguttatum.*

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The Texas cichlid *Cichlasoma cyanoguttatum* is a native species of North of Mexico and South of USA. This species has been studied on its territorial behavior. In Mexico the studies about this cichlid are scarce and of course the reproductive aspects are little elaborate.
In this work we observed the reproductive behavior in the Texas cichlid, the points investigated were changes in coloration patterns, nest preparation and size, territory defense, spawning size, hatching time, fry development, offspring care, mortality and survive rate.

PII-31

YOUNG MURRELS ARE LESS FATTY

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Channa spp. (Murrels) of Distt. Meerut and Ghaziabad were collected and studied and it was observed that fat accumulation and age of the fish were highly correlated with each other. The fatness varies from species to species. The intensity of energy and protein metabolism in fishes decreases with age that leads to a shift towards fat accumulation. Young fish cannot attain same fatness due to vigorous energy and protein metabolism for its growth and development. When fishes Young most of the energy is utilized for the synthesis of genital products. So the genadal development period is intensive energy expenditure time. Due to accumulation of fat the appearance and texture of fish changes. Just by seeing thefish. Although Channa spp have less fat yet by measureing the fat content contents the age of the fish can also be determined.

PII-32

ANNUAL CHANGES IN REPRODUCTIVE PARAMETERS AND PLASMA STEROID HORMONES IN FEMALE CATFISH *Clarias macrocephalus* (GUNTHER)

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Reproductive parameters and accompanying steroid hormones were examined in the captive female catfish *C. macrocephalus* during an annual reproductive cycle to establish its breeding season. Gonadosomatic index (GSI), egg size and fecundity were lowest while the percentage of atretic oocytes highest in January-March. Plasma testosterone (T) were lowest in February-April (36-37 ng/ml), and reached its peak in July-September (54-59 ng/ml). Estradiol-17β (E_2) was lowest in January(7 ng/ml), and highest in December (20 ng/ml). Postvitellogenic oocytes and very low levels of 17α,20β-dihydroxy-4-pregnen-3-one (DHP; < 0.18 ng/ml) were observed throughout the year. These results suggest that off season, before, peak and end of the natural breeding season of catfish in the Philippines correspond to the months of January-March, April-June, July-September, and October-December, respectively.

PII-33

STEROID CONCENTRATIONS AND REPRODUCTION IN THE BLUEGILL SUNFISH

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With recent interests in the interactions between the endocrine and immune systems and between the endocrine system and reproductive behavior we have begun a study that looks at each of these three parameters within the same system. Blood samples and morphological measurements were taken from a population of bluegill sunfish (*Lepomis macrochirus*) throughout their 1994 breeding season in Lake Austin, Austin, Texas. Blood samples were analyzed for testosterone, 11-ketotestosterone, and cortisol and these results were then correlated with individual behavioral and morphological parameters, including proximity to day of synchronized spawning, reproductive condition, male secondary sexual characteristics, and parental strategy. Overall, androgen levels differed significantly among reproductive adults. Females were characterized with high testosterone levels and little to no 11-ketotestosterone. Paternal males, in contrast, displayed low levels of testosterone and high 11-ketotestosterone, whereas non-paternal males showed both low testosterone and low 11-ketotestosterone. These titers were seen to vary within the different reproductive adults with respect to condition factor and breeding-cycle stage. These correlations can now be used in our continued study of the interactions of the endocrine and immune sytems and reproductive behavior within this intersexually and intrasexually dimorphic species.

PII-34

DEPLETION OF FATTY ACIDS DURING EMBRYONIC DEVELOPMENT IN THE MEDAKA

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The Japanese medaka *Oryzias latipes* is a convenient model for the study of teleost embryonic physiology. However, lipid utilization during embryonic development has not been extensively investigated in this species. Eggs were removed from females within an hour of fertilization and incubated at 25 ± 1° C. Samples for lipid analysis were taken at fertilization, 96 hours of incubation and at hatching. Total fatty acid content was 43 μg/egg at fertilization, of which 2/3 was in the neutral lipid fraction. Polar lipid content declined slowly but significantly throughout incubation; a large decrease in neutral lipid content occurred between 96 hours and hatching. Unlike many teleost embryos, medaka did not preferentially retain (n-3) polyunsaturated fatty acids or preferentially deplete monounsaturated fatty acids during development. Linoleic acid (18:2(n-6)) was the preferred fatty acid for catabolism.

PII-35

FEMALE GOLDFISH RELEASE PHEROMONALLY ACTIVE F-PROSTAGLANDINS VIA TWO ROUTES AND MAY CONTROL THEIR RELEASE

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Although it is well established that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and its metabolite 15-keto prostaglandin $F_{2\alpha}$ (15K-$PGF_{2\alpha}$) are potent olfactory stimulants for male goldfish (*Carassius auratus*), and that these compounds as well as several other non-stimulatory F-prostaglandins (PGFs) are produced and released by ovulated females (Sorensen, this symposium), the routes by which these compounds are released is unknown. This is an important question because the manner by which these compounds are released directly determines how they function as behavioral stimuli. We sought to answer this question by injecting radio-labelled $PGF_{2\alpha}$ and tracing its fate using gill dams, urinary plugs, and catheters. Two-thirds of all PGFs were released in urine. Significantly, the most potent olfactory stimulant, 15K-$PGF_{2\alpha}$, was released almost exclusively in urine while $PGF_{2\alpha}$ was released via both urine and gills. The ratio of 15K-$PGF_{2\alpha}$ to $PGF_{2\alpha}$ released via the urine (1.59±0.56, Mean±S.E.M.) was significantly larger than that released via the gills (0.17±0.17, $P<0.01$). Preliminary studies also found that female goldfish release urine at 3-5 min. intervals, suggesting that urinary release of 15K-$PGF_{2\alpha}$ might have a function in chemical signalling. In this way, female goldfish may control the release of pheromonal PGFs, permitting those compounds to function as a communicatory signal.

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PII-36

SPERMIATED MALE SEA LAMPREY RELEASE A POTENT SEX PHEROMONE

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Behavioral studies of the anadromous sea lamprey, *Petromyzon marinus*, have indicated that it is attracted to different conspecific odors depending on its stage of sexual maturity. At the start of their spawning migration immature lamprey are attracted to washings of conspecific larvae which contain unique bile acids, but later they are attracted to washings of mature adults. The present study sought to determine if the latter attraction is attributable to a specific sex pheromone by examining the olfactory sensitivity and behavioral responsiveness of adult sea lamprey to washings of conspecifics and various hormonal compounds. Electro-olfactogram recording (EOG) demonstrated that only males release potent odorant(s) and then only when spermiated: water which has contained a spermiated male for 4 hours is detected by conspecific adults at dilutions of 1:10,000,000. Chemical characterization of this putative sex pheromone suggested that the active component(s) is conjugated with a sulfate group. This finding is complemented by other EOG results showing that of 68 hormonal compounds tested on this species, only 7 are detected at micromolar concentrations, and all of these are sulfated gonadal steroids. Interestingly, EOG sensitivity to the unpurified male odorant was not influenced by gender, sexual maturity, or life stage. Finally, behavioral studies found that sexually mature females are attracted by the odor of mature males whereas sexually mature males are repelled. In conclusion, this study indicates that sexually mature male sea lamprey release a conjugated steroid pheromone(s) to attract females to their spawning nest. This is the first characterization of a sex pheromone in a primitive, cartilaginous fish.

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PII-37

OLFACTORY HYPERSENSITIVITY TO SEX PHEROMONES IN BLIND CAVE FISH

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We previously demonstrated developmental differences among male *Puntius schwanenfeldi* in the magnitude of peripheral olfactory response to a prostaglandin pheromone (PGF), and that these differences are under endocrine control. More recently, we have found that similar differences exist between adult males and females of the same species, and in the South American blind cave tetra (*Astyanax fasciatus*). Behavioral investigations suggest that, as in *Puntius* (Cypriniformes), PGF is a sex pheromone in *Astyanax* (Characiformes). Interestingly, the magnitude and sensitivity of olfactory responses to PGF in male *A. fasciatus* far exceed those in *Puntius* or other previously tested species. Data from comparative electro-olfactogram studies of *Astyanax* and related Characiform species suggests that olfactory hypersensitivity to sex pheromones is extremely rare. However, sighted, river-dwelling populations of *A. fasciatus* also exhibit extremely sensitive olfactory responses to PGF. Thus, the phenomenon may represent either a trait that pre-adapted *Astyanax* for cave life, or an adaptation to reproducing in the dark that has introgressed to sighted populations through occasional interbreeding. These findings are discussed in relation to hypotheses about the evolution of hormonal pheromone systems in fish.

PII-38

SEASONAL CHANGES IN OLFACTORY SENSITIVITY OF MATURE MALE ATLANTIC SALMON (*Salmo salar* L.) PARR TO PROSTAGLANDINS.

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The olfactory sensitivity of mature male Atlantic salmon parr to four F series prostaglandins (prostaglandin-$F_{1\alpha}$, prostaglandin-$F_{2\alpha}$, 15-ketoprostaglandin-$F_{2\alpha}$ and 13,14-dihydro-15-ketoprostaglandin-$F_{2\alpha}$) was studied using an electrophysiological technique. Mature male parr were acutely sensitive to $PGF_{1\alpha}$ and $PGF_{2\alpha}$, less sensitive to 15-keto$PGF_{2\alpha}$ and did not respond at all to 13,14-dihydro-15-keto$PGF_{2\alpha}$. The sensitivity of male salmon parr to prostaglandin-$F_{2\alpha}$ increased both with maturity and as the reproductive season progressed, with maximum responses recorded in spermiating parr. There was however, a more rapid increase in the olfactory response of spermiating male parr to prostaglandin-$F_{1\alpha}$ which occurred over a 10 day period. Pre-ovulated and ovulated female Atlantic salmon were significantly less responsive to the F-series prostaglandins than the male parr. The results are discussed in relation to the synchronisation of spawning in the Atlantic salmon.

PII-39

CHARACTERIZATION OF STEROIDAL SEX PHEROMONES IN THE ROUND GOBY, (*Neogobius melanostomus*)

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The round goby (*Neogobius melanostomus*) was recently introduced from the Black and Caspian Seas into the Great Lakes, and, possibly through competition for spawning habitat, the increase in *N. melanostomus* populations has led to a decline in numbers of indigenous sculpins (*Cottus bairdi*). Previous studies suggest that both gobiids and cottids employ chemical signals (pheromones) during mating, and that these pheromones may be hormone metabolites released by males; this study was designed to characterize the nature and biology of sex pheromones in the round goby as part of an effort to investigate pheromonal interactions between the two species. We used electro-olfactogram recordings to screen several steroids, prostaglandins and their metabolites as possible hormonal pheromones. While *N. melanostomus* exhibited no olfactory response to prostaglandins, a finding predicted from their mating system, this species detected a diverse array of free and conjugated C18, C19 and C21 steroids. Cross-adaptation and dose-response studies suggest that discrete receptors exist for unconjugated C18 steroids (eg. estrone) and 5(α, or β) reduced androstan compounds with sensitivities ranging from 10^{-10}M to 10^{-8}M. We are currently investigating the likely existence of additional receptor types. While the pheromonal functions of these compounds remains to be determined, the present data strongly suggest that the round goby has evolved a complex sex pheromone system; complete understanding of this system may indicate avenues of intervention for influencing reproduction in *N. melanostomus*.

PII-40

GAMETOGENESIS OR BEHAVIOR? THE ROLE OF SEX STEROIDS IN HERMAPHRODITE REPRODUCTION

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The relationship between alternative spawning behaviors, aggression, circulating sex steroids, and testis allocation in the belted sandfish (*Serranus subligarius*) was examined. This simultaneous hermaphrodite rapidly alternates between male and female spawning roles. Two male spawning behaviors occur, pair spawning and streak spawning. Female spawns are always pair spawns. Within individuals, frequency of streaking and of female pair spawning decreases with body size, while male pair spawning increases with size. Aggressive behavior increases with size and with frequency of male pair spawning. Frequency of aggression received increases with streak spawning. Individual mating and aggressive behaviors were recorded in the field and blood and gonad samples were taken. We quantified estradiol-17ß (E2), testosterone (T), 11-ketotestosterone (11KT), 17α,20ß-dihydroxyprogesterone (DHP), and 17α,20ß,21-trihydroxyprogesterone (20ßS). Plasma levels of E2, T, 11KT, and 20ßS did not differ with size or spawning behavior, indicating that these steroids are more important for gametogenesis than for behavior. Circulating DHP levels were significantly lower in small streakers ($\leq$ 72 mm SL) than in small pair spawners, suggesting an initiating, 'activational' effect of DHP on courtship behavior in small fish. Plasma 11KT levels increased significantly with size and showed an increasing trend with male pair spawning behavior. Plasma T and 11KT were not significantly correlated with frequency of initiating or receiving aggressive behavior or with the ratio of testis to ovary. Although mating and aggressive behaviors are clearly correlated, they are not dependent upon circulating androgen concentrations or upon testis size. These results are consistent with long term, 'organizational' effects of 11KT on development of mating and aggressive behaviors.

PII-41

RELATIONS BETWEEN MATERNAL BEHAVIOUR, OVARIAN DEVELOPMENT, AND ENDOCRINE STATUS, IN THE MOUTHBROODING FEMALE OF *OREOCHROMIS NILOTICUS*.

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In order to study hormonal changes and ovarian development during parental cares, two groups of females of the cichlid fish *Oreochromis niloticus*, allowed or not to perform mouthbrooding behavior, were compared on the following criteria: ovarian development, plasma and pituitary levels of prolactin (each of two isoforms ti-PRLI and II), growth hormone (ti-GH), and plasma levels of estradiol and testosterone. The ovarian cycle duration is shortened in non mouthbrooding females : 15 days instead of 27 days for a normal cycle. In females exhibiting parental behavior, the development of vitellogenic follicles is slower, particularly during the guarding phase which lasts from day12 to day 18. Plasma GH levels (up to 30 ng/ml) were significantly increased,compared to non mouthbrooding females. Moreover, the steroid increase (4,5 fold for estradiol), at the beginning of the sexual cycle, is delayed of 6 days in mouthbrooders. Plasma levels of both isoforms of PRL do not show significant differences between the two groups of females, but the pituitary level of ti-PRLII is significantly lowered in mouthbrooding females during the first 3 days of the incubation period. Our results show that vitellogenesis can be accelerated when parental cares are interrupted, suggesting some functionnal relationship between the two process.

PII-42

A SEXUAL PARADOX: ANDROGEN AND ESTROGEN SYNTHESIS IN TILAPIA

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Several teleost species release steroids, with pheromone-like activities, which are suggested to regulate behavioral or physiological responses related to reproduction. Because of its timely and predictable reproductive periodicity, the tilapia *Oreochromis niloticus* is a good model in which to study the relation of steroids and reproduction. In this study, adult tilapia were exposed to various social arrangements in individual recirculating aquaria including single males or females (SM, SF, 2wk), grouped males or grouped females (GM, GF, 2 wk), or individually paired males with females (PM, PF, 2 d). Total kidney tissues were removed and incubated in ^{3}H-androstenedione for up to 3 hr. Synthesized metabolites were recovered by organic or aqueous extractions and tentatively identified by TLC and other criteria. Within 3 hr, ca. 30% of the precursor was incorporated into unidentified water-soluble metabolites in SM and GM; less than 13% was incorporated in all other male or female groups. In organic extracts, male kidneys, regardless of social group, produced high quantities of estrone (not observed in female kidneys or in male testes). Female kidneys, regardless of social group, produced high quantities of unknown steroid (an unidentified metabolite produced normally in high quantities in male testes, minimally in female ovaries, and is not 11-ketotestosterone). We hypothesize that the paradoxical production of sex-related steroids in kidneys is related to either (i) an undefined intra-organismal endocrine function and/or (ii) pheromonal processes associated with, but not confined to, sexual identification and receptiveness. Further confirmation studies are needed.

PII-43

CHARACTERIZATION OF A cDNA ENCODING A PUTATIVE TESTICULAR GONADOTROPIN RECEPTOR FROM THE AFRICAN CATFISH

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A cDNA encoding a putative gonadotropic hormone (GTH) receptor in the testis of the African catfish, *Clarias gariepinus*, was cloned via a series of polymerase chain reactions (PCRs). Using (degenerate) primers based on conserved amino acid sequences in transmembrane domains of the mammalian glycoprotein (GTHs and thyrotropin) receptors, we were able to amplify a cDNA fragment derived from a mRNA encoding a putative glycoprotein hormone receptor. In order to clone the corresponding 3'-end of this cDNA, PCRs based on the principle of 'rapid amplification of cDNA ends (RACE) were performed. A random primed cDNA library was constructed from testis poly(A)$^+$-RNA to obtain sequence information of the 5'- part of the cDNA. The deduced amino acid sequence of the complete cDNA revealed that the putative transmembrane domains and interconnecting intra- and extracellular loops display a high sequence identity with the mammalian glycoprotein receptors. The N-terminal part of the putative catfish GTH receptor protein shares sequence characteristics with both the mammalian luteinizing hormone/choriogonadotropin (LH/CG) receptors and the mammalian follicle stimulating hormone (FSH) receptors, thus indicating that the putative catfish GTH receptor might be a hybrid receptor with respect to the mammalian GTH receptors. The C-terminal part of the putative catfish GTH receptor protein displays a much lower degree of sequence homology with the mammalian glycoprotein receptors. This is consistent with the low sequence homology found within the C-terminal part of all members of the glycoprotein receptor family.

PII-44

EFFECTS OF 17α,20β-DIHYDROXY-4-PREGNENE-3-ONE ON TESTICULAR ANDROGENS IN ATLANTIC SALMON MATURE PARR *IN VIVO* AND *IN VITRO*.

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During natural sexual maturation in male salmonids a peak in plasma androgen levels is followed by a decline in androgens and a consomitant rise in 17α, 20β-dihydroxy-4-pregnene-3-one (17,20P). Is this decline in androgens due to a suppressive effect of 17,20P?

Mature Atlantic salmon (*Salmo salar*) male parr were implanted in May and August with Silastic capsules filled with 17,20P or empty capsules. The experiments were terminated in September, when natural androgen levels are at their highest. RIA measurements showed that the 17,20P treatment raised plasma levels of 17,20P, whereas levels of testosterone (T) and 11-ketotestosterone (11KT) were not influenced. Testicular fragments from mature salmon where incubated with 17,20P in combination with either T, 17α-hydroxy-4-pregnene-3-one (17-P) or medium alone. Considerable amounts of 11KT were formed in incubates containing T or 17-P, but not with medium alone. A very high dose of 17,20P (3000 ng/ml) suppressed 11KT production, whereas 3, 30 or 300 ng/ml were without effect. It was concluded that physiological levels of 17,20P suppress 11KT production neither on central nor on testes level.

PII-45

THYROID HORMONE INDUCES THE SYNTHESIS OF A 52k PROTEIN IN PERCH LEYDIG CELL WHICH STIMULATED ANDROGEN RELEASE

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Leydig cells from a freshwater perch, Anabas testudineus, testis were isolated and incubated in vitro ($1x10^6$ cells/incubation) where addition of T_3(3,5,3'-triiodothyronine) caused stimulated release of androgen in a dose dependent manner. Stimulation of androgen release by T_3 was significantly inhibited by a protein synthesis inhibitor, cycloheximide. Perch Leydig cell nuclei have T_3 receptor and binding of T_3 resulted significant increase in protein synthesis. Therefore it appears that this increased protein synthesis by T_3 has relationship with the stimulation of androgen release. Subcellular fractions obtained from T_3-treated Leydig cell showed an increase in protein synthesis in mitochondrial and soluble supernatant fractions(100k sup) and it was only 100k sup which stimulated androgen release. T_3-induced protein(TIP) responsible for stimulated androgen release was purified and found to be a 52k monomer protein. Its addition to Leydig cell incubation resulted a remarkable increase in androgen release.

PII-46

STEROIDOGENESIS DURING ESTROGEN-INDUCED SEX INVERSION IN THE SEA BREAM, *SPARUS AURATA.*

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In order to understand the role of steroids in the process of sex inversion in the protandrous seabream, juveniles were fed oestrogen for periods of up to 3 months. Gonadal steroidogenesis was monitored by incubating in vitro gonadal fragments with androstenedione (0.5-2μg/ml) and assaying the production of testosterone (T), 11-ketotetosterone (11KT) and estradiol-17β (E2). In a first experiment fish were fed 17α-ethynilestradiol ($ethE_2$) at a rate of 15mg kg^{-1} food for 37 days (T1) or 112 days (T2) while controls (CTL) did not receive $ethE_2$. At the end of the experiment at least 50% of the volume of the gonad was filled by undifferentiated tissue in all groups of fish. A larger part of the gonad was filled with male tissue and male germ cell development was more advanced in CTL than in T1 or T2 fish. As $ethE_2$ caused a decrease in appetite, in a second experiment fish were fed with estradiol-17β at a rate of 2 mg kg^{-1} (T3) and 15mg kg^{-1} (T4). Sex inversion was fully accomplished in T4 (90.3±2.1% female tissue) while testes were vestigial (2.6±0.8%). The rate of in vitro 11-KT production was high but not significantly different between groups. T and E2 production was generally significantly higher in estrogen treated groups than in controls and were both correlated to the volume of the female portion of the gonad. However, T production was three orders of magnitude higher.

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PII-47

CLONING AND FUNCTIONAL EXPRESSION OF THE COHO SALMON (*ONCORHYNCHUS KISUTCH*) GONADOTROPIN II RECEPTOR

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The functional effects of the salmon gonadotropins, GTH I and GTH II are thought to be mediated via G protein-coupled glycoprotein hormone receptors located in the gonads. Based on previous binding studies in our laboratory, we have proposed a two-receptor model for gonadotropin action wherein there are two GTH receptors with distinct spatial and temporal distribution: 1) GTH-RI, which binds both GTH's but with higher affinity for GTH I and 2) GTH-RII which is highly specific for GTH II. The objectives of this study were to further characterize the distinct properties of these receptors by cDNA cloning and functional expression of receptor proteins. Probes for the salmon GTH receptors were prepared by PCR amplification of reverse-transcribed mRNA from immature coho salmon testes using degenerate primers derived from conserved regions of the mammalian glycoprotein hormone receptors (LH/CG-R,FSH-R ,TSH-R). The resulting 250 bp probe was used to screen a coho salmon gonadal cDNA library enriched in granulosa cell mRNA. Three overlapping clones were identified and used to generate a full-length 2.7 Kb cDNA encoding a putative GTH receptor which is 40-60% identical to mammalian LH, FSH, and TSH receptors. To determine whether this cDNA encoded a functional GTH receptor, human embryonic kidney 293 cells were transiently transfected with an expression vector containing the full-length cDNA and assayed for responsiveness to purified coho salmon GTH I and II. In HEK-293 cells expressing the putative receptor, cAMP production increased in a dose-dependent manner in response to GTH II but was unaffected by GTH I. Cyclic AMP production in control cells transfected with vector alone was unaffected by either GTH I or II. These results suggest that the cloned cDNA encodes for the coho salmon GTH II receptor.

PII-48

GONADAL ANDROGEN RECEPTORS IN FISHES

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We have begun to characterize gonadal androgen receptors from coho salmon, rainbow trout, Nile tilapia, and Pacific lamprey. The receptor characterized from salmonids and tilapia binds the synthetic androgen mibolerone with high affininty. The steroids that displaced ^{3}H-mibolerone binding were other 17α-alkylated steroids that have been shown to be potent masculinizing agents. Lamprey testes bind testosterone much more than mibolerone. In other studies of fish in which synthetic and natural androgen binding have been compared, the receptors described to date bind natural androgens better than synthetic androgens, and bind testosterone better than 11-ketotestosterone. The androgen binding site from coho salmon ovaries differs in character from these general findings and may be a new receptor. Thus, study of the distribution and characteristics of androgen receptors may provide insights into the mechanism of action of masculinizing androgens, and perhaps help to determine what role, if any, steroids play in natural gonadal differentiation.

PII-49

BINDING CHARACTERISTICS OF 20β-S TO ATLANTIC CROAKER SPERM MEMBRANES.

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A plasma membrane receptor for the maturation-inducing-steroid (MIS), 20β-S, has previously been characterized in the ovaries and detected in the testes of spotted seatrout . We have also found 20β-S (natural MIS) binding activity in the ovaries and testes of a closely related species, Atlantic croaker. In the present study, the presence of membrane binding sites for 20β-S in croaker sperm was investigated. Milt collected from spermiating males was homogenized in buffer and subjected to sequential centrifugation and the resulting fractions were assayed for 20β-S binding. Significant specific binding (approximately .055 pmol/mg protein) was confined to the plasma membrane fraction (final 20,000xg pellet) and was not present in any of the other subcellular fractions. Saturation and Scatchard plot analyses revealed the presence of a single class of saturable and displaceable binding sites with high affinity (Kd 10^{-8}M). The binding sites are specific for 20β-S and have 100 times lower affinity for 17,20β-P and do not bind testosterone or estradiol. These preliminary observations suggest that the specific binding sites for 20β-S in croaker sperm membranes have similar characteristics to the 20β-S plasma membrane receptor in seatrout ovaries. Progesterone has been shown to directly act on the plasma membrane of human sperm to induce capacitation. The identification of membrane binding sites for 20β-S in croaker sperms raises the possibility that 20β-S may have a similar role in this teleost species.

PII-50

EXPRESSION OF KALLIKREIN GENE FAMILY MRNAS IN THE TROUT OVARY

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The kallikrein system (kallikrein, kininogen, kinin) has been implicated in the control of ovulation in vertebrates. However, to our knowledge, the molecular biology of the ovarian kallikrein gene family has not been studied. During the characterization of several complementary DNA (cDNA) clones, obtained from screening a brook trout ovarian cDNA library, we obtained a sequence for a 1.8 kb clone that was homologous with several members of the mammalian kallikrein family. Using the 5' end of this clone we rescreened the library and obtained a larger, 2.4 kb clone, that appeared to have a complete open reading frame containing 801 nucleotide bases. In addition, it has an extended 5' region containing 673 nucleotide bases and approximately 1.0 kb of untranslated 3' region. The open reading frame encodes a protein of 267 amino acids that is very homologous with several related mammalian serine proteases including adipsin/complement factor D and tissue kallikrein. On Northern blots of brook trout ovarian RNA, this clone hybridizes with two primary RNAs of 2.4 and 3.2 kb. Using an *in vitro* incubation assay we have found that kallikrein is a potent stimulator of the contraction of brook trout follicles. Our results indicate that there are transcripts of the kallikrein gene family expressed in the preovulatory brook trout ovary and that differential regulation of these transcripts during reproduction may occur in certain ovarian tissue. Supported by NIH grant #HD25924-02.

PII-51

5β-PREGNANE-3α,17α,20β-TRIOL: A MAJOR METABOLITE OF THE OOCYTE MATURATION-INDUCING STEROID IN PLAICE, *PLEURONECTES PLATESSA*.

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During oocyte maturation in female plaice, plasma levels of 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P) are very low. The presence of abundant 5β-reducing and sulfating enzymes in the ovaries, however, suggests that 17,20β-P may be metabolized before it is released into the blood. In order to study this possibility, plasma samples from female plaice, grouped according to their stage of maturity, were examined for the presence of the free, sulphated and glucuronidated forms of 5β-pregnane-3α,17α,20β-triol (3α,17,20β-P5β). This steroid was initially measured with a radioimmunoassay which cross-reacts with all steroids having a 5β-pregnane,3α-hydroxyl configuration. Because of the high amounts of 3α,17α,21-trihydroxy-5β-pregnan-20-one found in plaice plasma, however, it was necessary to chromatograph the samples prior to assay. A relatively more specific radioimmunoassay has now been developed for 3α,17,20β-P5β, which cross-reacts with the 17α,20β-dihydroxyl part of the molecule and, when used to measure the sulfated steroid, does not require chromatography. Levels of sulfated 3α,17,20β-P5β are undetectable in pre-spawning females and between 50 and 400 ng/ml in spawning females. Levels also rise in response to HCG injection.

PII-52

ANDROGEN BINDING IN THE STICKLEBACK KIDNEY

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Several studies have shown that 11-ketotestosterone (11KT) is more effective than testosterone (T) in stimulating secondary sexual characters. In teleosts, however, no receptors for 11KT have been found. In the present study binding of 11KT and T was studied in the kidney of the male three-spined sticklebacks, *Gasterosteus aculeatus*. The kidney of the male hypertrophies under androgen stimulation in the breeding season and produces a "glue" which is used in nest-building. Kidney tissue pieces were incubated with tritiated 11KT, with or without unlabelled steroids present in varying amounts. A specific binding of 11KT was found. This was displaceable with 11KT (ED50 c. 28 nM) and, less effectively, with T. Specific binding of 11KT was not found in liver and muscle. Also, no specific binding of tritiated T was observed. Cytosolic androgen binding was studied in stickleback kidney and brain and in trout (*Salmo trutta*) skin. A receptor-like binding of T, but not of 11KT, was found in brain (stickleback) and skin (trout) cytosols. Neither androgen bound to the cytosol of the kidney. These results indicate that there is a specific binding of 11KT in the stickleback kidney, but that the nature of the binding component may be different from traditional steroid receptors.

PII-53

EFFECTS OF SUBSTRATE CONCENTRATIONS ON STEROIDOGENESIS IN OVARIES AND TESTES OF THREE SPECIES OF AMBISEXUAL FISHES.

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Gonads (100 mg) of sheim (*Acanthopagrus latus*), sobaity (*Sparidentex hasta*) and hamoor (*Epinephalus coioidus*) were incubated with radiolabelled 17-hydroxyprogesterone together with 0, 0.2, 2 or 20 μg unlabelled 17-hydroxyprogesterone. In males, 11β-hydroxyandrostenedione and polar reduced products predominated at low substrate concentrations while at high levels, 17,20αP predominated. In females, aetiocholanolone and polar reduced metabolites predominated at low substrates, and 17,20αP was again the major product at high substrate levels. The results confirm those found in recent studies with cyprinid fish in showing that precursor substrate may affect *in vitro* steroidogenesis, and in particular increased substrate may induce a steroidogenic switch from androgen to progestogen synthesis which is characteristic of the *in vivo* plasma steroid changes during final maturation of the gametes in both sexes.

PII-54

OVARIAN RECEPTORS FOR 17α,20β,21-TRIHYDROXY-4-PREGNEN-3-ONE (20β-S) IN STRIPED BASS

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Plasma 17α,20β-dihydroxy-4-pregnen-3-one (DHP) and 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S) levels increase in striped bass undergoing final oocyte maturation (FOM) and both hormones are produced by ovarian fragments undergoing hCG-induced germinal vesicle breakdown (GVBD) in vitro. In the present study, we investigated the binding of DHP and 20β-S to ovarian membranes prepared from striped bass undergoing FOM. Saturable binding sites for DHP were not detected on ovarian membranes. Saturation of 20β-S binding sites occurred within 30 minutes at 0° C and the binding was pH dependent. Scatchard analysis revealed the presence of a single class of high affinity, limited capacity 20β-S binding sites. Only low levels of specific binding were detected on membranes from testes, muscle, liver, and brain. Ovarian membranes prepared from vitellogenic females also had low levels of specific 20β-S binding. DHP was ~200 times less effective than 20β-S for displacing 20β-S from the ovarian membranes. In contrast, 20β,21-dihydroxy-4-pregnen-3-one was very effective, although it is only a weak inducer of oocyte GVBD in vitro. Of several other steroids tested, only progesterone showed any affinity for the 20β-S binding site within a physiological range of concentrations. Taken together with previous studies of striped bass FOM, these findings indicate that 20β-S is a maturation-inducing steroid hormone in striped bass. This work was supported by a grant from the University of North Carolina Sea Grant College Program (NA90AA-D-SG-062) and a PHS grant (ESO 4216).

PII-55

PURIFICATION AND CHARACTERIZATION OF A PLASMA SEX-STEROID BINDING PROTEIN IN THE SPOTTED SEATROUT (*Cynoscion nebulosus*)

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A plasma sex-steroid binding protein (SBP) was purified and characterized in the spotted seatrout, *Cynoscion nebulosus*. Competition studies demonstrated that the seatrout SBP had high affinities for a number of estrogens and androgens, with exception of 11-ketotestosterone, and little affinity for C_{21}-steroids including cortisol and the maturation-inducing steroid, 20β-S. The rates of steroid association and dissociation were very rapid with a $t_{½}$ of less than 30 seconds for ligand association and 90 seconds for ligand dissociation.

Plasma SBP concentration and binding affinity did not differ significantly between male and female spotted seatrout. SBP levels increased with the stage of ovarian recrudescence in the females, with the lowest levels (approx. 300 nM) in regressed females and the highest levels of (approx. 470 nM) in females with fully developed ovaries. In addition, the steroid dissociation constant (K_D) increased from less than 5 nM in fish with regressed ovaries to greater than 6.5 nM in vitellogenic fish.

The seatrout SBP was purified to homogeneity by acetone and ammonium sulphate precipitation, anion exchange chromatography, gel filtration chromatography, preparative native PAGE and reverse phase HPLC. The purified SBP migrated as a dimeric protein with a molecular mass of 135 *kDa* on native PAGE and dissociated into subunits with molecular masses of 49 to 52 *kDa* on SDS-PAGE. The SBP subunits consisted of a single protein with an N-terminus having little "homology" to any presently known protein sequences.

PII-56

IN VITRO STEROIDOGENESIS BY THE GONADS AND SPERMATOZOA OF THE GROUPER (*EPINEPHELUS TAUVINA*) IMPLANTED WITH 17α-METHYL-TESTOSTERONE.

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Steroidogenesis by the gonadal tissues of the protogynous grouper (*Epinephelus tauvina*) was examined during sex inversion induced by implantation with 17α-methyltestosterone. Incubations of gonadal tissues of the fish with [^{3}H]testosterone resulted in the biosynthesis of 5β-androstane-3β,17β-diol and 5β-dihydrotestosterone as free and conjugated steroids in the male and female phases. However, there is a shift towards the production of 11β-hydroxytestosterone and 11-ketotestosterone as the major free metabolites at the completion of sex inversion. The steroidogenic potential of the sperm isolated from these sex-inversed males was examined by incubating them with [^{3}H]17-hydroxyprogesterone. 17,20α-dihydroxy-4-pregnen-3-one (17,20α-P) and 5β-pregnane-triols were produced, indicating that 17,20α-P may play a role in regulating spermiation in the grouper.

PII-57

IDENTIFICATION OF OVARIAN STEROIDS PRODUCED DURING THE REPRODUCTIVE SEASON IN CHANNEL CATFISH (*Ictalurus punctatus*)

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To date, estradiol, testosterone and one of two maturation inducing steroids have been shown to be produced by teleost oocytes. These steroids are vital for successful reproduction. However, many teleost species produce a suite of steroids during oocyte development, many of which have no known biological function. The steroidogenic nature of the channel catfish has not been thoroughly investigated, in spite of its economic importance. This study reports the diversity of ovarian steroid products of the channel catfish. Oocytes of sexually mature catfish were crushed in Mg^{++}- and Ca^{++}-free Dulbecco's phosphate-buffered saline to obtain follicular envelopes free of yolk proteins. The follicular preparation was incubated in modified L-15 supplemented with tritiated steroids (pregnenolone, progesterone, 17α-hydroxy-progesterone, DHEA, androstenedione, or testosterone). The methylene chloride extracts of these incubations were analyzed by HPLC. In order to verify idenification, steroid metabolites were derivitized and subjected to TLC analysis. Catfish oocytes were evaluated during both vitellogenic growth and during gonadotropin-induced oocyte maturation. Androstenedione, testosterone, and estradiol were positively identified as major ovarian steroids produced during the vitellogenic phase of the reproductive season in channel catfish. Another major steroid product of pregnenolone metabolism has yet to be fully identified. There are four additional steroids for which incomplete structural information is available.

PII-58

SPAWN INDUCTION ON ROSY BARB *Barbus conchonius* WITH $F_{2\alpha}$ PROSTAGLANDIN.

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The prostaglandins, carboxylic acids with similar effects like hormones have been used to spawn induction. They have luteolitic effect and induce smooth muscle contraction. We compare the response of male and female *Barbus conchonius* (Rosy Barb) to immersion in $F_{2\alpha}$ prostaglandin $F_{2\alpha}$PG (0.1 mg/500 ml x 5 minutes) and the combination of human chorionic gonadotrophin HCG (50 UI oral) and the same immersion in $F_{2\alpha}$PG. On the other hand we compare the efficiency of local application on genital pore (0.4 µg) and immersion (0.0484 mg/500ml x 3 minutes). On first design there exist signifficative differences between $F_{2\alpha}$PG, HCG-F2αPG and control treatments on females; no differences were observed on males. On second design local application result less effective than immersion on males, no differences were observed on females.

PII-59

EPIDERMAL GROWTH FACTOR ENHANCES OVARIAN PROSTAGLANDIN SYNTHESIS IN THE GOLDFISH

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Earlier studies have shown that goldfish vitellogenic ovarian follicles are responsive to epidermal growth factor (EGF) and transforming growth factor alpha (TGFα). These peptides inhibited gonadotropin stimulated steroid production while enhancing DNA synthesis. EGF and TGFα) may play a role in other aspects of ovarian function as these growth factors affect prostaglandin (PG) biosynthesis in a variety of mammalian tissues. A series of experiments were conducted to test the effects of munine EGF on PG E_2 synthesis by vitellogenic ovarian follicles of the goldfish. EGF alone had no effects on PG products but caused a dose related increase in the conversion of exogenous arachidonic acid to PG E_2. By comparison, no effect on PGE_2 production was observed when EGF was added to follicles stimulated with calcium ionophore- A23187, alone or in combination with phorbol myristate acetate; together activators of the DAG/IP_3 signalling pathway. These findings suggest involvment of EGF in PG biosynthesis at a point post-AA liberation from phospholipid storage and in light of earlier studies implicate EGF as an integral regulator of ovarian development in the goldfish.

PII-60

IN VITRO STEROID BIOSYNTHESIS BY GONADS OF A HYBRID STURGEON, BESTER, AT DIFFERENT DEVELOPMENTAL STAGES

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Bester, a hybrid sturgeon, could not spermiate or ovulate in the absence of exogenous hormone treatment. In this study, *in vitro* steroidogenic ability of gonads to produce 11-Ketotestosterone(11KT) in male and E2 in female and 17α,20β-dihydoroxy-4-pregnen-3-one(DHP) in both sexes was examined. In addition, LHRH induced spermiation response in male and ovulation in female as well as *in vitro* induction of oocyte maturation were studied to look for clues in endocrine control of gonadal maturation. Testicular fragments or ovulation follicles at different developmental stages were incubated in the presence of five precursors. Steroid production in the media was measured by radioimmunoassay. Male and female fish were injected with LHRH, and the occurrence of spermiation and ovulation was examined while the serum DHP levels were measured. Full grown oocyte were incubated in the presence of steroids(10 kinds), forskolin, HCG and salmon pituitary extract(SPE) and the status of germinal vesicle breakdown(GVBD) observed.

Higher concentrations of 11KT during late spermatogenesis in male and E2 during vitellogenesis in female and DHP at the final stage of gonadal development in both sexes were detected in media incubated with precursors. LHRH treatment caused spermiation and ovulation in majority of fish with gonads at late stage of its development and serum concentration of DHP were elevated in these spermiated and ovulated fish. GVBD occurred in oocytes in the presence of DHP and several other preparations. These results indicated a good correlation between gonadal developmental stage and the ability to produce hormone and that, exogenous induction of final maturation could took place when gonads are at the late stage of their development.

PII-61

GONADOTROPIC AND cAMP CONTROL OF STEROIDOGENIC ENZYMES IN MALE CHANNEL CATFISH, *Ictalurus punctatus*

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Appropriate and timely changes in steroidogenesis are vital for reproductive success of all vertebrates. Changes in steroid secretion are a consequence of changes in the activity of steroidogenic enzymes. In spite of the economic importance of channel catfish, gonadal steroidogenesis and its control have been poorly studied. This study determined the induced changes in mRNA abundance of steroidogenic enzymes in catfish testes. Incubations of catfish testicular tissue in modified L-15 media supplemented with hCG or dibutryl-cAMP stimulated androgen synthesis in a dose related manner. Total RNA was isolated from control and induced testicular tissue, and subjected to northern and slot blot analysis. The blots were evaluated for abundance of mRNA encoding for key steroidogenic enzymes: cholesterol side chain cleavage (P450scc), 17α-hydroxylase (P450c17), and 3β-hydroxysteroid dehydrogenase (3β-HSD). Results indicated that specific changes in mRNA levels of steroidogenic enzymes were correlated with induction of steroidogenesis by gonadotropin and cAMP. The molecular control of steroidogenic enzymes has not been studied in fish to date and only poorly studied in other non-mammalian vertebrates.

PII-62

PEROXISOME INVOLVEMENT IN OVARIAN STEROIDOGENESIS IN TELEOSTS

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Peroxisomes are organelles present in most eukaryotic cells and are important in the metabolism of long chain polyunsaturated fatty acids (PUFAs), eicosanoids, and cholesterol. Peroxisomal activity is abundant in rat Leydig cells and inducible by gonadotropin. PUFAs and peroxisome proliferator (PP) compounds induce transcription of peroxisomal enzymes by way of a receptor of the steroid-retinoic acid superfamily; the peroxisome proliferator activated receptor (PPAR). Treatment of goldfish ovarian follicles *in vitro* with a range of PUFAs (arachidonic, eicosapentaenoic, docosahexaenoic, linolenic, and linoleic acids) attenuated gonadotropin-stimulated steroid production independent of their conversion to eicosanoids. The action of PUFAs was rapid, reversible, independent of cAMP levels, but affected the supply of cholesterol to the steroidogenic pathway. Treatment of ovarian follicles with the hypolipidemic drugs clofibrates and Wy-14643 which are PP attenuated gonadotropin-steroid production similar to studies with PUFAs. The presence of peroxisomes in goldfish ovarian follicles was confirmed using catalase activity as marker. These results suggest involvement of peroxisomes in ovarian steroidogenesis and underscore the importance of PUFAs as regulators of ovarian function.

PII-63

STEROID LEVELS DURING OVULATION AND SPERMIATION IN TOADFISH (*HALOBATRACHUS DIDACTYLUS*)

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Steroid levels were investigated during ovulation and spermiation in toadfish. Two groups of mixed sex toadfish were injected either with 100μg/fish LHRHa in saline or saline only. Most females in both groups ovulated and most males in both groups produced some sperm during the experiment. Females plasma levels of 17α,20β,21-trihydroxy-4-pregnen-3-one, which had been previously identified as the major ovarian steroid metabolite, ranged from 1 to 5 ng/ml and levels were not related to ovulation time. Similar levels were found for 17α,20β-dihydroxy-4-pregnen-3-one and its 3α,5β-reduced metabolite. In males, low levels of these hormones were also obtained. However, an increase in 11-ketotestosterone plasma levels (from 1-2 to 8-9 ng/ml) was observed in males of the control group within 24 hr prior to ovulation of females kept in the same tank. In order to test for possible pheromonal effects, females were injected with toadfish pituitary extract and males were placed isolated in tanks receiving water from these females. Males exposed to water from ovulated females did not show any significant changes in hormone levels, although they produced noticeable amounts of sperm, in comparison to males smelling non-ovulating females. In conclusion, we suspect that toadfish produce steroid hormone metabolites that we were not able to detect with our RIAs and there was no clear evidence for the use of sex pheromones by this species.
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PII-64

IMMUNOLOCALIZATION OF STEROIDOGENIC CELLS THROUGHOUT GONADAL RESTRUCTURING IN THE PROTOGYNOUS HERMAPHRODITE *THALASSOMA DUPERREY*.

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Sex change, a common phenomenon in fishes, requires significant reorganization of gonadal anatomy and reproductive physiology. Throughout the course of protogynous sex change, ovarian tissue degenerates as testicular tissue develops. The gonadosomatic index decreases considerably after sex-change yet total steroid production, particularly 11-KT production, significantly increases. To identify the source of several steroid hormones during sex change, we utilized immunocytochemistry methods to detect P450scc, 3β-HSD and P450arom in the protogynous hermaphrodite *Thalassoma duperrey*. In the early stages of sex change, immunoreactive P450scc localizes in thecal cells. As gonadal restructuring progresses, P450scc becomes localized in interstitial (Leydig) cells, with a marked increase in immunoreactive cell number during the period of active spermatogonial proliferation. Similarly, 3β-HSD is initially localized in thecal cells and later in interstitial cells. In contrast, P450arom is initially localized in granulosa cells. In latter stages of sex change, however, P450arom could not be detected. Very low concentrations of circulating estradiol in sex changed fish support this observation. A preliminary comparison of the total number of steroid producing cells between sex-changed and initial sex individuals suggests sex-changed individuals actively produce more steroid hormones, a conclusion also supported by circulating steroid hormone concentrations. In conclusion, these results describe changes in the localization of key steroidogenic enzymes throughout the process of sex change and represent a critical step in elucidating the endocrine mechanisms of sex change.
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PII-65

MATURATION INDUCING STEROID IN TURBOT, *SCOPHTHALMUS MAXIMUS L.*

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In vitro steroidogenesis was examined using ovaries of turbot, a multiple spawning fish. Ovarian fragments have been sampled during the spawning season and incubated with tritiated or radioinert 17α-hydroxy-4-pregnene-3,20-dione (17-P). 17,21-dihydroxy-4-pregnene-3,20-dione (S) and 17,20β,21-trihydroxy-4-pregnene-3-one (20β-S) have been found in both types of incubation, using thin layer chromatography, high pressure liquid chromatography, microchemical reactions and atmospheric pressure chemical ionization coupled with mass spectrometry. 17,20β-dihydroxy-4-pregnene-3-one (17,20β-P) was not detected.
Biological activity of 15 steroids has been tested *in vitro* on ovarian fragments. 20β-S was the most effective stimulator of oocyte maturation and ovulation (less than 1 ng/ml induced 50% ovulation). S showed a significant activity but it was weaker than 20β-S.
We suggest that 20β-S is a Maturation Inducing Steroid in turbot.

PII-66

REGULATION OF STEROIDOGENESIS IN THE STINGRAY INTERRENAL

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Steroidogenesis of the elasmobranch interrenal is regulated by both adrenocorticotrophic hormone (ACTH) and angiotensin II. However, the molecular mechanism involved in this control is unknown. As a first step, this study describes cholesterol side chain cleavage (cytochrome P450scc) and 3β-hydroxysteroid dehydrogenase (3β-HSD), and their role in the control of steroidogenesis. These enzymes are crucial to the synthesis of steroids hormones in all vertebrates. Degenerate primers and RT-PCR were used to generate specific, although short (284 bp), cDNAs that were used as probes for screening a cDNA library made from the southern stingray (*Dasayatis americana*) interrenal. Partial-length cDNAs were isolated for both enzymes. The deduced amino acid sequence of the stingray forms of P450scc and 3β-HSD were only 35-38% identical to bovine forms and 44-45% identical to trout forms of these enzymes. *In vitro* studies showed that the ACTH-induced synthesis of 1α-hydroxycorticosterone in the Atlantic stingray (*Dasayatis sabina*) could be blocked by the protein synthesis inhibitor, cycloheximide. Northern blot analysis illustrates that the ACTH- and angiotensin-induced stimulation of steroidogenesis of the stingray interrenal was correlated with an increase in mRNA abundance of both P450scc and 3β-HSD.

PII-67

17α,20β-DIHYROXY-4-PREGNEN-3-ONE STIMULATES CORTISOL PRODUCTION BY RAINBOW TROUT INTERRENAL TISSUE *IN VITRO*: MECHANISM OF ACTION

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The teleost oocyte-maturation-inducing steroid 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P) stimulated a dose-dependent, and highly reproducible, increase in cortisol production by rainbow trout head kidney preparations (interrenal tissue) *in vitro*. The effect of 17,20β-P was rapid, with cortisol production significantly enhanced above control levels within one hour. Estradiol-17β, testosterone, and 11-ketotestosterone had no effect on cortisol production, whereas 17α,20α-dihydroxy-4-pregnen-3-one was 50% as effective as 17,20β-P. [3H]17,20β-P was not converted by head kidney preparations to [3H]17α-hydroxyprogesterone or [3H]cortisol. Neither actinomycin-D nor cycloheximide (10, 100, or 1000 ng/ml) inhibited the stimulatory effects of 17,20β-P on cortisol production. The general calcium channel blocker $CoCl_2$ (0.1, 1 or 5 mM) or incubation in low calcium medium also had no effect on 17,20β-P-stimulated cortisol production. H-89, a potent inhibitor of cAMP-dependent kinase (PKA), inhibited the effects of 17,20β-P on cortisol production in a dose-dependent manner (doses ranging from 1 to 100 μM), whereas the less potent PKA inhibitor H-8 was ineffective in this regard. Taken together, the results suggest that 17,20β-P may be a physiological regulator of cortisol secretion in peri-ovulatory fish, and that 17,20β-P may act on interrenal cells to enhance cortisol production by a rapid, cAMP-mediated mechanism. The stimulation of cortisol secretion by 17,20β-P may explain the hypercortisolism associated with programmed death in semelparous salmonids.

PII-68

RADIOIMMUNOASSAY OF 5β-PREGNANE-3α,17α,20β-TRIOL IN SPERMIATING MALE PLAICE, *PLEURONECTES PLATESSA*.

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In male salmonids, it is possible to monitor the effects of injected hormones (such as Gonadotropin Releasing Hormone; GnRH) and pheromones by measuring the levels of 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P) in blood plasma. In male plaice, however, levels of this steroid are 100 times lower than those in male trout and only show a slight response to GnRH. We have now found, however, that the plasma of spermiating male plaice contains substantial quantities of sulphated 5β-pregnane-3α,17α,20β-triol (a metabolite of 17,20β-P). We have developed two radioimmunoassays for this compound - one of which, though direct, has the disadvantage that it cross-reacts with sulphated 3α,17α,21-trihydroxy-5β-pregnan-20-one - the other of which, though more specific, requires the samples to be solvolysed prior to assay. Using either assay, large differences in steroid levels are found between male plaice caught on or off their spawning grounds in the North Sea. A substantial and prolonged rise in steroid levels is also found in males treated with GnRH analogue via sustained-release delivery systems.

PII-69

CHARACTERIZATION OF AN ANDROGEN RECEPTOR IN SALMONID LEUKOCYTES: LINK TO ANDROGEN INDUCED IMMUNOSUPPRESSION.

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Cytosol of rainbow trout (*Oncorhynchus mykiss*) leukocytes demonstrated specific and saturable binding of ^{3}H-testosterone (K_d = 1.98 nM ± 0.46 nM and B_{max} = 17.8 ± 5.31 fmol/mg protein). Specific binding of ^{3}H-testosterone was restricted to leukocytes and other tissues with known androgen binding affinity. Specific binding of ^{3}H-testosterone was displaced by testosterone and dihydrotestosterone. Androstenedione effectively displaced bound ^{3}H-testosterone between 10 and100-fold excess, while 17α-methyltestosterone, 11-ketotestosterone, and progesterone were effective only between 100 and 500-fold excess. Cortisol, 17β-estradiol, 17α,20β-dihydroxyprogesterone, the synthetic androgen mibolerone, and the synthetic estrogen ethenylestradiol did not significantly displace ^{3}H-testosterone binding, even at 500-fold excess. Treatment of cytosol with proteolyic enzyme significantly reduced the specific binding of ^{3}H-testosterone. These data strongly suggest that androgen receptors exists in salmonid leukocytes and support the hypothesis that these receptors play a role in androgen induced immunosuppression. In vitro experiments with trout leukocytes indicate that cell death, rather than reversable deactivation, may be responsible for androgen induced immunosuppression.

PII-70

ISOLATION AND CHARACTERIZATION OF THE cDNA ENCODING THESPINY DOGFISH SHARK (*Squalus acanthias)* FORM OF CYTOCHROME P450c17.

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Cytochrome P450c17 is a key steroidogenic enzyme for the production of steroids in gonadal and adrenal tissue. This single protein possesses two enzymatic activities (17α-hydroxylase and C17,20-lyase). A cDNA library was constructed from poly(A)-enriched mRNA isolated from spiny dogfish shark (*Squalus acanthias*) testis and ligated into *Eco*RI-cut lambda arms. Five positive clones were isolated from the amplified library using a bovine P450c17 cDNA probe. The shark cDNA encompasses 23 bp of the 5'-untranslated region, a 1527 bp open reading frame, and 414 bp of the 3'-untranslated region. A putative polyadenylation signal (AATAAA) is 18 bp from the poly(A) tail. Northern blot analysis showed a single transcript of 1.9 kb, thus indicating the isolated clone is a full-length cDNA. The deduced amino acid sequence of the shark form of P450c17 is 59% and 57% identical to the rainbow trout and chicken forms, respectively. The shark form is 43% to 46% identical to mammalian forms. The deduced protein is 509 residues in length with a predicted weight of 57.2 kDa. The shark protein, heterologously expressed in yeast and COS cells, was capable of both 17α-hydroxylase and C17,20-lyase activities using pregnenolone and progesterone as initial substrates. The shark cDNA was modified to facilitate production in *E. coli* and purification of the resultant protein by a single column technique. This purified protein was used to raise antisera against dogfish shark P450c17 for western blot analysis.

PII-71

EFFECT OF LHRH ON SEX REVERSAL AND STEROID LEVELS IN GILTHEAD SEABREAM (*SPARUS AURATA*)

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The initial objective of the work was to study the long term effect of luteinizing hormone-releasing hormone analog (LHRHa) on spermiation and steroid levels in male gilthead seabream. Ten one-year-old fish 450g average weight received fortnightly 3 injections of 4μg/kg of des-Gly^{10},[D-Ala^{6}]-Neth-LHRH in 250 μl of 0.8%NaCl, over a period of 50 days. Another group of 10 fish received saline only (control). The experiment started at the end of September at the begining of the spawning season. At the end of experiment there were no significant differences in the number of spermiating fish and gonadossomatic indexes between groups. However, the average percentage volume of male gonadal tissue was 70% in the control group as compared to 30% in the LHRHa group where female tissue predominated. No significant changes in steroid levels (free and conjugated) were observed: estradiol-17β, 17α,20α-dihydroxy-4-pregnen-3-one, 17α,20β-dihydroxy-4-pregnen-3-one, testosterone and 11-ketotestosterone. Steroid differences between groups could be ascribed to two fish and were not related to the effect of treatment. The results indicate that LHRH is involved with the process of sex change in gilthead seabream. The question of whether LHRH acts directly or indirectly in the gonads is under investigation.
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PII-72

OVARIAN DEVELOPMENT IN TRIPLOID BROOK TROUT (Salvelinus fontinalis).

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Numerous studies have demonstrated that triploid males undergo substantial testicular growth, but that ovarian growth is greatly retarded in triploid females. These studies have generally been terminated at the time of first sexual maturation in diploid controls. The purpose of this research was to test the hypothesis that ovarian growth is delayed in triploid females due to an abnormally long period of vitellogenesis. Ovarian development was followed in triploid and control diploid brook trout for the 6-month periods leading up to first sexual maturation (for diploids) as 2-year-olds and second sexual maturation as 3-year-olds. Most triploids had very small ovaries at the time of ovulation in diploids (gonadosomatic indices <0.1%, compared to 20-25% for diploids). Small numbers of early vitellogenic oocytes were observed in triploids, with no difference in the maximum size of these oocytes in the two year-classes. The exception was a single 3-year-old triploid which ovulated a substantial number of large, vitellogenic oocytes that were variable in size and had apparently undergone asynchronous development. A model for ovarian growth in triploids will be presented, suggesting that estrogen-mediated vitellogenesis progresses at a very slow rate, a reflection of the small number of developing oocytes and associated follicles.

PII-73

ISOLATION AND CHARACTERIZATION OF A VITELLOGENIN RECEPTOR IN WHITE PERCH, *MORONE AMERICANA*

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Receptors for white perch vitellogenin (wVTG) were characterized using wVTG, labeled in vivo with ^{3}H-leucine or in vitro with ^{125}I, and semipurified ovarian membranes. Specific binding of wVTG to the membranes was temperature-dependent, proportional to the amount of membrane and saturable. Scatchard analyses revealed a single class of binding sites of high maximum binding capacity (MBC ~35 pmol VTG / mg membrane protein) and low affinity (Kd ~400 nM), consistent with wVTG levels (540 to 2700 nM) circulating in maturing females. Ligand blotting revealed a receptor protein of $M_r \simeq 157{,}000$ and a smaller protein likely to be its degradation product. Striped bass vitellogenin, chicken egg yolk very low density lipoprotein and suramin displaced wVTG from its receptor, but bovine serum albumin did not. No change in Kd was noted over the course of vitellogenesis in maturing perch and MBC increased only slightly very late in the gametogenic cycle. The wVTG bound specifically to membranes prepared from liver, muscle, and mesenteric fat, but not to erythrocyte membranes. The Kd for ovary (394 nM) and liver (345 nM) were similar, but the Kd for muscle (1440 nM) was much lower. This work was supported by grants from the University of North Carolina Sea Grant College Program (NA86AA-D-SG062 and NA90AA-D-SG062) and the National Coastal Resources Research and Development Institute (NA87AA-D-SG065, #2-5606-22-2).

PII-74

VITELLOGENIN RECEPTOR EXPRESSION IN THE MEDAKA, *ORYZIAS LATIPES*

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An ovary developmental time course was performed with medaka to look at the changes in the ovary with regard to oocyte development and vitellogenin receptor expression after exposure to spawning conditions (14 h L, 10 h D, 27°C) or non-spawning conditions (14 h L, 10 h D, 22°C) for 0, 4, 8, 12, 16 and 42 days. Analysis of variance was used to determine the effect of temperature and days at temperature on body weight, gonosomatic index (GSI), and vitellogenin receptor density. Vitellogenin receptor density was measured as vitellogenin binding per μg of solubilized oocyte membrane protein in a ligand blotting assay and adjusted for the yield of membrane proteins per ovary. There were significant differences in body weight ($P<.05$) and GSI ($P<.0001$) between temperature treatments over the time course. At 22°C, the vitellogenin binding per ovary increased 5-fold and reached a plateau by day 12. At 27°C, there was an 11-fold increase in vitellogenin binding per ovary by day 16. The differences in receptor densities between the two temperatures were significant ($P<.0001$). By 42 days, the fish at 27°C spawned daily, while a few fish at 22°C spawned sporadically. The GSI of the fish at 27°C was 4 to 8 fold larger then the fish at 22°C, however, the vitellogenin binding per ovary was only a 2 to 3 fold greater in the fish at 27°C than 22°C. These results indicate that receptor synthesis occurs early in oocyte development prior to vitellogenic growth. Water temperature can be used to separate the two processes for studies of vitellogenin receptor expression and vitellogenic growth in the medaka.

PII-75

IN VITRO HORMONAL CONTROL OF VITELLOGENIN SYNTHESIS IN TWO MARINE SPECIES *DICENTRARCHUS LABRAX* AND *SPARUS AURATA*

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Vitellogenin (VTG) is the precursor of the yolk proteins lipovitellin and phosvitin in all oviparous vertebrates so far studied. The synthesis of VTG is a hormone-dependent process, and estradiol-17ß seems to be the factor mainly responsible for synthesis and release in the blood circulation. At present, the involvement of pituitary hormones in inducing hepatic vitellogenin synthesis must be considered. In the present study, the effects of homologous pituitary homogenate (HPH, 1/20 eq gland), estradiol 17-ß at different doses (10-4 to 10-8 M), as well as different doses of bream GH (10 ng to 10 µg), were tested in both hepatic tissue as well hepatocytes cultures during spawning and non-spawning time in two marine species: the European sea bass *Dicentrarchus labrax* and the gilted seabream *Sparus aurata*. VTG titers in the media were assayed by ELISA. The levels of VTG released in the medium varies depending on the season, since higher levels were found during spawning time in both species studied and very low levels in the non-spawning one. In addition to the expected stimulatory action exerted by estradiol-17ß in both male and female liver, HPH and GH have been found, as well, very significant to increase VTG synthesis and release, compared with the medium. These results indicate that in sea bass and seabream a multihormonal control of vitellogenin synthesis may occur.

PII-76

WILL PUBERTY BE REGULATED BY SEXUAL STEROIDS IN MALE AFRICAN CATFISH (*Clarias gariepinus*)?

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In male African catfish, pubertal development occurs between 10 and 20 weeks of age. During this period testicular development takes place, starting with the proliferation of spermatogonia, and ending with the appearance of spermatozoa. The endocrine system of prevailing importance for the regulation of reproductive processes and hence for puberty is the brain-pituitary-gonad-axis (BPG-axis). In fish sexual steroids can induce precocious sexual maturation. Therefore the hypothesis was adopted that steroids have a positive and morphogenetic effect on the developing BPG-axis. The origin of the steroids could be the immature gonads or the interrenal tissue. In a first approach steroidogenesis in testis and interrenal tissue during different stages of pubertal development was investigated *in vitro*. In all stages, 11ß-hydroxyandrostenedione (OHA) and cortisol were the main products of testes and interrenal tissue, respectively. To check if the *in vitro* results reflect the *in vivo* situation, plasma was analyzed for the presence of OHA and 11-ketotestosterone (11KT). In all stages OHA could be detected, but always on a rather low level (0.15 - 0.88 ng/ml). 11KT, however, was not always detectable during the proliferation of spermatogonia; sometimes low levels could be detected (0.2 - 0.6 ng/ml). With the appearance of spermatids, the levels of 11KT increases about 10 fould (3.7 ng/ml). In order to test steroidal induction of puberty, animals at the onset of puberty were treated with the above mentioned steroids, to monitor the effects on gonadal development and gonadotropic capacity of the pituitary.

PII-77

INHIBITION OF *FUNDULUS* OOCYTE MATURATION *IN VITRO* BY SEROTONIN

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The effects of serotonin (5-hydroxytryptamine, 5-HT) on oocyte maturation *in vitro* were tested on *Fundulus heteroclitus* prematurational ovarian follicles (1.3-1.5 mm in diameter). *F. heteroclitus* pituitary extract (FPE)- and steroid (17α,20β-dihydroxy-4-pregnen-3-one, DHP)-induced oocyte maturation were both inhibited by 5-HT in a dose-dependent and reversible manner. Maximum inhibition was observed at concentration of 0.5 and 5 μM 5-HT, respectively. The inhibition by 5-HT was independent of FPE-induced steroid production by the follicle, since the levels of 17β-estradiol and DHP released into the culture medium were not modified by the presence of 5-HT. When follicles were preincubated with DHP for increasing times, subsequently washed, and transferred to steroid-free, 5-HT-containing medium, inhibition was still observed in follicles preincubated for up to 4 hr; however, inhibition by 5-HT was reduced or eliminated after 8-hr and 16-hr preincubation with DHP, respectively. These results suggest the presence of putative 5-HT receptor(s) that do not affect steroidogenic pathways in the ovarian follicle yet inhibit steroid-induced oocyte maturation at some point downstream in the steroid transduction pathway of the oocyte.

PII-78

THE EFFECTS OF EXOGENOUS ESTRADIOL-17β IN JUVENILE MALES OF PROTANDROUS BLACK PORGY, ACANTHOPAGRUS SCHLEGELI

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Juvenile protandrous black porgy were divided into 4 groups, fed with diet mixed with E2 (0, 0.25, 1 or 4 mg/kg feed) for 6-7 mo. Higher GSI was observed in low dose E2 (0.25 or 1 mg) groups. E2 (4 mg) suppressed spermiation while low dose E2 stimulated spermiation. At 10 mo or older, gonads in all fish except E2 (4 mg)-treated group underwent spermatogenesis while spermatids were not observed until at 11 mo in low dose E2 groups. Primary oocytes in testicular tissues were observed in the 1 and 4 mg E2 groups. After 5 mo treatment, ovary developed in 4 mg E2 group while testis developed in control and low dose E2 groups. Elevated plasma E2 levels were observed only in 4 mg E2 group while low E2 and testosterone (T) levels were observed in all other groups. Higher 11-ketotestosterone (11-KT) levels were observed in low dose E2 groups. Plasma vitellogenin showed a dose response to E2 treatment. The data suggest that exogenous E2 stimulates the development of either testicular or ovarian tissues depending on the dosage of E2. Plasma 11-KT but not T correlates with testicular development and spermiation in protandrous black porgy.

PII-79

UNIVERSAL VERTEBRATE VITELLOGENIN ANTIBODIES.

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Monoclonal antibodies (mAb's) were generated in Balb/C mice using routine methods and vitellogenin (Vg) purified from either rainbow trout (Oncorynchus mykiss) or striped bass (Morone saxatilis) as the anitigens. Those mAb's showing specific cross-reactivity to Vg from both trout and bass were selected for further screening. ELISA and Western blot analyses showed that the mAb's specifically recognize Vg in representative species from 4 vertebrate classes (fish, amphibians, reptiles and birds), including several species of teleost fish. All the mAb's were IgM class. N-terminal amino acid sequence analysis of Vg showed extensive homologies within and across vertebrate classes. A rabbit polyclonal antiserum was raised against a synthetic peptide representing the consensus N-terminal Vg sequence. Western blots indicated that the antiserum specifically recognizes Vg from teleost fish of diverse families. Our results indicate that it is feasible to generate antibodies capable of recognizing Vg without regard to species and that development of a 'universal' Vg assay is an achievable goal. This research was supported by a grant from the N.C. Biotechnology Center (#9113-ARIG0803), a graduate fellowship (for SAH) from the Electric Power Research Institute, and a cooperative agreement (#CR821437) between the U.S. Environmental Protection Agency and the University of Florida.

PII-80

THREE EGG YOLK PROTEINS ARE DERIVED FROM SALMON VITELLOGENIN

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Vitellogenin (Vg) and its 3 egg yolk protein products lipovitellin (Lv), phosvitin (Pv) and β'-component (β'), were isolated from mature female huchen (*Hucho perryi*). Vg was purified by precipitation in distilled water followed by column chromatography on Sepharose 6B. Yolk proteins were isolated according to Markert & Vanstone (1971, J. Fish Res. Bd. Can., 28, 1853-1856) and rechromatographed on Superose 6 and Sephadex G-75 to estimate mol. weight. Vg had an apparent mol. wt. of 540 kDa after FPLC on Superose 6, appeared as a major 240 kDa band in SDS-PAGE which resolved into two major bands (165 and 125 kDa) after reduction. The mol. wt. of purified Lv, Pv and β' were 330, 23 and 30 kDa, respectively. Lv appeared as a main band of 150 kDa in SDS-PAGE which resolved into two smaller bands (92 and 29 kDa) after reduction. β' appeared as a 34 kDa band and a 17kDa band after reduction. Lv and β' reacted with antiserum against Vg , but Pv did not. In SDS-PAGE, Pv appeared as two main bands (23 and 20.5 kDa) and a diffuse band (< 6.5 kDa) which stained with CBB + aluminum mordant. Purified yolk proteins were injected into rabbits to prepare antisera. Only Pv had no antigenicity. When Pv was dephosphorilated by alkaline phosphatase (Ap), an antiserum to it was prepared. The antiserum (pre absorbed by Ap) reacted with dephosphorilated Pv and Vg, but not with Lv, β' and Ap in Western blotting. These results are the first immunological proof that 3 egg yolk proteins (Lv, Pv and β') are derived from Vg in fish.

PII-81

Influence of natural vitellogenesis and estradiol-treatment on hepatic protein synthesis and gluconeogenesis in *Zoarces viviparus*.

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Administration of estradiol to male fish is known to induce profound metabolic changes, which are normally related to the process of vitellogenesis in sexually mature female fish.

The interaction between amino acids and glucose in gluconeogenesis was investigated in estradiol-treated males and females of the viviparous blenny *Zoarces viviparus* in comparison with females in natural vitellogenesis. A steady increase was observed in the capacity of the liver to incorporate ^{14}C-phenylalanine into protein during the course of natural vitellogenesis of the female fish. No significant changes could be observed in the rate of gluconeogenesis or the oxidative metabolic flux of isolated hepatocytes in these fish. Estradiol-treatment of females in vitellogenesis and of males resulted in a marked increase in the polyphenylalanine synthesizing capacity of the liver and a concomitant decrease in the rate of gluconeogenesis as indicated by a decrease in the rate of *de novo* glucose synthesis from labeled alanine and by a decrease in activity of enzymes associated with the process of gluconeogenesis and amino acid metabolism.

Estradiol-treatment of males performed early in the reproductive season resulted in a marked reduction of the testicular size and weight. When the hormone treatment was initiated later during the reproductive season no such effect could be observed.

PII-82

CORRELATION BETWEEN PLASMA AND EGG STEROID HORMONE CONTENT OF ARCTIC CHARR

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As part of ongoing studies into the physiological significance of yolk hormones in salmonid species, we examined the steroid hormone profile (including total, sulfated and glucoronidated) of plasma and eggs of Arctic charr, *Salvelinus alpinus*, using HPLC methods that permit the separation and identification of 20 hormones and metabolites. All major steroid hormone classes were present in both the plasma and yolk; although ratios of different hormones differed reflecting either relative differences in the efficiency of transfer of the different molecules into the egg, or the timing of the collection of plasma relative to the time at which major steroid hormone incorporation occurred. Changes in the content of steroid hormones in the yolk of embryos is described.

PII-83

ANALYSIS OF DIFFERENTIALLY EXPRESSED mRNAs DURING SPERMATOGENIC DEVELOPMENT IN THE SHARK TESTIS

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Spermatogenesis is a unique developmental sequence, involving the functional interdependence of germ cells and Sertoli cells. Using the shark testis which allows isolation of germ cell/Sertoli cell units (spermatocysts) at defined stages, we have begun to characterize developmentally-expressed known and unknown genes by analysis of their mRNAs. In one approach, using a rockcod B-tubulin cDNA to screen a shark specific cDNA library, we isolated and characterized a shark specific B-tubulin clone. Northern analysis of tissues in premeiotic (PrM), meiotic (M) and postmeitoic (PoM) stages, revealed stage-specific (2.9 kb: PrM) and stage-dependent (1.4 kb, M>>PrM=PoM; 2.6 kb, PrM=PoM>>M) transcripts. Analysis of additional tubulin clones is in progress. In a second approach, we used the polymerase chain reaction-differential display (PCR-DD) technique and multiple primer pairs to obtain a series of cDNA banding patterns or "fingerprints" representative of expressed mRNAs at each stage. Of 180 total bands, 17% were reproducibly stage-specific or -dependent. A subset of constant vs. stage-related cDNA bands was selected for cloning, sequencing and use as hybridization probes. Although their germ cell vs. Sertoli cell origin remains to be detemined, results show this panel of cDNAs has utility as maturation markers for <u>in vivo</u> and <u>in vitro</u> regulatory studies. Supported by NICHD16715 (GVC) and the Endocrine Society (HK).

PII-84

REGULATION OF HYDROMINERAL BALANCE AND SPERMIATION BY mGtH AND PROLACTIN IN FRESHWATER CATFISH, HETEROPNEUSTES FOSSILIS

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Apparently no regulatory mechanism of testicular hydration prior to spermiation is available in teleost. Therefore, to explore the factors responsible for testicular hydration, seasonal variation in water content, Na^+ & K^+ concentrations and Na^+K^+ ATPase activity in testes and role of ovine prolactin & <u>Mystus</u> GtH in their regulation were studied. Testicular water, Na^+ & K^+ concentrations exhibited seasonal fluctuations with their peak values in spawning phase coinciding with spermiation. Hypophysectomy caused significant reduction in testicular levels of water, Na^+ & K^+ as well as Na^+K^+ ATPase activity and inhibited spermination. Prolaction treatment recovered water and K^+ levels partially but Na^+ and Na^+K^+ ATPase activity fully. Administration of mGtH also recovered water and K^+ partially, and had no effect on Na^+ and Na^+K^+ ATPase activity. Combined treatment of PRL and mGtH restored normal levels of all parameters. Thus, it appears that water intake by testes occurs due to high osmotic pressure caused by Na^+ and K^+ influx under the control of prolactin and mGtH.

PII-85

ESTRADIOL-17β INDUCES VITELLINE ENVELOPE PROTEINS IN 14 TELEOST SPECIES

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Induction of vitelline envelope proteins by estradiol-17β was investigated in 18 teleost species from five systematic groups. Homologous antisera were produced against purified vitelline envelope proteins from rainbow trout, brown trout, turbot, European sea bass and gilthead sea bream. Using Western blot technique, vitelline envelope proteins were detected in plasma from fish injected with estradiol in all five species. Estradiol induction experiments were also conducted with 13 additional species utilizing three heterologous antisera directed against vitelline envelope proteins from rainbow trout, turbot or halibut for immuno detection. Vitelline envelope proteins were induced and detected in plasma from 9 of these species, but the immunoreactivity of the proteins varied considerably within and between species. Of totally 18 species investigated, induction of vitelline envelope proteins was demonstrated in 14 species. In 9 of these species males were used, which demonstrates that the synthesis of vitelline envelope proteins is not confined to the ovaries. It is suggested that the synthesis of vitelline envelope proteins is controlled by estradiol-17β in the majority of teleost species.

PII-86

EARLY GONADAL DEVELOPMENT AND SEX DIFFERENITATION IN MUSKELLUNGE (*ESOX MASQUINONGY*)

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We studied the early gonadal development and sex differentiation by histological methods in muskellunge (*Esox masquinongy*). Samples were collected periodically from fish after hatching until they reached 50 mm and from fish larger than 82 mm in total body length in 1992 and 1993, respectively. The primordial germ cells (PGCs) were first identified in fish of 21 mm. The PGCs were accompanied with a few somatic cells. Gonads did not form yet a complete string at this stage. At a fish size of 32 mm, gonads formed a typical spherical shape in cross sections and the PGCs were completely enveloped by somatic cells. Gonads hung on the dorsal peritoneum at the lateral side of the swimming bladder and the gonadal strings were complete. Blood vessels were first found inside the gonads with Crossman staining at a fish size of 50 mm. Some of the PGCs underwent mitotic division at this stage. Gonads of the fish up to 82 mm were still considered as sex undifferentiated. At a fish size of 138 mm, one type of the gonad contained dispersed germ cells among stromal cells, similar to that of the early stages, whereas the other contained clusters of the germ cells. Gonads with clusters of germ cells developed into ovaries. This stage marked the onset of the morphological sex differentiation. Germ cells had proliferated in both sex of fish examined at a size of 211 mm. Female gonads contained lobes of germ cells, some of them enlarged. Germ cells of testes still resembled the PGCs in morphology.

PII-87

LIPID CONTENTS OF FEMALE STRIPED BASS PLASMA AND OOCYTES EXHIBIT SEASONAL CHANGES ASSOCIATED WITH OOCYTE MATURATION.

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Total protein and lipid concentrations in plasma of eight male and eight female captive striped bass (*Morone saxatilis*) were monitored monthly over the course of two reproductive cycles as part of an effort to investigate the time course of lipid mobilization and subsequent deposition in the oocytes of mature females. Vitellogenin levels in the plasma of these fish had been previously determined for each sampling date used in the study. Total lipid concentrations in the plasma of both males and females showed seasonal fluctuations with the highest levels in the late Spring after spawning and during the Fall. Total lipid concentrations in the plasma of females were significantly lower than those of the males during the four months preceding spawning when vitellogenin levels were at their highest. Analysis of the lipid class composition of the plasma lipids revealed that the decrease in plasma lipid concentrations in females prior to spawning is primarily due to a decrease of up to 50% in the phospholipid content of the plasma relative to males. The fatty acyl composition of plasma lipids in these fish did not vary seasonally or with sex. Total protein levels showed seasonal fluctuation, but did not vary with sex. Changes in lipid content of oocytes of captive female striped bass were followed from the onset of vitellogenesis through spawning. The total lipid content, lipid class composition and fatty acyl composition of each lipid class in the ova were determined and correlated with data from histological studies to provide a biochemical time course of lipidation in developing oocytes.

PII-88

PROTEOLYTIC CLEAVAGE OF YOLK PROTEINS DURING OOCYTE MATURATION IN BARFIN FLOUNDER

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Structural and quantitative changes in three classes of vitellogenin (Vg)-derived yolk proteins during oocyte maturation were examined in barfin flounder, *Verasper moseri*, a marine teleost which lays pelagic eggs. Native dimeric lipovitellin (Lv), estimated to be a 410 kDa protein, proteolytically cleaved into two homologous 170 kDa monomeric Lv during the course of oocyte maturation. An additional Vg-derived yolk protein, β'-component, at 19 kDa in native form decreased in amount to less than 12 % in ovulated eggs when compared with that in vitellogenic oocytes. A highly phosphorylated 38 kDa band of phosvitin (Pv) in SDS-PAGE also disappeared during oocyte maturation. Results of quantitative analysis of free amino acids in oocytes showed a reverse correlation with the changes in these three classes of yolk proteins, and showed close similarity to the change in water content of oocytes. The oocyte maturation of this species takes about 7 days at 6 ℃ and all these changes occur mostly during the period between the 4 th and 6 th days. We thus propose that all three classes of yolk proteins (Lv, Pv and β'-component) undergo proteolytic cleavage during the latter half of oocyte maturation and contribute to provide free amino acids utilizing the osmotic effecter for oocyte hydration, which causes the buoyancy of eggs, and so ensures a stock of nutrient during early development.

PII-89

EFFECT OF GnRH PEPTIDES ON HISTONE H-1 KINASE ACTIVITY IN THE FOLLICLE-ENCLOSED GOLDFISH OOCYTES, *IN VITRO.*

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Gonadotropin hormone (GTH) stimulates synthesis of maturation inducing steroid (17α,20β-dihydroxy-4-pregnen-3-one; DHP) which in turn stimulates production of maturation promoting factor (MPF). MPF mediates germinal vesicle break down (GVBD) and reinitiation of oocyte meiosis in goldfish and other vertebrates. Recently, we have shown that GnRH peptides such as salmon GnRH (sGnRH) and chicken GnRH-II (cGnRH-II) which are native in goldfish brain effect oocyte meiosis and follicular steroidogenesis. Treatment with GnRH peptides alone, stimulated the reinitiation oocyte meiosis as indicated by GVBD. However, sGnRH was found to inhibit GTH-induced oocyte meiosis while cGnRH-II had no effect on GTH-induced response. In the present study we investigated the effect of both sGnRH and cGnRH-II on MPF activity in the follicle-enclosed goldfish oocytes as determined by histone H-1 kinase activity. Treatment with DHP, GTH, sGnRH or cGnRH-II alone significantly increased histone H-1 kinase activity. Concomitant treatment with sGnRH significantly reduced GTH-induced H-1 kinase activity, but had no effect on DHP-induced response. In accordance with the observed GVBD response, treatment with cGnRH-II had no effect on GTH-induced H-1 kinase activity. Subsequent time course studies revealed that the effect of GTH on H-1 kinase activity is significantly slower than GnRH and DHP by approximately 4 hours. Even in the presence of GTH, sGnRH initially stimulated H-1 kinase activity, followed by a reduction corresponding to its inhibitory effect on GVBD response. In this regard, the time course of the inhibitory action of sGnRH on GTH-induced GVBD response is consistent with the reduced histone H-1 kinase activity. These findings indicate the effect of GnRH peptides on reinitiation of oocyte meiosis involves activation of MPF in goldfish

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PII-90

NEGATIVE REGULATION OF DNA SYNTHESIS BY PHOSPHODIESTERASE INHIBITORS IN TESTES OF THE SHARK SQUALUS ACANTHIAS

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Regulation of DNA synthesis was investigated in the premeiotic spermatocysts of the shark, *Squalus acanthias*. DNA synthesis, as indicated by the incorporation of 3H-thymidine, was stimulated by insulin and inhibited by IBMX and other phosphodiesterase (PDE) inhibitors. This suggests an inhibitory role for cAMP or cGMP. When tested alone, cAMP inhibited DNA synthesis only at very high doses , but at lower levels it potentiated the inhibitory effects of IBMX. Forskolin, a potent agonist of cAMP, was ineffective as was cGMP, alone or in combination with IBMX. The general ineffectiveness of cyclic nucleotides suggests that other mechanisms may mediate the inhibitory effects of PDE inhibitors. PDE inhibitors also inhibit extracellular adenosine receptors. Preliminary evidence supports a role for adenosine in the negative regulation of DNA synthesis in the shark testis.

PII-93

SPERMATOGENESIS IN THE YELLOW PERCH (*PERCA FLAVESCENS*) - COMPARISON OF RELATIVE GERM CELL TYPES IN 2 SUBPOPULATIONS OF YOUNG-OF-YEAR FISH. G.P. Toth[1], S.A. Christ[1], R.E. Ciereszko[2], and K. Dabrowski[2]. [1]U.S. Environmental Protection Agency, Cincinnati, OH, 45268,[2]School of Natural Resources, The Ohio State University, Columbus, OH, 43210.

Young-of-year (YOY) stock were produced via artificial fertilization in April 1994; eyed eggs were stocked in ponds in early May. Beginning mid-July, juvenile yellow perch were sampled every 3-6 weeks; data on weight, length, GSI, plasma steroid concentrations (estradiol (E_2), testosterone (T), 11-ketotestosterone (11-kT)) and *in vitro* gonadal steroid production were collected for both sexes (R.E. Ciereszko, et al., unpublished). A histological evaluation of the testes was made for males. By the end of August, it was evident that two subpopulations existed based on size (NGR, normal growth rate; AGR, accelerated growth rate). To avoid cannibalism of the NGR by the AGR fish, NGR fish remained in the pond while AGR fish were moved to an indoor tank under ambient conditions and fed a semi-natural diet. Results from the histological evaluation of the hematoxylin/eosin stained testis samples show that a greater percentage of AGR males had a full range of germ cell types (spermatogonia (spg) through spermatozoa (spz)) earlier in the season (Oct 4, 100%) than NGR males (Oct 7, 44%). In AGR males, spz comprised 90-95% of the germ cells beginning at the Nov 15 sampling, while a comparable percentage of spz was not observed in the NGR males until Dec 7. The possibility of quantifying germ cell types via flow cytometry methods of ploidy determination will be addressed. (This research was supported in part by U.S. EPA (Grant No. 823720-01-1) and does not necessarily reflect EPA policy.)

PII-94

EFFECT OF SOME STEROIDS AND PROSTAGLANDINS ON GVBD AND OVULATION IN CATFISH, HETEROPNEUSTES FOSSILIS

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Apparently no work is available on the effect of prostaglandins (PGs) when administered simultaneously with maturation inducing steroids (MIS) on germinal vesicle breakdown (GVBD) and ovulation. Present experiment deals with the impact of PGs when given in combination with the main MIS, 17a,20B-dihydroxy-4-pregnen-3-one (17a,20B-DHP). In vitro effect of various steroids on GVBD were investigated after 18 hrs of incubation. Several steroids were tested and 17a,20B-DHP was found to be the most effective, while other progestins and corticoids were also effective but their response was significantly lower than that of earlier. PGF2a (5ug/ml) increased ovulation. PGE1 & PGE2 stopped completion of GVBD and ovulation. In experiment oocytes obtained from carp gonadotropin injected fish after GVBD and incubated along with PGs, then only PGF2a increased the percentage of ovulation. Indomethacin was partially effective in blocking 17a,20B-DHP induced ovulation did not affect increased ovulation when given alonqwith 17a,20B-DHP and PGF2a.

PII-95

EFFECTS OF ESTROGEN ON RED DRUM (*SCIAENOPS OCELLATUS*) THYROID FUNCTION

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Seasonal changes in fish thyroid hormone levels indicate that gonadal hormones may influence the thyroid axis at several locations. To determine if estrogen works via alterations in thyroid hormone transport or metabolism, red drum received silastic implants containing 0, 5 or 50mg/g estrogen for up to 16 days. Electrophoretic analysis of blood revealed substantial amounts of circulating vitellogenin by day 16 in estrogen-treated animals. At day 16, both thyroxine (T_4) and triiodothyronine (T_3) levels for fish implanted with estrogen were significantly higher than control levels. Associated with increased levels of vitellogenin, there was a significant increase in both T_4 and T_3 binding to blood proteins. These results indicate that elevated thyroid hormone levels were due, in part, to increased capacity for thyroid hormone binding in the circulation. Although there was a significant increase in total T_3 levels, 5'-outer ring deiodinase activity was significantly reduced, indicating that the increased total thyroid hormone levels may inhibit T_4 to T_3 conversion.

PII-96

ASPECTS OF SPERMATOGENESIS AND SPERMIOGENESIS IN OCEAN POUT *Macrozoarces americanus*

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Spermatogenesis and spermiogenesis in ocean pout have been examined by histological and ultrastructural approaches in this study. The testis of ocean pout is paired and identified as the tubular type. The vas deferens, which is enlarged during spermiation, functions as a spermatozoa reservoir. Spermatogonia are restricted at the blind end, apex, of tubules through the whole testis. They then migrate to the centre of the testis in cysts, as spermatogenesis progresses. While spermatocytes develop into spermatids, cysts break down to discharge spermatids into the tubular cavity. Spermiogenesis takes place in the cavity of tubules and the canal of the testis, resulting in vas deferens and milt containing spermatids at different stages of spermiogenesis and mature spermatozoa. Spermatozoa, which are composed of an elongated head, well developed mid-piece and biflagellated tail, are free and motile in the vas deferens, rather than grouped together in a spermatophore. It is suggested that spermatogenesis and spermiogenesis in ocean pout are semi-cystic type resulting in free spermatozoa in the vas deferens, a distinct characteristic compared with some internal fertilizing teleosts.

OR-48

INSULIN-LIKE GROWTH FACTOR I (IGF-I) IN THE FISH OVARY

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We have investigated the potencial roles of insulin and IGF-I in regulating ovarian function in fish. First, insulin and IGF-I receptors were semipurified from brown trout (*Salmo trutta*) ovaries with wheat germ agglutinin. Binding characteristics and tyrosine kinase activity were examined at various stages of the reproductive cycle. Specific receptors for insulin and IGF-I were found. The number of IGF-I receptors and affinity of the IGF-I receptor exceeded that of insulin at all stages. Significant changes in binding for both peptides were found during the reproductive cycle. Maximum binding was found at the beginning of the reproductive cycle for both IGF-I and insulin, however there was also a peak in IGF-I binding during the prespawning period. Receptors for IGF-I were found in both isolated theca-interstitial and granulosa follicular cell layers. Once functional receptors were identified in the ovarian follicle, effects of IGF-I on *in vitro* steroid production by isolated theca and granulosa cell-layers of the preovulatory coho salmon ovary were examined. IGF-I decreased theca-interstitial layer production of both testosterone and 17α-hydroxyprogesterone (17-OHP). In contrast, IGF-I enhanced the stimulatory effects of gonadotropin II on conversion of 17-OHP to 17α,20β-dihydroxy-4-pregnen-3-one during final oocyte maturation. The abundance of IGF-I receptors in the ovarian follicle and observed effects of IGF-I on ovarian steroidogenesis suggest an important role of IGF-I in fish ovarian function.

OR-49

ACTIVIN B IS A MAJOR MEDIATOR OF HORMONE-INDUCED SPERMATOGONIAL PROLIFERATION IN THE JAPANESE EEL

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In the cultivated male Japanese eel, spermatogonia are the only germ cells present in the testis. A single injection of human chorionic gonadotropin (HCG) can induce all stages of spermatogenesis. A subtractive hybridization method was used to identify genes that are expressed differentially in eel testes in the first 24 hr following HCG treatment *in vivo*. We extracted mRNA from testes with or without a single injection of HCG for 24 hr and cloned specific cDNAs expressed at each stage. Two up-regulated and six down-regulated cDNA were obtained. From its deduced amino acid sequence, one of the up-regulated cDNAs was identified to be activin β_B subunit. Chinese hamster ovarian cells (CHO cells) transfected with this clone secreted activin B (the β_B-β_B homodimer) which had EDF (Erythroid differentiation factor) activity. Activin β_B mRNA expression was restricted to Sertoli cells in testes treated with HCG for one to six days. This stimulation of activin β_B mRNA was accompanied by spermatogonial proliferation.

We have previously shown that HCG-induced spermatogenesis in eel testes is mediated by testicular production of 11-ketotestosterone (11-KT), a potent androgen in teleosts. To determine whether HCG action on activin B production is direct or mediated through 11-KT, we investigated the effects of HCG and 11-KT on activin B mRNA expression and protein secretion using an organ culture system for eel testis. Both HCG and 11-KT induced activin B mRNA expression and protein secretion. Furthermore, eel activin B extracted from cultured medium induced spermatogonial proliferation *in vitro*. These results strongly suggest that 11-KT production stimulated by HCG induces Sertoli cells to produce activin B, which, in turn, initiates spermatogonial proliferation in eel testis.

OR-50

OVULATION SPECIFIC TRANSCRIPTION OF AN ANTILEUKOPROTEINASE-LIKE mRNA IN THE BROOK TROUT OVARY

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An ovulation specific cDNA clone (TOP-1; Trout Ovulatory Protein) was isolated from a brook trout (Salvelinus fontinalis) ovarian cDNA library by subtractive screening with preovulatory specific probes. On Northern blots, the cDNA hybridized with RNAs of 0.8, 1.1, 1.4, 1.7 and 2.4 kilobases and the expression of these transcripts increased greatly at the time of ovulation. No hybridization was observed on Northern blots of total RNA from heart, kidney, liver, gills, skin, gut, brain or muscle, indicating that it is ovarian specific. The TOP-1 cDNA contains an open reading frame which encodes a protein of 121 amino acids with a high cysteine content. Comparison of the deduced amino acid sequence of TOP-1 with all sequences in GenBank indicate that the TOP-1 protein is most similar to mammalian antileukoproteinases (ALPs). A distinct feature shared by TOP-1 and ALPs is the presence of two consecutive repeating domains. ALPs are inhibitors of leukocytic proteases such as elastase and cathepsin. Given the structural similarity between TOP-1 and ALPs, it is possible that the function of TOP-1 is to modulate proteolysis in the ovary at the time of ovulation. (supported by NIH grant #HD25924).

OR-51

SEX STEROIDS DURING THE OVULATORY CYCLE OF GILTHEAD SEABREAM (*SPARUS AURATA*).

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The seabream is a protandrous hermaphrodite and a serial daily spawner. Steroidogenesis was studied by incubating biopsy fragments of ovarian tissue with tritiated progesterone. The major metabolites identified were 3α,17α,21-trihydroxy-5β-pregnan-20-one (3α,17α,21-P-5β), 17α,20β,21-trihydroxy-4-pregnan-3-one (17α,20β,21-P), 3α,17α,20β,21-tetrahydroxy-5β-pregnane, estradiol-17β and 11-desoxycortisol. The effect of the a LHRH analogue on steroid plasma levels was studied in 6 females receiving implants of analogue (150μg/kg) and 3 females with implants only (control). Levels of free and conjugated 17α,20β,21-P and 3α,17α,21-P-5β reached more than 100 ng/ml in some individuals. However, a clear pattern of steroid changes was not observed. In intact spawning females sampled at 9.00 a.m. and 3.00 p.m. only low levels (<10ng/ml) of the above hormones were detected and there were no differences between sampling times. These results suggest that 17α,20β,21-P is the likely maturation-inducing steroid in gilthead seabream and that marked short term changes in hormone levels occur daily.
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OR-52

REGULATION OF THE AROMATASE ACTIVITY IN RAINBOW TROUT, *ONCORHYNCHUS MYKISS*, OVARIAN FOLLICLES.

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The maturational gonadotropin (GtH2) and, in some cases, growth hormone (GH) stimulate estradiol production by vitellogenic ovarian follicles of rainbow trout. This could be due to an increase in precursors (androgens) availability, an increase of the aromatase activity, and a decrease of a further metabolism of estradiol. These various steps of estradiol production have been investigated *in vitro* during both vitellogenesis and last stages of the female reproductive cycle.
Two kinds of methods have been used for the analysis of aromatase activity: quantification of tritiated estradiol synthesised from tritiated androgens, and quantification of tritiated water released from 1,2β^{3}H-androstenedione. Data will be compared.
It has been shown that GtH2 stimulates androgen production but inhibits aromatase activity. No inhibition could be detected with GH.
We conclude that the increase in estradiol production under GtH2 stimulation is not due to an activation of aromatase. Mechanisms involved in this process will be discussed.

OR-53

REGULATION OF OVARIAN STEROIDOGENESIS *IN VITRO* BY GONADOTROPINS DURING SEXUAL MATURATION IN COHO SALMON (*ONCORHYNCHUS KISUTCH*)

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The progression of oocyte maturation is associated with marked changes in the production of reproductive steroids by the salmonid ovarian follicle. A developmental shift from the production of estrogens to the production of progestogens has been described and is thought to be regulated in part by gonadotropins. Despite information on changes in the nature and distribution of gonadotropin receptors during oocyte maturation, not much is known about the relative steroidogenic effects of gonadotropins, GTH I and GTH II, during oocyte maturation. In this study, the changes in sensitivity of coho salmon ovarian tissue to the steroidogenic effects *in vitro* of GTH I and GTH II during oocyte maturation were investigated. Concentration-response experiments with GTH I and GTH II were performed with intact ovarian follicles or isolated follicular layers from fish at different stages during oocyte maturation. In intact ovarian follicles, the sensitivity (as indicated by the minimal effective concentrations of GTH) to the effects of GTH II on 17α-hydroxyprogesterone (17OH-P) and 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P) production increased during oocyte maturation. On the other hand, the sensitivity of intact ovarian follicles to the effects of GTH I decreased during oocyte maturation. In isolated granulosa layers (incubated in the presence of steroidogenic precursors), the sensitivity to the effects of GTH II on 17,20β-P production increased until the germinal vesicle breakdown stage; however, the sensitivity to the effects of GTH I decreased markedly during oocyte maturation. In isolated theca layers, the production of 17OH-P gradually increased during oocyte maturation and at all stages was stimulated more effectively by GTH II that by GTH I. These results support the hypothesis that changes in ovarian steroidogenesis during oocyte maturation are associated with changes in the relative activity of gonadotropins and in gonadotropin receptor distribution.

OR-54

STEROIDOGENESIS BY MILT & TESTIS OF ROACH IS AFFECTED BY SUBSTRATE CONCENTRATION

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The effect of incubation time and substrate concentration on the *in vitro* metabolism of 17-hydroxyprogesterone by testes of the roach has been examined. There was a shift from synthesis of the 11-oxygenated androgens, 11-ketotestosterone and androstenetrione, at low substrate concentration to 17,20α-dihydroxy-4-pregnen-3-one (17,20αP) at high substrate. Glucuronides and sulfates were of significant importance only at low substrate and long incubation times. There was a shift from 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) to 17,20αP with increased substrate. The results confirm that substrate affects the steroidogenic profile and that 3 hr is optimal time for such studies. Incubations of sperm with 17-hydroxyprogesterone gave predominantly 11-ketotestosterone at low substrate concentrations and 17,20αP at high substrate. The synthesis of 11-ketotestosterone is demonstrated for the first time in teleost sperm.

OR-55

STUDIES ON A NUCLEAR PROGESTOGEN RECEPTOR IN THE OVARY OF THE SPOTTED SEATROUT, *CYNOSCION NEBULOSUS*.

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A nuclear progestogen receptor has previously been characterized in the ovary of the spotted seatrout, *Cynoscion nebulosus*, which has a high affinity for the two teleostean maturation-inducing steroids (MIS's), 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-P) and 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S). Receptor levels gradually increased on a whole ovarian basis during gonadal recrudescence. Twelve hour incubations of vitellogenic ovarian fragments with a variety of steroids (290 nM) or hCG (20 IU/ml) resulted in significant decreases in receptor levels only in the tissues treated with 17α,20β-P and 20β-S. Further studies demonstrated that 17α,20β-P-induced receptor decrease was maximal within 4 h ($T_{1/2}$ = 2 h) and at a steroid concentration of 5 nM (EC_{50} = 2.4 nM). Incubation of ovarian fragments with hCG (12 h) followed by MIS (12 h) results in germinal vessicle breakdown (GVBD) in this species. Our studies indicate that exposure to MIS for an additional 12 hours results in the appearance of loose oocytes in the culture medium. Histological examination of the loose oocytes revealed that they lack a follicular layer, although this layer is present in pre- and post-GVBD oocytes. We conclude that these eggs have been induced to ovulate by the MIS and we are currently investigating the possible requirements for RNA and protein synthesis, as well as prostaglandin synthesis, in the steroid-induced ovulatory process.

OR-56

VITELLINE ENVELOPE PROTEINS IN TELEOST FISH

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During oocyte development in vertebrates, an acellular envelope is formed outside the plasma membrane of the oocyte. In teleosts, the envelope is often referred to as the vitelline envelope and forms a tough, protective coat around the egg and embryo. Recent research has provided insight into the composition, regulation, origin and formation of the vitelline envelope in teleost fish. The vitelline envelope is mainly composed of two to four major proteins. These proteins share a characteristic amino acid composition, i.e. a high proline and glutamine/glutamic acid content, and may represent a separate class of structural proteins. Estradiol-17ß induces the major vitelline envelope proteins in most of the investigated species. The proteins are found in plasma of female fish during sexual maturation and the amount is positively correlated to the plasma level of estradiol-17ß and the stage of oocyte development. Teleost fish is the first vertebrate group where an endocrine regulation of the protein constituents of the egg envelope has been described. Recent evidence strongly suggests that the major teleost vitelline envelope proteins originate in the liver and are transported by the blood to the ovary, where the proteins assemble into the vitelline envelope. The formation of the vitelline envelope may even start before the active uptake of vitellogenin and appears to continue until ovulation.

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OR-57

LIVER-DERIVED cDNAs: VITELLOGENINS AND VITELLINE ENVELOPE PROTEINS

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We have substantially increased our chances of isolating cDNAs that code for reproductively significant proteins by constructing a lambda gt10 liver library from the poly A^+RNA of *Fundulus heteroclitus* treated with estradiol-17β. To date, two vitellogenin (Vtg) and two putative vitelline envelope protein (VEP) cDNAs have been isolated. Deduced primary structures of the two Vtg cDNAs share 25-30% sequence identity with other reported vertebrate Vtgs. Compared to each other, the *F. heteroclitus* Vtgs are 60% identical, but they share no overlapping stretches of nucleotide sequence, indicating that they are expressed from two separate Vtg genes. The deduced a.a. sequence of our first putative VEP cDNA is 50% identical to a flounder zp gene product (Lyons *et al.* 1993, *J. Biol. Chem.* **268**:21351-21358) while the other cDNA is 70% identical to a medaka ZI-3 precursor, the L-SF protein (Murata *et al.* 1995, *Dev. Biol.* **167**:9-17). The first VEP cDNA includes a prominent repeat region consisting of several (PQQ PQQ PQY PSK) casettes, reminiscent of a repeating PXX motif reported in other extracellular matrix proteins. The second cDNA contains a less apparent PQQ region. In contrast to the two closely related Vtg isoforms, these two *F. heteroclitus* VEP isolates are not isoforms, but rather represent related but separate molecular lineages that have been maintained from teleosts to mammals. N-terminal a.a. data from isolated yolk proteins have confirmed proteolytic products of the first Vtg cDNA, and studies to confirm the other three cDNA products are currently underway.

OR-58

Towards the development of genetic probes to the rainbow trout vitellogenin receptor.

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Vitellogenin (VTG) sequestration is central to the oocyte growth process in teleosts and in the rainbow trout may account for as much as 80% of the final egg size. In fish, as in other oviparous vertebrates, VTG is sequestered by receptor mediated endocytosis involving specific cell surface receptors.The expression and modulation of the VTG receptor are key determinants in the oocyte growth process. Establishing what controls VTG receptor function, hormonal or otherwise, is likely to come from studies on the expression of the receptor gene and this in turn requires specific genetic probes.

In birds, where developing oocytes sequester significant amounts of lipoproteins other than VTG, it has been established that a single receptor effects the ovarian uptake of both very low density lipoprotein (VLDL) and VTG. There is little evidence of yolk proteins derived from lipoprotein precursors other than VTG in fish, however, and none in salmonids. Our previous work on the rainbow trout has indicated that there is more than one oocyte membrane protein capable of interacting with VTG. This study set out to establish clearly the number of oocyte membrane proteins capable of interacting with VTG and further whether these proteins were also able to bind other homologous serum lipoproteins. Isolated receptor proteins binding vitellogenin alone were subjected to trypsin digests and selected peptides were sequenced for use in the genesis of specific genetic probes.

OR-59

Connexin Genes, Gap Junctions, and Ovarian Maturational Competence

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Gonadotropin (GtH) induces oocyte maturational competence (OMC). Our earlier studies with fishes and amphibians suggested that OMC is at least partly due to increased synthesis or activity of maturational steroid receptor. Induction of OMC in teleosts also seems to require *de novo* gene activation. In this regard, our recent studies with Atlantic croaker suggest that expression of connexin (Cx; gap junction protein) genes may be important for OMC. We cloned and sequenced two Cx cDNAs from croaker ovary: Cx32.2 and Cx32.7. Northern blots showed a negligible level of Cx32.2 mRNA in incompetent ovaries, but this level increased with the GtH induction of competence. However, Cx32.7 mRNA levels were similar in incompetent and competent ovaries. Also, conductance assays using paired *Xenopus* oocytes showed that Cx32.2, but not Cx32.7 produced functional, homotypic channels, and that Cx32.2 and Cx32.7 could not form heterotypic channels. These findings indicate that increased Cx32.2 synthesis can lead to the new formation of homotypic, intercellular channels in the ovary. Indeed, ultrastructural studies showed that GtH stimulates granulosa cell-oocyte gap junction coupling concomitantly with OMC. We are also studying the regulation of Cx32.2 gene expression by GtH. We cloned and sequenced the Cx32.2 gene, and identified cAMP responsive element consensus sequences in the first intron and the 5' upstream region. Thus, selective activation of ovarian Cx32.2 gene (possibly via cAMP-dependent pathway) and formation of granulosa cell-oocyte channels correlate with the GtH-dependent acquisition of OMC. This enhanced heterocellular coupling may be necessary for the establishment of maturational signal transduction pathways.

OR-60

EFFECTS OF INSULIN-LIKE GROWTH FACTOR-I ON FINAL MATURATION OF OOCYTES OF RED SEABREAM, *PAGRUS MAJOR, IN VITRO*

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Previous experiments have shown that HCG induces both production of maturation-inducing steroid (MIS) and maturational competence (responsiveness to MIS) through a mechanism dependent on RNA and new protein synthesis during the course of oocyte maturation of red seabream, a daily spawning marine teleost. In the present study, the effects of insulin-like growth factor I (IGF-I) on *in vitro* germinal vesicle breakdown (GVBD) were examined in oocytes of the red seabream. IGF-I (10 nM) alone induced GVBD in both MIS (17α,20β-dihydroxy-4-pregnen-3-one, DHP)-insensitive (maturational incompetent) and DHP sensitive (maturational competent) oocytes. Cyanoketone (0.1 μg/ml), an inhibitor of 3β-hydroxysteroid dehydrogenase, blocked HCG-induced GVBD, but did not block IGF-I-induced GVBD in DHP sensitive oocytes. These data indicate that IGF-I acts directly on oocytes to induce GVBD, not via MIS production. Moreover, DHP insensitive oocytes underwent GVBD in response to DHP when oocytes were incubated with low doses of IGF-I, which alone were ineffective. GVBD also occurred in response to DHP after oocytes were preincubated with IGF-I. Actinomycin D blocked HCG-induced maturational competence, but not IGF-I-induced maturational competence. These results show the possibility that IGF-I can induce maturational competence of oocytes of the red seabream, through a mechanism different from HCG-induced maturational competence.

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A PERTUSSIS TOXIN SENSITIVE GTP-BINDING PROTEIN IS INVOLVLED IN THE SIGNAL TRANSDUCTION PATHWAY OF THE MATURATION-INDUCING HORMONE (17α,20β-DIHYDROXY-4-PREGNEN-3-ONE) OF RAINBOW TROUT (*Oncorhynchus mykiss*) OOCYTES

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Rainbow trout oocyte maturation is induced by 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP) acting upon an oocyte surface receptor. To investigate the signal transduction pathway of 17α,20β-DP, we examined the interaction between the 17α,20β-DP receptor and GTP-binding protein (G-protein) using oocyte cortex preparations. Pertussis toxin (PT) catalyzed ADP ribosylation of a 40 KDa protein, and cholera toxin (CT) labeled several bands including a 43 KDa protein. These 40 and 43 KDa proteins were immunoprecipitated by an antibody against the α1 and α2 subunits of inhibitory G-protein (anti-Giα1,α2) and an anti-Gs antibody, respectively. The 40 KDa protein was present in immature oocytes, but disappeared during oocyte maturation. The ADP ribosylation of the 40 KDa protein with PT was suppressed by preincubation of membrane preparations with 17α,20β-DP. The specific binding of 17α,20β-DP to membrane preparations was inhibited by PT, but not by CT. Non-hydrolizable GTP analogs, GTPγS and GppNHp, inhibited 17α,20β-DP binding, while GDPβS and ATP did not. Scatchard analysis revealed that the GppNHp-induced decrease in 17α,20β-DP binding is due to a decrease in binding affinity. Our findings demonstrate that a 40 KDa PT-sensitive G-protein is involved in the signal transduction pathway of maturation-inducing hormone.

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INHIBITORY REGULATION OF SPERMATOGENESIS IN SHARK TESTIS

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Using the testis of the shark *Squalus acanthias*, which allows isolation of germ cell/ Sertoli cell clones in defined stages of spermatogenesis, we obtained evidence of an intratesticular gradient of growth inhibitory bioactivity (mature > > immature regions) when DNA synthesis by premeiotic stages was used as a bioassay. Inhibitory bioactivity was greater in the epigonal organ, a lymphomyeloid tissue adjacent to the mature region. The epigonal growth inhibitory factor (EGIF) also was detected in media from epigonal cultures and in fractions of epigonal homogenates. EGIF effects were dose- and time-dependent, had a short response latency, were reversible and counteracted the effects of insulin on premeiotic DNA synthesis. Initial characterization shows EGIF is water- and acid-soluble, heat stable, trypsin and pronase insensitive and < 10 kDa. Inhibitory responses to epigonal-derived bioactivity were constant throughout the year, whereas maximal testes-derived bioactivity *in vitro* coincided with the end of the *in vivo* period of spermatogenic proliferation. These results suggest that a growth inhibitory signal secreted by lymphomyeloid tissue into the blood entering the testis may regulate the number of germ cell clones which enter meiosis and account for the diametric arrangement of spermatogenesis in this animal model (Supported by NIH grant HD 16715).

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INSULIN LIKE GROWTH FACTOR EXPRESSION AND ACTION IN TROUT TESTIS.

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Few data exist concerning the occurrence.and potential role of an IGF system in fish gonads. Using RT-PCR and Northern blot hybridisation with a specific salmon IGF-I cDNA (Duguay et al. 1992), we found that IGF transcription occurs in trout testes . Recombinant human IGFs bound with high affinity to crude trout testis preparation, to cultured isolated testicular cells and to a membrane fraction of these cells (IGF-I Ka $\approx 0.5 \times 10^{10}$ M^{-1}; Bmax = 10 to 20 fmoles / 10^7 cells). The binding site was identified as type1 IGF receptor by its binding specificity (IGF-I > IGF-II >>> Insulin) and the molecular size of its α-subunit labelled with ^{125}I-IGF-I (M_r 125 000 - 140 000). ^{125}I-IGF-II also bound to the type1 receptor whereas an IGF-II /mannose 6 phosphate receptor was not detected. Using populations enriched with different cell types from the seminiferous tubules, we found that IGFI-mRNA and IGF-receptor were preferentially observed in a Sertoli cell enriched population and in spermatogonia with primary spermatocytes (GO+CI). When GO+CI were cultured for 3 days in the presence of different IGF analogs, these factors stimulated the incorporation of ^{3}H-thymidine in a dose-dependant manner and with the following order of potency: hIGF-I ≈(QAYL)IGF-I > hIGF-II >> salmon or bovine Insulin. Human IGF binding protein inhibited the stimulatory effect of IGF-I but not that of the QAYL analog.

IGF-I acts directly on the germ cells through type1 receptors and is a potential paracrine/autocrine regulator in the spermatogenetic compartment.

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