PROGRAM AND ABSTRACTS

6TH INTERNATIONAL SYMPOSIUM ON REPRODUCTIVE PHYSIOLOGY OF FISH

BERGEN, JULY 4-9, 1999

INSTITUTE OF MARINE RESEARCH
AND

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MEETING PROGRAMME

SUNDAY, JULY 4TH

12.00 - 18.00	Registration desk open at Fantoft Sommerhotell
19.00 - 22.00	Reception at the Institute of Marine Research / Bergen Aquarium

MONDAY, JULY 5TH

08.30 - 12.00	Registration desk open at Fantoft (conference venue)
09.00 - 09.30	Opening addresses
09.30 - 12.30	**Sesson: Brain/Hypothalamus** *Session chairs: Richard E. Peter (Edmonton, Canada) and Lin Hao Ren (Guangzhou, People's Republic of China)*
09.30 - 10.10	State of the Art: Kah, O. (Rennes, France): What's new in the reproductive brain in teleost fish? **OP-1**
10.10 - 10.30	Fradinger, E.A., von Schalburg, K. and Sherwood, N.M. (Victoria, Canada): An evolutionary perspective on GnRH in fish **OP-2**
10.30 - 10.50	González, A. and Piferrer, F. (Barcelona, Spain): Characterization of cytochrome P450 aromatase enzyme activity in the European sea bass (*Dicentrarchus labrax*) **OP-3**
10.50 - 11.10	*Coffee Break*
11.10 - 11.30	Parhar, I. (Tokyo, Japan): Hormonal regulation of GnRH gene expression **OP-4**
11.30 - 11.45	Ookura, T.[1], Okuzawa, K.[2], Tanaka, H.[3], Gen, K.[2] and Kagawa, H[3]. ([1]Tsu; [2]Tamaki; [3]Nansei, Japan): The ontogeny of gonadotropin-releasing hormone neurons in the red seabream, *Pagrus major* **OP-5**
11.45 - 12.00	Elofsson, U.O.E.[1], Winberg, S.[2]; Francis, R.C.[3] and Nilsson, G.E.[4] ([1,2]Uppsala, Sweden; [3]Berkeley, USA; [4]Oslo, Norway): Gonadotropin releasing hormone (GnRH) producing cells in the brain of sex changing fish **OP-6**
12.00- 12.15	Larson, E.T.[1,2], Norris, D.O.[1], Grau, E.G.[2] and Summers, C.H.[3] ([1]Boulder; [2]Kaneohe; [3]Vermillion, USA): Central monoaminergic changes accompany sex reversal in the saddleback wrasse **OP-7**
12.15 - 12.30	Holland, M.C.H., Hassin, S. and Zohar, Y. (Baltimore, USA): Seasonal variations in the levels of the three native forms of GnRH during juvenile and pubertal development in the striped bass, *Morone saxatilis* **OP-8**
12.30 - 15.00	*Lunch* and **Poster Session**

15.00 - 17.10 **Session: Reproduction in Wild Populations**
Session chairs: Peter R. Witthames (Lowestoft, UK) and Olav Sigurd Kjesbu (Bergen, Norway)

15.00 - 15.40 State of the Art: Lambert, Y. and Dutil, J.-D. (Mont-Joli, Canada):
Nutritional condition and reproductive success in wild fish populations **OP-9**

15.40 - 15.55 Kurita[1,2], Y., Thorsen[2], A., Fonn[2], M., Svardal[2], A. and Kjesbu[2], O.S. ([1]Aomori, Japan; [2]Bergen, Norway):
Oocyte growth and fecundity regulation of Atlantic herring (*Clupea harengus*) **OP-10**

15.55 - 16.15 Wiegand, M.D., Johnston, T.A. and Brooks, J. (Winnipeg, Canada):
Egg neutral and polar lipid fatty acid compositions from seven reproductively isolated populations of walleye **OP-11**

16.15 - 16.30 Pickova, J.[1], Larsson, P.-O.[2] and Kiessling, A.[3] ([1]Uppsala; [2]Lysekil, Sweden; [3]Matredal, Norway):
Possible explanations to Baltic cod reproduction problems - a short review **OP-12**

16.30 - 16.50 Ueda, H.[1], Urano, A.[2], Zohar, Y.[3] and Yamauchi, K.[4] ([1]Abuta; [2]Sapporo, Japan; [3]Baltimore, USA; [4]Hakodate, Japan):
Hormonal control of salmon homing migration **OP-13**

16.50 - 17.10 Pankhurst, N.W., Pankhurst P.M., Hilder, P.I. and Hilder, M.L. (Launceston, Australia):
In vivo and *in vitro* ovarian steroid production by fish from a natural population of the brooding tropical damselfish *Acanthochromis polyacanthus* **OP-14**

TUESDAY, JULY 6TH

09.00 - 10.45 **Session: Reproductive Behaviour**
Session chairs: Bertil Borg (Stockholm, Sweden) and Alexander P. Scott (Lowestoft, UK)

09.00 - 09.40 State of the Art: Kobayashi, M.[1] and Stacey, N.E.[2] ([1]Tokyo, Japan; [2]Edmonton, Canada):
Sexual plasticity of behavior and gonadotropin secretion in goldfish and crucian carp **OP-15**

09.40 - 10.00 Sorensen, P.W.[1], Scott, A.P.[2], Appelt, C.W.[1], Kihslinger, R.L.[1] and Stacey, N.E.[3] ([1]St Paul, USA; [2]Lowestoft, UK; [3]Edmonton, Canada):
Natural modulation of hormonal pheromone action in the goldfish **OP-16**

10.00 - 10.15 Volkoff, H. and Peter, R.E. (Edmonton, Canada):
Effects of two forms of gonadotropin releasing hormone and a GnRH antagonist on the spawning behavior of the female goldfish, *Carassius auratus* **OP-17**

10.15 - 10.30 Poncin, P.[1], Binda, O.[1], Termol, C.[1], Chenuil, A.[2], Rinchard, J.[3], Kestemont, P.[3], Berrebi, P.[2] and Ruwet, J.C.[1] ([1]Liège, Belgium; [2]Montpellier, France; [3]Namur, Belgium):
Spawning tactics and reproductive success in two European cyprinids: the bream (*Abramis brama*) and the barbel (*Barbus meridionalis*) **OP-18**

10.30 - 10.45 Tolstoganova, L. (Moscow, Russia):
Acoustic behaviour of Russian sturgeon during prespawning period **OP-19**

10.45 - 11.00 *Coffee break*

11.00 - 15.00 **Session: Gonadal Physiology**
Session chairs: Alexis Fostier (Rennes, France) and Kohei Yamauchi (Hakodate, Japan)

11.00 - 11.40 State of the Art: Thomas, P. (Austin, USA):
Nuclear and membrane steroid receptors and their functions in teleost gonads **OP-20**

11.40 - 11.55 Morrey, C. and Nagahama, Y. (Okazaki, Japan)
11-β-hydroxylase and androgen receptor mRNA expression in the ovary, testis and brain of the protogynous hermaphrodite *Thalassoma duperrey* **OP-21**

12.00 - 13.30 *Lunch*

13.30 - 13.50 Coffman, M.A. and Goetz, F.W. (Notre Dame, USA):
Characterization of ovarian- and ovulation-specific proteins in the brook trout (*Salvelinus fontinalis*) **OP-22**

13.50 - 14.05 Oba, Y.[1], Hirai, T.[2], Yoshiura, Y.[1] and Nagahama, Y.[1] ([1]Okazaki; [2]Yamanashi, Japan):
Fish pituitary glycoprotein hormone receptors: cDNA cloning and characterization of two different gonadotropin receptors from gonads and two thyrotropin-like receptors from thyroid of amago salmon (*Oncorhynchus rhodurus*) **OP-23**

14.05 - 14.20 Bogerd, J., Andersson, E., Blomenröhr, M., Tensen, C.P., Van der Putten, H.H.A.G.M., Vischer, H., Granneman, J.C.M., Janssen-Dommerholt, C., Goos, H.J.Th. and Schulz, R.W. (Utrecht, The Netherlands):
Discrepancy between structural characteristics and ligand specificity of an African catfish gonadotropin receptor **OP-24**

14.20 - 14.35 Trant, J.M., Kumar, S. and Ponthier, J. (Baltimore, USA):
Seasonal changes in ovarian expression of steroidogenic enzymes and gonadotropin receptors in the channel catfish, *Ictalurus punctatus* **OP-25**

14.35 - 14.50 Mugnier, C.[1], Scott, A.P.[1], Vermeirssen, E.L.M[1] and Rand-Weaver, M.[2] ([1]Lowestoft; [2]Brunel, UK):
Two pathways for c21 steroid biosynthesis in North Sea plaice *Pleuronectes platessa*: what controls them and what is their function? **OP-26**

14.50 - 15.30 *Coffee break*

15.30 - 16.30 **Special Plenary Lecture:**
Session chair: Birgitta Norberg (Austevoll, Norway)

Nagahama, Y. (Okazaki, Japan):
Gonadal steroid hormones: major regulators of gonadal sex differentiation and gametogenesis in fish **OP-27**

19.00 - 22.00 **Poster Session**

WEDNESDAY JULY 7TH

09.00 - 15.50 **Session Gametogenesis and Sex Differentiation**
Subsession: Spermatogenesis and Sex Differentation
Session chairs: Francesc Piferrer (Barcelona, Spain) and Jennifer Specker (Narragansett, USA)

09.00 - 09.40 State of the Art: Schulz, R.W., Bogerd, J. and Goos, H.J.Th. (Utrecht, The Netherlands):
Spermatogenesis and its endocrine regulation **OP-28**

09.40 - 10.00 Grier, H.J[1]. and Lo Nostro, F.[2] ([1]Palmetto, USA; [2]Buenos Aires, Argentina):
The germinal epithelium in fish gonads **OP-29**

10.00 - 10.20	Socorro, S., Power, D.P. and Canario, A.V.M. (Faro, Portugal): Expression of two estrogen receptors during induced sex reversal in seabream, *Sparus aurata* **OP-30**
10.20 - 10.35	Guiguen, Y.[1], Govoroun, M.[1], D'Cotta, H.[2], McMeel, O.M.[3] and Fostier, A.[1] ([1]Rennes; [2]Montpellier, France; [3]Galway, Ireland): Steroids and gonadal sex differentiation in the rainbow trout, *Oncorhynchus mykiss* **OP-31**
10.30 - 10.50	D'Cotta, H.[1], Guiguen, Y.[2], Govoroun, M.S.[2], McMeel, O.[3] and Baroiller, J.F.[1] ([1,2]Rennes, France; [3]Galway, Ireland): Aromatase gene expression in temperature-induced gonadal sex differentiation of tilapia *Oreochromis niloticus* **OP-32**
10.50 - 11.10	*Coffee break*
11.10 - 11.25	Nakamura, M.[1], Yoshiura, Y.[2], Kobayashi, T.[2] and Nagahama, Y.[2] ([1]Tokyo; [2]Okazaki, Japan): Role of endogenous steroid hormones on gonadal sex differentiation in fish **OP-33**
11.25 - 11.40	Contreras-Sanchez, W.M., Fitzpatrick, M.S., Milston, R.H. and Schreck, C.B. (Corvallis, USA): Masculinization of nile tilapia with steroids: alternate treatments and environmental effects **OP-34**
	Subsession Oogenesis: *Session chairs: Bernard Jalabert (Rennes, France) and Frederick W. Goetz (Notre Dame, USA)*
11.40 - 12.20	State of the Art: Tyler, C.R., Santos, E.M. and Prat, F. (Brunel, UK): Unscrambling the egg: cellular, molecular and endocrine advances in oogenesis **OP-35**
12.20 - 14.30	*Lunch* and **Poster Session**
14.30 - 14.50	Le Menn, F.[1], Davail, B.[1], Bennetau, C.[1], Bon, E.[1] and Nunez Rodriguez, J.[2] ([1]Bordeaux; [2]Montpellier, France): New approach to fish oocyte vitellogenesis **OP-36**
14.50 - 15.05	Lethimonier, C., Flouriot, G., Tujague, M., Kern, L., Valotaire, Y., Kah, O. and Ducouret, B. (Rennes, France): Glucocorticoids inhibit the expression of estrogen receptor (rter) and vitellogenin (Vg) in the liver of rainbow trout **OP-37**
15.05 - 15.20	Finn, R.N.[1], Fyhn, H.J.[1], Norberg, B.[2], Munholland, J.[3] and Reith, M.[3] ([1]Bergen; [2]Storebø, Norway; [3]Halifax, Canada): Oocyte hydration as a key feature in the adaptive evolution of teleost fishes to seawater **OP-38**
15.20 - 15.35	Patiño, R.[1], Chang, X.[1], Yoshizaki, G.[2], Thomas, P.[3] and Kagawa, H.[4] ([1]Lubbock, USA; [2]Tokyo, Japan; [3]Port Aransas, USA; [4]Nansei, Japan): Role and regulation of gap junctions during oocyte maturation in teleosts **OP-39**
15.35 - 15.50	Wood, A.W. and Van Der Kraak, G.J. (Guelph, Canada): Apoptotic cell death in rainbow trout (*Oncorhynchus mykiss*) and goldfish (*Carassius auratus*) ovarian follicles *in vivo* and *in vitro* **OP-40**
15.50 - 16.10	*Coffee break*

16.10 - 17.00 **Session: Natural Environmental Influences on Reproduction**
Session chairs: Silvia Zanuy (Torre de la Sal, Spain) and Wolfgang Holtz (Göttingen, Germany)

16.10 - 16.30 Porter, M.J.R.[1], Roed, A.J.[1], Duncan, N.[2], Oppedal, F.[3], Taranger, G.L.[3] and Bromage, N.R.[1] ([1]Stirling, UK; [2]Mazatlan, Mexico; [3]Matredal, Norway):
Differential effects of light intensity on growth, maturation and plasma melatonin in Atlantic salmon and its importance in aquaculture **OP-41**

16.30 - 16.45 Randall, C.F., Bromage, N.R. and Porter, M.J.R. (Stirling, UK):
Circannual rhythms of reproduction in rainbow trout **OP-42**

16.45 - 17.00 Karlsen, Ø.[1], Taranger, G.L.[2], Dahle, R.[1] and Norberg, B.[1] ([1]Storebø; [2]Matredal, Norway)
Effect of exercise and continuous light on early sexual maturation in farmed Atlantic cod (*Gadus morhua* L.) **OP-43**

19.00 - 21.00 **Reception at Håkonshallen (Medieval Castle)**

THURSDAY, JULY 8TH

09.00 - 11.20 **Session: Anthropogenic Environmental Influences on Reproduction**
Session chairs: David E. Kime (Sheffield, UK) and Carl Schreck (Corvallis, USA)

09.00 - 09.40 State of the Art: Sumpter, J.P. (Brunel, UK):
Endocrine disrupting chemicals in the aquatic environment **OP-44**

09.40 - 10.00 Van Der Kraak, G.J., Tremblay, L. and Wells, K. (Guelph, Canada):
Suitability of testing strategies to evaluate endocrine disrupting chemicals (EDCs) in fish **OP-45**

10.00 - 10.15 Pakdel, F., Petit, F., Le Guével, R., Madigou, T., Métivier, R., Le Goff, P., Flouriot, G., Kah, O. and Valotaire, Y. (Rennes, France):
Estrogenic potency of xenobiotics: determination of the molecular mechanisms of action using two complementary bioassays **OP-46**

10.15 - 10.30 Fent, K.[1], Ackermann, G.[1] and Schwaiger, J.[2] ([1]Dubendorf, Switzerland; [2]Wielenbach, Germany):
Long-term effects of nonylphenol on vitellogenin and estrogen receptor expression determined by quantitative competitive RT-PCR and on sex determination in juvenile rainbow trout **OP-47**

10.30 - 10.50 *Coffee break*

10.50 - 11.05 Katsiadaki, I.[1], Scott, A.P.[1] and Matthiessen, P.[2] ([1]Lowestoft, [2]Burnham, UK):
The three-spined stickleback as a biomarker for androgenic xenobiotics **OP-48**

11.05 - 11.20 Parkkonen, J.[1], Larsson, D.G.J.[1], Adolfsson-Erici, M.[2], Petterson, M.[2], Berg A.H.[3], Olsson, P-E.[3] and Förlin, L.[1] ([1]Gothenburg; [2]Stockholm; [3]Umeå, Sweden)
Contraceptive pill residues in sewage effluent are estrogenic to fish **OP-49**

12.00 - 21.30 **Excursion to the Institute of Marine Research, Austevoll Aquaculture Research Station**

FRIDAY, JULY 9TH

09.00 - 12.05	**Session: Aquaculture** *Session chairs: Niall Bromage (Stirling, UK) and Tom Hansen (Matredal, Norway)*
09.00 - 09.40	State of the Art: Zohar, Y. (Baltimore, USA): Endocrine and molecular strategies for the manipulation of spawning in farmed fish: current and future perspectives **OP-50**
09.40 - 09.55	Carrillo, M.[1], Mañanós, E.[1], Sorbera, L.[1], Mylonas, C.C.[2], Cuisset, B.[3], Zohar, Y.[2] and Zanuy, S.[1]. ([1]Torre de la Sal, Spain; [2]Baltimore, USA; [3]Talence, France): Effects of sustained administration of GnRHa on gonadotropin-2 (GtH-2) and gonadal steroid levels in adult male sea bass (*Dicentrarchus labrax*) **OP-51**
09.55 - 10.10	King, H.R.[1] and Pankhurst, N.W.[2] ([1]Wayatinah; [2]Launceston, Australia): Ovulation of Tasmanian Atlantic salmon maintained at elevated temperatures: implications of climate change for sustainable industry development **OP-52**
10.10 - 10.25	Vermeirssen, E.L.M.[1,2], Mazorra de Quero, C[3], Shields, R[4], Norberg, B[5], Scott, A.P.[1] and Kime, D.[6] ([1]Lowestoft; [2]Norwich; [3]Stirling; [4]Ardtoe, UK; [5]Storebø, Norway; [6]Sheffield, UK) Fertility and motility of sperm from male Atlantic halibut (*Hippoglossus hippoglossus*) treated with gonadotrophin-releasing hormone agonist **OP-53**
10.25 - 11.00	*Coffee break*
11.00 - 11.15	Linhart, O. and Rodina, M. (Vodnany, Czech Reprublic): Cryopreservation of common carp *Cyprinus carpio* and tench *Tinca tinca* for gene resources conservation **OP-54**
11.15 - 11.30	Lahnsteiner, F.[1], Weismann, T.[2] and Berger, B.[3] ([1]Salzburg; [2]Mondsee; [3]Thalheim, Austria) Prolongation of sperm motility in the rainbow trout (*Oncorhynchus mykiss*) and its consequences for artificial insemination **OP-55**
11.30 - 11.45	Trippel, E.A., Castell, J.D., Neil, S.R.E. and Blair, T.J. (St. Andrews, Canada) Assessment of egg quality of haddock (*Melanogrammus aeglefinus*) in paired matings **OP-56**
11.45 - 12.05	Hansen, T.[1], Stefansson, S.O.[2], Taranger, G.L.[1] and Norberg, B.[3] ([1]Matredal; [2]Bergen; [3]Storebø, Norway) Aquaculture in Norway **OP-57**
12.05 - 13.45	*Lunch*
13.45 - 16.40	**Session: Pituitary** *Session chairs: Bernard Breton (Rennes, France) and Henk J. Th. Goos (Utrecht, The Netherlands)*
13.45 - 14.25	State-of-the -Art: Dufour, S.[1], Huang, Y-S.[1,2], Rousseau, K.[1], Sbaihi, M.[1], Le Belle, N.[1], Vidal, B.[1], Marchelidon, J.[1], Chang, C-F.[3] and Schmitz, M.[4] ([1]Paris, France; [2,3]Keelung, Taiwan; [4]Umeå, Sweden): Puberty in teleosts: new insights into the role of peripheral signals in the stimulation of pituitary gonadotropin **OP-58**
14.25 - 14.45	Elizur, A.[1] , Zmora, N.[1], Meiri, I.[1], Kasuto, H.[1], Rosenfeld, H.[1], Chaouat, S.[1], Kobayashi, M.[2], Zohar, Y.[3] and Yaron, Z.[4]. ([1]Eilat, Israel; [2]Tokyo, Japan; [3]Baltimore, USA; [4]Tel Aviv, Israel): Gonadotropins - from genes to recombinant proteins **OP-59**

14.45 - 15.00	Gur, G.[1], Meiri, I.[2], Elizur, A.[2] and Yaron, Z.[1] ([1]Tel Aviv; [2]Eilat, Israel): Hypothalamic control of glycoprotein-hormone α subunit mRNA level in tilapia **OP-60**
15.00 - 15.15	Sohn, Y.C., Kobayashi, M. and Aida, K. (Tokyo, Japan): Distinct expression and structure of the goldfish GTH-Iβ and GTH-IIβ genes **OP-61**
15.15 - 15.35	*Coffee break*
15.35 - 15.50	Chyb, J.[1,2] and Breton, B.[1] ([1]Rennes, France; [2]Krakow, Poland): Effects of recombinant human inhibin on GTHI and GTHII secretion from dispersed pituitary cells of female rainbow trout during the reproductive cycle **OP-62**
15.50 - 16.05	Weltzien, F.-A[1]., Andersen, Ø.[2], Kobayashi, T.[2], Swanson, P.[3] and Norberg, B[1]. ([1]Storebø; [2]Ås, Norway; [3]Seattle, USA): Isolation and molecular characterization of LHα and β subunits from Atlantic halibut (*Hippoglossus hippoglossus* L.) **OP-63**
16.05 - 16.20	Baker, D.M. [1], Davies, B.[2], Dickhoff, W.W.[1,2] and Swanson, P.[2] ([1,2]Seattle, USA): Effects of fasting and metabolic hormones on the hypothalamic-pituitary-gonadal axis of coho salmon, *Oncorhynchus kisutch* **OP-64**
16.20 - 16.40	Swanson, P.[1,2], Dickey, J.T.[2], Davies, B.[1] and Baker, D.M.[2] ([1,2]Seattle, USA): Regulation of gonadotropins during gametogenesis in salmon **OP-65**
16.45 - 17.00	*End of Conference*
19.00 -	**SYMPOSIUM BANQUET**

Footnote:

OP = Oral Presentation; **PP** = Poster Presentation.
The following Book of Abstracts contains 65 OP's and 227 PP's (excluding 3 that have been withdrawn and 4 reserved but not used) giving a total of 292 contributions.

Minor errors might be present in the abstracts due to examples of problems during electronic transfer of files. We apologize if we have overlooked such errors.

Bergen, Norway 18 June 1999

Brain/Hypothalamus

OP-1

WHAT'S NEW IN THE REPRODUCTIVE BRAIN OF TELEOST FISH

O. Kah, I. Anglade, D. Mazurais, A. Teitsma, E. Mañanos, T. Bailhache, F. Pakdel, B. Ducouret, P. Jégo and C. Saligaut
Endocrinologie Moléculaire de la Reproduction, UPRES-A CNRS 6026,
Institut Rennais de Biologie et Ecologie des Poissons, 35042 Rennes Cedex, France

Reproduction is a highly integrative function whose success depends throughout the life cycle on an appropriate balance between the hormonal status of the individual and the surrounding environment. A suitable condition will lead to gonadal development and eventually the triggering of a preovulatory surge of LH via activation and/or desinhibition of the GnRH system. Among the brain factors that have been shown to relay the information to the pituitary, the primary target organ, GnRH, monoamines, aminoacids and several other neuropeptides have been identified as the main actors controlling LH secretion. Although considerable progress has been made with respect to the brain factors and neuronal systems controlling the release of pituitary gonadotropins, mainly that of LH, there is still little understanding of how the neuroendocrine brain integrates all the inputs from the peripheral organs and from the external environment. Such mechanisms involve a complex interplay mainly between gonadal steroids and metabolic hormones under the influence of environmental signals. This lecture will try to address those questions by reviewing recent data from the literature and our own work with special emphasis on what we have recently learnt concerning the brain as an integrative reproductive organ.

OP-2

AN EVOLUTIONARY PERSPECTIVE ON GnRH IN FISH

E.A. Fradinger, K. von Schalburg and N.M. Sherwood
Department of Biology, University of Victoria, Victoria, B.C. V8W 3N5, Canada

There are fourteen distinct forms of gonadotropin-releasing hormone (GnRH) at present; two are identified in jawless fish, two in cartilaginous fish and seven in bony fish. All forms are decapeptides with positions 1, 4, 9 and 10 conserved. Two or more forms of GnRH are found in the brain for fish species studied to date. In cartilaginous and bony fish, one form is chicken (c)GnRH. The second form in cartilaginous fish is dogfish GnRH; and in bony fish is mammalian GnRH, salmon (s)GnRH or catfish GnRH. In teleosts that have three forms of GnRH, herring, seabream or pejerrey GnRH have been identified and appear to displace the second form in the preoptic-hypothalamic region. Forms of GnRH identical to those in the brain have been identified in the ovary and testis. We isolated two cDNAs encoding sGnRH and one cDNA encoding cGnRH-II from rainbow trout gonad. One of the sGnRH mRNAs was found to use an upstream promoter and alternative splice site in gonads compared to brain. In the trout embryo three transcripts were expressed as early as embryonic day 14. One transcript was identical to that found in the brain, whereas the other two transcripts used an alternate promoter and/or splice site. In the first two years of life, GnRH mRNA was expressed in the gonads intermittently, but continuously in the last few months before spawning. Our results show that the tetraploid trout has duplicate genes encoding identical peptides (sGnRH) that are differentially controlled.

OP-3

CHARACTERIZATION OF CYTOCHROME P450 AROMATASE ENZYME ACTIVITY IN THE EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*)

A. González and F. Piferrer
Institut de Ciències del Mar, CSIC. Passeig Joan de Borbó, s/n. 08039, Barcelona, Spain

This study characterized aromatase activity in the sea bass as a part of a project aimed at understanding sex steroid-regulated processes in the brain-gonadal axis, including steroid feedback and sex differentiation. Incubations of brain homogenates were optimized for time, substrate (1-^{3}H -androstenedione; ^{3}HA4), cofactor and tissue concentration. Controls included blanks, heated samples, omission of cofactors, inclusion of specific inhibitors (CGS 16949 and 4OH-A4) and use of authentic ^3H_2O to calculate recovery. Results showed that aromatase activity was linear with respect to the amount of tissue (2.5-20 mg FW) and time up to 60 min. Substrate >100 nM was saturating (Km 5-10 nM). Ten milligram FW tissue incubated for 30 min in the presence of 1 mM NADP and 150 nM ^{3}HA4 fulfilled the criteria for appropriate assay conditions. Subcellular fractionation showed that aromatase activity was 10-fold higher in microsomes than in cytosol, with specific activity 3-fold higher in the microsomal fraction than in crude homogenates. Aromatase was high in brain and ovary, detectable in liver but negligible in all other organs tested, including testis. Peak aromatase levels coincided with the spawning season (Jan-Feb). In mature fish, aromatase was highest in brain areas implicated in reproduction (i.e., the olfactory bulb, pituitary, telencephalon and hypothalamus), with males consistently exhibiting higher activity than females. These results demonstrate sex- and seasonally-related variations in aromatase activity in the sea bass and establish the basis for further studies of certain androgen-mediated actions through locally formed estrogen in both central and peripheral targets.

OP-4

HORMONAL REGULATION OF GNRH GENE EXPRESSION

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In the tilapia *Oreochromis mossambicus*, immunocytochemistry and *in situ* hybridization studies showed neurons synthesizing gonadotropin-releasing hormone (GnRH) mRNAs have three separate embryonic origins from progenitor cells in the olfactory placodes (salmon-GnRH), basal diencephalon (seabream-GnRH), and the midbrain ventricles (chicken II-GnRH). The salmon-GnRH and the chicken II-GnRH synthesizing neurons develop on day 10 ± 1 after fertilization (DAF). The appearance of seabream-GnRH neurons (26 DAF) coincide with the onset of gametogenesis and steroidogenesis. The seabream-GnRH synthesizing neurons appear to be the main contributors of GnRH fiber projections to the pituitary. No change in salmon-, seabream-, and chicken II-GnRH synthesizing neuronal numbers and neuronal sizes were observed when juvenile fish were treated with estrogen or triiodothyronine or castrated sexually immature and mature adults treated with ketotestosterone, or castrated adult males treated with testosterone or progesterone. However, castration alone of mature animals significantly increased seabream- and chicken II-GnRH neuronal size. In juveniles and castrated sexually immature and mature adults hormone treatments had no effect on chicken II-GnRH mRNA levels. Castration alone or with testosterone treatment significantly increased salmon-GnRH mRNA levels. Seabream-GnRH mRNA levels decreased following testosterone treatment. Our results suggest that the three molecular forms of GnRH in tilapia have three different embryonic origins, their neuronal numbers change independent of sexual maturity, instead they are established early during development. The three molecular forms of GnRH are differentially regulated and probably have distinct roles in fish reproductive physiology.

OP-5

THE ONTOGENY OF GONADOTROPIN-RELEASING HORMONE NEURONS IN THE RED SEABREAM, *PAGRUS MAJOR*

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In most vertebrates, gonadotropin-releasing hormone (GnRH) neurons are considered to be formed in the olfactory placode and migrate into the forebrain during development. However, limited information is available about the origins of three GnRH neuronal systems in evolutionary advanced teleosts, namely, terminal nerve (TN), preoptic, and midbrain GnRH systems producing salmon (s) GnRH, seabream (sb) GnRH, and chicken (c) GnRH-II respectively. Therefore, we investigated the ontogeny of all three GnRH systems in the red seabream using immunocytochemistry. Salmon GnRH-immunoreactive (ir) neurons were first detected on one day after hatching (the day 1) in the olfactory organs. During days 4 - 8, sGnRH neurons were observed along the olfactory nerve and in the most rostral area of the ventral forebrain. After the day 27, they were found only in ganglia of the terminal nerve. Chicken GnRH-II-ir neurons were first observed in the midbrain tegmentum on the day 6, and they have been observed in the same area thereafter. The immunoreactivity of sbGnRH first emerged in cell bodies in the preoptic area (POA), in axons from the POA to the pituitary, and in the pituitary around days 37 - 44. The number of sbGnRH-ir neurons and the intensity of the immunoreactivity in the pituitary increased with development. This result shows that the preoptic sbGnRH system, which regulates gonadotropin secretion, is already developed much earlier than the puberty. Taken together, the present results suggest that TN (sGnRH), preoptic (sbGnRH), and midbrain (cGnRH-II) systems originate in the olfactory organ, the POA, and the midbrain respectively.

OP-6

GONADOTROPIN RELEASING HORMONE (GnRH) PRODUCING CELLS IN THE BRAIN OF SEX CHANGING FISH

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Sequentially hermaphroditic fish offer unique possibilities to study neuroendocrine mechanisms underlying vertebrate sexuality. In the present study the distribution of brain gonadotropin releasing hormone (GnRH) cells was examined in sex changing fish representing different sexual phases. The brain GnRH system, has been implicated in the control of reproductive behaviour and physiology throughout the vertebrates, and is thought to have a role in socially induced sex change as well. In the female-to-male sex-changing ballan wrasse (*Labrus berggylta*) and bridled goby (*Coryphopterus glaucofraenum*), male fish was found to have greater mean GnRH-immunoreactive (GnRH-ir) cell numbers in the preoptic area (POA) than females, a difference not found in GnRH-ir cells in other brain regions. Similarly, male fish had more POA GnRH-ir cells than females in the male-to-female sex changing anemonefish (*Amphiprion melanopus*). In contrast, although closely related to the sex-changing bridled goby, no sex difference in POA GnRH-ir cell number was found in the non-sex changing goldspot goby (*Gnatholepis thomsoni*). Maturational state did not correlate with GnRH-ir cell numbers in the ballan wrasse. However, post-spawning males tended to have larger GnRH-ir cells in all brain regions relative to both pre-spawning males and all females. Further, both the GnRH-ir cell number (in POA) and cell size correlated with gonad size in pre-spawning males, indicating a relationship between both size and number of GnRH cells and male gonadal development. These results suggest that temporary changes in the size of brain GnRH neurones are coupled to the male spawning cycle, and that permanent changes in POA GnRH cell number are involved in the process of sex change in sequential hermaphrodites.

OP-7

CENTRAL MONOAMINERGIC CHANGES ACCOMPANY SEX REVERSAL IN THE SADDLEBACK WRASSE

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Monoamines play a role in reproduction and sexual behavior throughout the vertebrates. They are the first endogenous chemical signals regulating the HPG axis. In teleosts with behavioral sex determination, much is know about the behavioral cues that induce sex reversal. The mechanisms by which these external behavioral cues are converted to an internal chemical cue are largely unknown. The protogynous Hawaiian saddleback wrasse, *Thalassoma duperrey*, was used to investigate how monoamines are associated with sex reversal. Large outdoor tanks were set up with 1 male, 1 large female and 4 small females. Although gonadal sex reversal can take 8 weeks, the largest female begins to exhibit aggressive behaviors the first day after male removal. On day 3 post-removal, large females begin to exhibit courting behaviors. By day 7, large females are not behaviorally different from terminal phase males. These behavioral changes mirror a number of complex neuroendocrine changes associated with sex reversal. There is a marked reorganization of monoaminergic activity in both the preoptic area (POA) and the raphe nucleus (RN) associated with behavioral sex reversal. The changes happen on day three and coincide with the initiation of courting. In the POA, serotonergic activity drops on day 3 allowing for an increase in noradrenergic activity. This is a likely trigger for the reorganization of the HPG axis. In the RN, there is a marked decrease in serotonergic activity which could drive the transition from female to male sexual behaviors. This study suggests a link between monoamines and behavioral sex reversal.

OP-8

SEASONAL VARIATIONS IN THE LEVELS OF THE THREE NATIVE FORMS OF GNRH DURING JUVENILE AND PUBERTAL DEVELOPMENT IN THE STRIPED BASS, *MORONE SAXATILIS*

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It has been hypothesized that the inactive state of the reproductive axis in juvenile fish is the result of an insufficient production or release of GnRH. In the present study we investigated the relationships between the pituitary levels of each of the three native GnRH forms and pubertal development in striped bass. Regardless of sex or stage of maturity,seabream (sb) GnRH, chicken (c) GnRH-II and salmon (s) GnRH were present in the pituitary in a ratio of 100:10:1. Puberty was initiated in 70% of the females during the third year of life and the levels of sbGnRH and cGnRH-II increased concomitantly to the increases in oocyte diameter. In addition, the pituitary levels of all three GnRH forms showed seasonal fluctuations during the first two years of life, when all females were still immature. When standardized for pituitary protein content, maximum GnRH levels weresignificantly higher during the first year than in subsequent years. In maturing two- and three-year-old males elevations in the pituitary levels of sbGnRH and cGnRH-II coincided with increases in GSI. Juvenile two-year-old males, however, displayed a similar increase in pituitary sbGnRH and cGnRH-II levels as those observed in precocious two-year-old males. Taken together these findings suggest that 1) sbGnRH and cGnRH-II, but not sGnRH, may be involved in the regulation of early gonadal development, and 2) the onset of puberty is not limited by an insufficient production of GnRH in this species. We are currently studying the expression levels of the three forms of GnRH in the brain.

PP-1

withdrawn

PP-2

NEUROENDOCRINE REGULATION OF GROWTH HORMONE (GH) RELEASE IN TELEOSTS: PHYLOGENETIC CONSERVATIONS AND VARIATIONS

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In fish, GH is implicated in many functions such as growth, reproduction, metabolism, immunity, and osmoregulation. We studied the regulation of GH release in two teleosts, a primitive one, the European eel (Elopomorphs) and a recent one, the turbot (Pleuronectiforms). We used long-term serum-free primary cultures of pituitary cells. In both species, somatotrophs are highly active *in vitro*, suggesting the existence of a predominant inhibitory control of GH release *in vivo*. Our results showed strong inhibitory effects of somatostatin (SRIH) and insulin-like growth factor (IGF1), in agreement with studies done in other teleosts. Concerning the stimulatory control, in the eel, pituitary-adenylate-cyclase-activating-peptide (PACAP) which is encoded by the same gene as somatoliberin (GHRH) in teleosts, was found to stimulate GH release, whereas GHRH had no action. PACAP was also much more active than GHRH on GH release in the goldfish and the salmon. In the eel, corticoliberin (CRH) stimulated GH release, whereas TRH, GnRH, CCK and NPY, found to be active in some other teleosts, were without effect. In the turbot, none of the neuropeptides tested was able to stimulate GH release. These data, associated with those obtained in different vertebrates, demonstrate a large diversity of factors implicated in the stimulatory control of GH release and a total conservation of the inhibitory control exerted by SRIH and IGF1. This inhibitory control could represent the basal regulation of GH release, set up early in vertebrate evolution.

PP-3

NEUROENDOCRINE CONTROL OF GONADOTROPIN II SECRETION IN THE ATLANTIC CROAKER (*MICROPOGONIAS UNDULATUS*): STEROID FEEDBACK INFLUENCES

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The effects of gonadal steroids on gonadotropin II (GTH II or LH) secretion were investigated in gonad-intact and gonadectomized (Gx) Atlantic croaker (*Micropogonias undulatus*) at different stages of their gonadal cycle. Gonadectomy in males and females during the late gonadal recrudescence phase elicited significant increases in the gonadotropin response to an LHRH analog (LHRHa), without altering basal GTH II secretion. This lack of gonadal negative feedback on basal GTH II secretion in croaker differs from most teleosts investigated to date. Testosterone or estradiol treatment significantly inhibited LHRHa-induced GTH II secretion during the late-recrudescence phase in gonad-intact and Gx male croaker, and in Gx females, whereas 5α-dihydrotestosterone, a non-aromatizable androgen, was ineffective. Pretreatment of fish with an aromatase inhibitor, 1,4,6-androstatrien-3,17-dione, 2 days prior to the administration of testosterone, completely blocked the negative feedback effect of testosterone on LHRHa-induced GTH II secretion in males, but only partially blocked it in females. This suggests that the negative feedback effect of testosterone in males is primarily mediated by its conversion to estradiol, whereas in females the androgen may also exert a direct inhibitory effect, probably mediated via binding to an androgen receptor. In addition, estradiol and testosterone exerted positive feedback influences on basal and LHRHa-induced GTH II secretion during the early-recrudescence phase of the gonadal cycle. Thus, steroid feedback influences in croaker show marked changes which are intimately linked to the stage of gonadal development. The role of GABA in mediating some of these influences on GTH II secretion will be discussed.

PP-4

TYROSINE HYDROXYLASE EXPRESSION IN THE PREOPTIC AREA IS REGULATED BY A POSITIVE GONADAL FEEDBACK IN RAINBOW TROUT

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Dopamine (DA) has been shown to play an important role in the control of fish reproduction, mainly by inhibiting gonadotropin (GtH II) secretion during vitellogenesis. DA inhibiting effects depends on the activation of both anabolic and catabolic enzymes activity, especially for Tyrosine Hydroxylase (TH), the rate-limiting anabolic enzymes of catecholamines. In the rainbow trout, the dopaminergic inhibitory tone is depending upon estradiol (E2), leading to a negative feedback of E2 on GTH II secretion. Moreover, most estrogen receptor-positive cells located in the NPOav, ventral to the large extensions of the preoptic recess, are also TH-positive, suggesting that this region is a major target for E2 feedback (Linard et al, 1996, Neuroendocrinology 63:156-165). Recently, a cDNA encoding the rainbow trout TH has been cloned (Linard et al., 1998, J. Neurochem. 71:920-928), providing a tool to study the regulation of TH gene expression. In order to investigate a possible gonadal feedback on TH expression, *in situ* hybridization was performed in brain of ovariectomized trout during vitellogenesis, implanted or not with steroids. TH mRNA levels were quantified in the NPOav and appeared to be greatly affected by gonadectomy. These results suggest a positive gonadal feedback on TH gene expression which is in agreement with our previous studies showing a positive correlation between blood E2 levels and DA turnover.

PP-5

NEUROPEPTIDE Y STIMULATES *IN VITRO* sbGnRH RELEASE FROM PREOPTIC ANTERIOR HYPOTHALAMUS AND PITUITARY OF PRE-PUBERTAL RED SEABREAM, *PAGRUS MAJOR*

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Our previous studies have shown that neuropeptide Y (NPY) stimulates *in vitro* release of seabream gonadotropin-releasing hormone (sbGnRH) from preoptic anterior hypthalamus (POA-H) and pituitary of about two-year-old but immature red seabream. To clarify the development of responsiveness of sbGnRH neurons to NPY, we examined the effects of NPY on the *in vitro* release of sbGnRH from the slices of POA-H and pituitary of red seabream from pre-puberty (one- and two-year-old) to adult (three-year-old). Concomitantly, changes in sbGnRH levels in brain and pituitary were also measured. Levels of sbGnRH were higher in both brain and pituitary of the three-year-old red seabream than in one- or two-year-old fish. Depolarizing concentration of K^+ ions (75 mM KCl) stimulated *in vitro* release of sbGnRH from POA-H and pituitary of two- and three-year-old fish but not in the pituitary of one-year-old fish. NPY stimulated *in vitro* release of sbGnRH from the slices of POA-H (at 0.1 and 1.0 microM) of all age groups and from the pituitary (at 2.5 and 5.0 microM) of two- and three- year-old fish. *In vitro* release of sbGnRH by NPY from POA-H was greatest in three-year-old fish. These results indicate that the responsiveness of sbGnRH neurons to NPY is already developed in pre-pubertal red seabream.

PP-6

MONOAMINE METABOLISM IN THE HYPOTHALAMUS OF THE CATFISH, *PIMELODUS BLOCHII*, DURING GONADAL DEVELOPMENT

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The catfish, *Pimelodus blochii*, is a seasonal breeder, distributed in rivers and lagoons of the Venezuelan plains. In the present study we have evaluated the changes in noradrenaline (NA), dopamine (DA) and serotonin (5HT) metabolism in a wild population. Amine levels have been correlated with gonadal stages (based on visual inspection). Levels of NA, DA, A, 5HT, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanilic acid (HVA), methoxyhydroxy-phenilglycol (MHPG), 3-methoxytyramine (3MT), and 5-hydroxyindoleacetic acid (5HIAA) were measured by HPLC-ED. Monoaminergic activity was calculated as the metabolite/amine ratios. All data were analysed with one-way ANOVA test. Significance was accepted at the 0.05 level. In all groups, a high NA concentrations were observed. In contrast, DA and 5HT levels were about 10-fold lower than NA concentration. Levels of 3MT and MHPG were under the detection limits. At stage 3, there was a significant decrease of DA and NA contents. An increase in the dopaminergic activity was observed during gonadal development, with a maximal value at gonadal stage 3 and a decrease at stages 4 and 5. The content of 5HT showed a decrease throughout gonadal development. The 5HIAA/5HT ratio showed an increase from stage 1 to stage 4, however a significant decrease was observed at stage 5.

PP-7

SEROTONIN METABOLISM IN THE BRAIN OF WILD AND CAPTIVE *CHAETODIPTERUS FABER* (PISCES: EPHIPPIDAE)

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It is well known that some teleosts do not spawn when held under captive conditions for fish culture. Nevertheless, no information exists on the possible mechanisms involved in the inhibition of reproduction in captive fish. In the present study, we present data on the metabolism of 5-hydroxytryptamine (5HT) in the hypothalamus (Hyp), olfactory bulb (OB), telencephalon (TEL), optic tectum (OT) and cerebellum (Cer) of two populations of adult *Ch. faber*: captives (growth under farm conditions) and wild: (caught in the Northeast coast of Venezuela). Concentrations of 5HT, and their metabolites, 5-hydroxyindoleacetic acid (5HIAA) were determined by HPLC-ED. The metabolite/amine ratio was used as index of serotonin activity. All data were analysed with nonpaired Student's t-test. Significance was accepted at the 0.05 level. In the Hyp, high concentrations of both 5HT and 5HIAA were observed in captive animals, however, serotonergic activity was similar in both groups. In the other brain areas 5HT were below of detection limit and levels of 5HIAA were significantly higher in captive population than in that of wild animals. The results of the present study show that 5HT metabolism in certain brain areas is increased in animals in captivity, indicating increased brain 5HT activity in these individuals.

PP-8

CATECHOLAMINE METABOLISM IN THE BRAIN OF WILD AND CAPTIVE, *CHAETODIPTERUS FABER*

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In the present study, we present data on the metabolism of noradrenaline (NA), dopamine (DA) and adrenaline (A) in the hypothalamus (Hyp), olfactory bulb (OB), telencephalon (TEL), optic tectum (OT) and cerebellum (Cer) of captives and wild *Ch. faber*. Concentrations of NA, DA, A, 3-methoxy-4-hydroxyphenilglycol (MHPG), 3,4-dihydroxyphenilacetic acid (DOPAC), homovanilic acid (HVA), and 3-methoxytyramine (3-MT) were determined by HPLC-ED. The metabolite/amine ratio was used as indexes of catecholamine activity. In the Hyp, TEL, OT and Cer concentrations of NA were significantly higher in captive animals than in wild populations. However, in the OB, levels of NA did not differ between wild and captive fish. With the exception of the Cer, levels of MHPG as well as MHPG/NA ratio were significantly higher in captive fish than in wild animals. Levels of DA as well as Met/DA ratio did not differ between wild and captive fish in both the Hyp and OB. In contrast, levels of DA, DOPAC and HVA were higher in the TEL of captive fish as compared to wild animals; however, Met/DA ratio was similar in both groups. Levels of 3MT were under the detection limit. Only in the TEL concentration of A was significantly higher in captive fish than in wild animals.

PP-9

THE GnRH/GTH SYSTEM OF CAPTIVE AND WILD STRIPED BASS AND ITS RESPONSE TO GnRHa STIMULATION

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The striped bass, *Morone saxatilis*, is an important anadromous teleost that supports a growing aquaculture industry as well as vigorous recreational and commercial fisheries. As with many cultured fish species the striped bass exhibits reproductive dysfunction upon captivity. This dysfunction can be alleviated upon sustained administration of gonadotropin-releasing hormone (GnRH) and/or its agonists to ripe broodstock. We have developed an RNAse protection assay (RPA) in order to quantify mRNA expression of the three GnRH forms found in striped bass, salmon GnRH, cGnRH-II, and seabream GnRH. Using this RPA in conjunction with ELISAs for the three GnRH forms, a GtH subunit RPA and a GtH-II RIA, we have conducted experiments designed to a) ascertain whether there are differences in GnRH expression between captive and wild striped bass sampled on their spawning grounds in the Chesapeake Bay, and b) what effects GnRHa treatment has on the GnRH/GtH system of captive striped bass. Our data indicate that sbGnRH mRNA is elevated in captive striped bass while there are no significant differences between the other two forms. This may reflect the higher levels of sbGnRH peptide found in the pituitaries of the wild fish which was paired with lower levels of cGnRH-II peptide. In the second experiment we observed GnRHa treatment induce a surge of plasma GtH II levels followed by final oocyte maturation. We are presently measuring concomitant changes in brain GnRH mRNAs and pituitary GnRH peptide as well as GtH I and II subunit mRNAs from this experiment.

PP-10

TWO DIFFERENT MESSENGER RNAs FOR SALMON GONADOTROPIN-RELEASING HORMONE (sGnRH) ARE PRESENT IN RAINBOW TROUT, *ONCORHYNCHUS MYKISS*: REGULATION BY GONADAL HORMONES

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It is well known that the decapeptide salmon gonadotropin-releasing hormone (sGnRH) plays a major role in the regulation of the hypothalamic-pituitary-gonadal axis leading to the control of reproductive events in salmonids. Two genes for two types of sGnRH precursors have been cloned in sockeye salmon (Ashihara et al., J. of Endocrinol., 15: 1-9; 1995) and masu salmon (Higa et al., J. of Endocrinol.; 19: 149-161; 1997) and the presence of two mRNAs for sGnRH was suspected in rainbow trout (Ashihara et al., J. of Endocrinol., 15: 1-9; 1995). However, the importance of the two sGnRH genes in relation to salmonid reproduction is not known and their regulation needs to be further defined. In this study, we obtained two pro-sGnRH cDNAs from rainbow trout, *Oncorhynchus mykiss*, by a cloning strategy based on reverse transcription-PCR (RT-PCR). Thereafter, we developed a relative-PCR technique in order to determine the relative abundance of the two forms of mRNA in the brain. We simultaneously amplified a control gene, b-actin, as a base line to semiquantitate sGnRH-I and -II genes expression. This technique allowed us to determine the relative abundance of the two forms of mRNAs in different parts of the brain and at different stages of the reproductive cycle, in males and females. Finally, we examined the regulation by gonadal hormones of the sGnRH genes expression in vitellogenic female rainbow trout castrated and implanted with steroids (estradiol and testosterone).

PP-11

TRANSGENIC RAINBOW TROUT EXPRESSING SGnRH ANTISENSE mRNA UNDER THE CONTROL OF SGnRH PAB PROMOTER OF ATLANTIC SALMON

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Recombinant vector containing antisense DNA complementary to Atlantic salmon sGnRH cDNA driven by specific promoter Pab derived from sGnRH gene (Klungland et al, 1992) was introduced into trout eggs and resulted in transgenic animals that have integrated several copies of transgene in their genome and can transmit it through generations. Expression of sGnRH antisense mRNA was detected in the brain of transgenic fish F1 and F2 generations by means of RT-PCR. However, this didn't change significantly the level of sGnRH in the brain and pituitary measured either in F1 adults or 2- or 3-month-old F2 transgenic animals comparing with control group. There was slight decrease of blood concentration of gonadotropins in F1 transgenic group, but this didn't result in alteration of spermatogenesis. Growth rates, gonadosomatic indexes of adult fish are similar in both groups. However in some transgenic males spermiation was delayed and it could be induced only after exogenous gonadotropic stimulation. In addition, GtH2 release after *in vivo* testosterone implantation (10 mg/kg) was smaller in transgenic males than in control fish. Thus F1 Pab-sGnRH-antisense population exhibits high variability of physiological responses, probably because of the individual variation of transgene expression. Expression of sGnRH antisense RNA is not sufficient for complete blocking of sGnRH synthesis in transgenic rainbow trout. The use of strong enhancer elements, as well as regulatory and coding sGnRH gene sequences derived from rainbow trout but not from salmon, might be more convenient to develop a model of underexpressing of sGnRH in rainbow trout.

PP-12

LOCALIZATION OF STEROID RECEPTORS IN THE BRAIN AND PITUITARY OF RAINBOW TROUT

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Steroid nuclear receptors are ligand-dependent transcription factors which one activated modulate the expression of certain target genes resulting in a physiological response. Such interactions with DNA mediate the genomic feedback effects of steroids at the brain and pituitary levels which are crucial for the synchronization of the reproductive axis. As a first step in understanding these interactions, it is essential to precisely localize steroid receptors in the brain and the pituitary. Our institute has developed molecular and/or immunological tools corresponding to estrogen receptors alpha (ER), glucocorticoid receptors (GR) and androgen receptors (AR) from rainbow trout. *In situ* hybridization and immunohistochemistry have allowed to demonstrate that, if ER expression is restricted to the ventral telencephalon, preoptic region, and mediobasal hypothalamus, GR and AR have a much larger distribution. Attempts to identify the nature of the corresponding target cells by means of double immunohistochemistry or double *in situ* hybridization have been successful in a number of cases. ER were found to be expressed in tyrosine hydroxylase and glutamate decarboxylase-expressing neurons and GTH2 pituitary cells, but not in GnRH neurons which were shown to express GR. Glucocorticoid receptors were also detected in all GTH1 and GTH2 cells. The nature of the AR-expressing cells is currently unknown. These studies will provide a basis for future investigations aiming at identifying the precise molecular targets of steroids in these cells.

PP-13

REGULATION OF GNRH MRNA SUBTYPES AND PEPTIDE BY 11-KETO-TESTOSTERONE IN SEXUALLY IMMATURE AND MATURE MALE TILAPIA

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We examined the regulation of three molecular variants of gonadotropin-releasing hormone (GnRH)-encoding mRNAs and peptide levels by 11-ketotestosterone (KT) in sexually immature and mature male tilapia *Oreochromis niloticus*. Tilapias castrated for two weeks were given two intraperitoneal injections of sesame oil or 5 and 10 mg/g KT over 7 days. Immunocytochemistry and *in situ* hybridization histochemistry was performed using specific GnRH antibodies and 35^S-labelled antisense oligonucleotide probes complementary to salmon-, seabream-, and chicken II-GnRH, to localize GnRH cells in the terminal nerve, preoptic area and the midbrain, respectively. GnRH mRNA and peptide levels were quantified using computerized image analysis system. KT treatment had no effect on the neuronal size, total neuronal numbers, mRNA and peptide levels of terminal nerve, preoptic or midbrain GnRH. Comparisons between sexually immature and mature animals resulted in no change in terminal nerve, preoptic and midbrain GnRH neuronal numbers. However, compared to immature, mature animals had significantly larger preoptic and midbrain GnRH neuronal cell size, and increased salmon- and seabream-GnRH mRNA levels. Our results demonstrate that the terminal nerve, preoptic and midbrain GnRH neuronal numbers are independent of sexual maturity, instead they are established early during development. It is likely that factor(s) derived from mature testes but not KT might regulate the size of preoptic and midbrain neurons, and gene expression of salmon- and seabream-GnRH.

PP-14

SEASONAL PHOTOPERIODIC EFFECTS ON GABA SYNTHESIS IN THE BRAIN OF FEMALE AND MALE GOLDFISH (*CARASSIUS AURATUS*)

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The neurotransmitter GABA stimulates GTH-II release in the goldfish by indirect actions on GnRH and DA neurons. Both temperature and photoperiod are important environmental factors regulating fish reproduction. Our earlier work demonstrated that increases in water temperature significantly increased GABA synthesis rates (GABA-SR) in the telencephalon (TEL) and hypothalamus (HYP). GABA-SR are measured following inhibition of GABA catabolism by g - vinyl GABA (GVG). Goldfish were held at 18°C under a natural simulated photoperiod (Aberdeen, Scotland). In females, TEL GABA-SR was 30% lower ($p<0.05$) in January when serum testosterone (T) levels were elevated compared to July. In males, TEL GABA-SR was 70% lower ($p<0.05$) in October when serum T levels were elevated compared to July. In contrast, HYP GABA-SR was 20% higher ($p<0.05$) in females in January when serum estradiol (E2) levels were elevated compared to July. In males HYP GABA-SR was 33% higher ($p<0.05$) in July when serum E2 was elevated compared to October. In agreement with these results, our previous work indicated that T decreases GABA-SR in the TEL whilst E2 implantation increases HYP GABA-SR in sexually regressed goldfish. Seasonal differences in the stimulatory effects of i.p injected GVG (300ug/g) on GTH-II release were noted. In females GVG stimulated GTH-II release in January (mid to late gonadal recrudescence) but not in July (sexually regressed). In contrast, in males GVG-injection stimulated GTH-II release in July but not in October (early recrudescent fish). This study demonstrates that natural changes in photoperiod modulate the brain-pituitary GABAergic system controlling GTH-II release in goldfish. Tissue specific changes in GABA-SR correspond to seasonal changes in serum sex steroid levels.

PP-15

MOLECULAR CLONING AND EXPRESSION OF GONADOTROPIN-RELEASING HORMONE RECEPTOR (GnRH-R) IN THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS)*

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Gonadotropin releasing (GnRH) hormone is the main factor responsible for the preovulatory surge of gonadotropin. The binding of this peptide to its receptor (GnRH-R) which belongs to the family of the G-protein-coupled receptors, triggers a cascade of events leading to the release of gonadotropin. cDNAs for this GnRH-R have been cloned in several mammalian species but little is known about GnRH-R in fish on general and rainbow trout in particular. In order to study the regulation of GnRH-R expression in the rainbow trout, we have cloned a partial cDNA by RT-PCR and used it as a probe to analyse the localisation of GnRH-R mRNA in different tissues by RT-PCR and southern blot. We showed that this mRNA was expressed in the pituitary, the brain, the gonad and the retina. A faint signal was also observed in the muscle and intestine. In the brain, a more detailed investigation was carried out by *in situ* hybridisation. A specific signal was found in the preoptic area, the mediobasal hypothalamus and the periventricular layer of the optic tectum. The strongest signal was observed in the nucleus lateralis valvulae. The probe was also used to screen a brain cDNA library to obtain the full length cDNA and a genomic library to clone the promoter region of the gene.

PP-16

SPECIFIC BINDING OF 17α-20β-DIHYDROXY-4-PREGNEN-3-ONE IN THE BRAIN MEMBRANE FRACTION FROM MATURE MALE ATLANTIC SALMON, *SALMO SALAR*

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In salmonids, plasma levels of 17α-20b-dihydroxy-4-pregnen-3-one, (17,20-P) are highest during the period of spawning and 17,20-P treatment has been found to stimulate spawning behaviour in castrated male rainbow trout. The purpose of the present study was to investigate the possible presence of receptor-like binding in the brain for 17,20-P. Therefore, membrane homogenates from anadromous mature male Atlantic salmon, *Salmo salar*, were incubated with different concentrations tritiated 17,20-P with or without unlabelled 17,20-P added. A specific saturable binding of 17,20-P was found with a Kd of 0.2 nM and Bmax of 2120 fmol/mg protein. In competetive binding studies the only tested substance able to displace tritiated 17,20-P was unlabelled 17,20-P, none of the other tested steroids, progesteron (P), 17α-hydroxypregnenolone (17α-P5), 17α-hydroxyprogesterone (17α-P4), testosterone (T) or 11-ketotestosterone (11KT) had the ability to displace 17,20-P at a concentration of 10^{-6} M. The present results suggests, though not proving, the presence of membrane receptors for 17,20-P in the salmon brain.

PP-17

DIFFERENTIAL EXPRESSION OF THREE GnRH-FORMS IN THE BRAIN OF THE SEA BASS (*DICENTRARCHUS LABRAX*): AN *IN SITU* HYBRIDIZATION STUDY

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Unique in vertebrates, the brain of highly evolved teleosts, such as perciforms, expresses three GnRH (gonadotrophin-releasing hormone) variants, salmon GnRH (sGnRH), sea bream GnRH (sbGnRH) and chicken GnRH-II (cGnRH-II), derived from three different genes. The full length cDNA encoding these three GnRH peptides have been recently cloned in the sea bass (N. Zmora, Y. Zohar and A. Elizur, unpublished data). We report here the central distribution of the cells expressing these different peptides as studied by *in situ* hybridization using ^{35}S-labeled probes corresponding to the GAP (GnRH Associated Peptide) sequences. Sequence identities between the three probes did not exceed 45% (cGnRH-II-GAP vs. sbGnRH-GAP), allowing a good specificity of the hybridization signal, as also confirmed by appropriate controls on adjacent sections. In general, the pattern of distribution was similar to that previously reported in the sea bream: a group of sGnRH-GAP-expressing neurons was consistently detected in the caudal olfactory bulbs. However, isolated cells were also observed in the ventral telencephalon and the preoptic region. Large neurons expressing chicken GnRH-II-GAP were restricted to the nucleus of the medial longitudinal fasciculus in the dorsal synencephalon. These results demonstrate that salmon GnRH expression is not restricted to the olfactory bulbs but also extends to the preoptic region.

Reproduction in Wild Populations

OP-9

NUTRITIONAL CONDITION AND REPRODUCTIVE SUCCESS IN WILD FISH POPULATIONS

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The production of a large number of high quality eggs is important both to farmers and natural populations in the wild. Parental genes, fish size and nutritional status are known to have major effects on egg production. While broodstocks are carefully selected and benefit from a controlled environment resulting in maximum reproductive output, fish in the wild are exposed to a host of environmental factors that fluctuate in an uncontrolled manner. Species respond to this situation by using a number of different reproductive strategies. American eel and pink salmon reproduce only once. They become very emaciated at spawning and no fish survive to a second reproduction. Lake whitefish and arctic charr, in contrast, may skip reproduction to enhance survival, and nutritional condition is thought to determine the length of the spawning interval. Atlantic cod reproduce annually when mature. However, nutritional condition may bear upon their reproductive success. While cod with fast growth rates do not experience poor individual condition, strong seasonal variations in condition occur in slow growing cod. We have examined the impact of poor condition on the survival and reproductive energetics of cod in the northern Gulf of St. Lawrence during a period when stock production was declining, presumably in response to a deteriorating environment. During that period, cod condition during spawning declined to levels close to the range where energy reserves are completely exhausted. Fecundity and total egg dry weight were significantly lower in poor condition females, particularly those of smaller size. Despite lower fecundity, estimated loss in somatic energy during reproduction was proportionally greater for cod with poorer levels of condition. This lower egg production per unit of spawning stock biomass may have seriously impeded the reproductive potential of the stock in the late 1980s and in the 1990s. The relationship between size of the spawning stock and recruitment to the fishery may thus vary considerably with the nutritional condition of the spawners.

OP-10

OOCYTE GROWTH AND FECUNDITY REGULATION OF ATLANTIC HERRING (*CLUPEA HARENGUS*)

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Effects of body weight and condition of Atlantic herring, *Clupea harengus*, on oocyte growth and fecundity were examined. About 120 adult females were caught at each time of sampling covering successively the months July, Oct, Nov, Jan, and Feb using a surface/midwater trawl. Peak feeding season of herring is spring and summer and spawning period is Feb-March. Oocyte diameter (volume) was measured by an image analyser, fecundity counted under a binocular microscope, and the percentage of atretic oocytes and oocyte developmental stage given histologically studying 40 repeat spawners (total length > 32 cm) at each occasion. Water content and lipid content of muscle and gonad were also examined, and solids calculated by subtraction. All oocytes from July were at the cortical alveoli or early yolk globule stage, indicating a surprisingly early start of the next vitellogenic period. Oocyte diameter and estimated oocyte volume increased linearly and according to a power function during vitellogenesis, respectively, but with a large deviation. These deviations were partially explained by body condition differences. Fecundity was strongly affected by body weight. From a period between July and October to January individual fecundity was found to decrease continuously, i.e. at a time when the fish feed very little and thereby rely on accumulated but declining body reserves. As a result, relative fecundity (fecundity/somatic body weight) decreased until Jan. Noticed seasonal changes in estimated values of oocyte volume x fecundity, and intensity of atresia are also discussed.

OP-11

EGG NEUTRAL AND POLAR LIPID FATTY ACID COMPOSITIONS FROM SEVEN REPRODUCTIVELY ISOLATED POPULATIONS OF WALLEYE

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Walleye (*Stizostedion vitreum*) produce eggs with a large oil globule composed of neutral lipid. The lipoprotein yolk (LPY) lipid is primarily polar lipid. The LPY is consumed during early development, whereas the oil globule is normally conserved until after the larva begins to feed. Ovulated eggs were sampled from seven reproductively isolated walleye populations in the Hudson Bay, St.Lawrence, and Mississippi drainages (41 females in total). Neutral and polar fractions were separated from total lipid extracts by silica gel column chromatography. Fatty acid methyl esters were prepared and analysed by capillary GC to determine relative abundances of 29 fatty acids. Three principal components (PCs) derived from the relative abundances accounted for > 80% of the among-fish variability in fatty acid composition. Analysis of these PCs (one-way ANOVA or Kruskal-Wallis test) indicated significant differences among populations in both neutral and polar lipid fatty acid composition. Analysis of four important individual fatty acids (arachidonic acid, oleic acid, docosahexaenoic acid, eicosapentaenoic acid) also revealed significant among-population differences for both polar and neutral fractions. We hypothesized that, since the LPY polar lipid is the principal source of essential fatty acid necessary for early development, its composition is likely to be more tightly regulated than that of the neutral lipid which is primarily used as an energy source. This hypothesis was supported by analysis of the among-female variance in fatty acid composition which indicated that the relative abundance of neutral lipid fatty acids was more variable than that of polar lipid fatty acids.

OP-12

POSSIBLE EXPLANATIONS TO BALTIC COD REPRODUCTION PROBLEMS - A SHORT REVIEW

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In this paper disturbances in Baltic cod reproduction, additional to the basic problems with low salinity and oxygen content, are discussed. Abnormal cod embryos appeared first in 1979 and have been observed since. Still recruitment has been relatively well related to "reproduction volume" (sufficient salinity and oxygen content) until year-class 1991. Since that year, the year-class strengths have been lower than expected considering available reproduction volume. The same year a remarkable change in timing of the spawning occurred, the spawning period being expanded several months with a spawning peak delayed more than one month. If the low reproduction success is primarily related to a mis-match of hatching and environmental factors, including food availability, or an insufficient hormone level needed for induction of spawning, is unknown. Many experimental investigations on Baltic cod reproduction have shown low hatching success of Baltic cod compared to other cod stocks. Simultaneously with cod, other fish species in the Baltic Sea have experienced reproduction problems, e.g. the M74 syndrome in salmon and totally failing reproduction of pike and perch in some coastal areas. Several hypotheses regarding the causes of the reproductive disturbances are discussed, e.g. the impact of xenobiotic substances, known for their oxidative impact, on cell functions via a number of pathways important for reproduction. Some cholesterol oxides and PCBs are known to induce desaturation of fatty acids. Oxidation of unsaturated lipids (polyunsaturated fatty acids and cholesterol) as well as other important molecules (e.g. DNA) and their effect on the quality of spawning products is discussed.

OP-13

HORMONAL CONTROL OF SALMON HOMING MIGRATION

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The salmon homing migration is closely related to gonadal maturation, which is regulated mainly by the brain-pituitary-gonadal axis. In particular, gonadotropin-releasing hormone (GnRH) produced from the preoptic area (POA), gonadotropins (GtHs) from pituitary gland, and gonadal steroid hormones seem to play important roles in the salmon homing migration. During upstream migration of chum salmon (*Oncorhynchus keta*), the signals for pro-salmon GnRH mRNA in neurons in the POA were strongest in fish on the spawning ground. Expression of pituitary GtH subunit genes (GtH a and IIb) was higher in fish on the spawning ground than in the coastal sea, but no significant changes were observed in the GtH Ib mRNA. Serum levels of estradiol-17ß in females and 11-ketotestosterone in males decreased rapidly on the spawning ground while serum 17α,20ß-dihydroxy-4-pregnen-3-one (DHP) level increased dramatically. The effect of intramuscular implantation of GnRH analog (GnRHa) on homing migration was examined using lacustrine sockeye salmon (*Oncorhynchus nerka*). GnRHa implantation induced shortening of homing duration in lacustrine sockeye salmon of both sexes in the early spawning period. The levels of GtH a and IIb mRNAs in GnRHa-implanted fish were higher than those in controls, but the level of GtH Ib showed no difference between GnRHa-implanted and control fish. The implantation caused significant increases of serum DHP levels in both sexes. These results indicate that GnRH stimulates GtH II release from the pituitary gland, and then GtH II enhances serum DHP levels. These hormonal changes may exert direct influence on the final stages of the salmon homing migration.

OP-14

IN VIVO AND *IN VITRO* OVARIAN STEROID PRODUCTION BY FISH FROM A NATURAL POPULATION OF THE BROODING TROPICAL DAMSELFISH *ACANTHOCHROMIS POLYACANTHUS*

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The spiny damselfish *Acanthochromis polyacanthus* is a brood-protecting species found on coral reefs throughout the Indo-Pacific basin. Its brooding habit offers an opportunity to assess endocrine function in relation to brood and egg stage in fish under natural conditions. This study investigated ovarian function across the boundary between regressed and reproductively active fish. Damselfish were captured by divers from coral reefs around Lizard Island on Australia's Great Barrier Reef, blood-sampled underwater and placed in keep-nets. At the end of the dive, fish were transferred to the surface, killed by spinal transection and the ovaries removed onto ice cold Leibowitz L15 medium. Ovaries and blood were transported on ice to the laboratory where plasma steroids were extracted for measurement by RIA. Individual ovarian follicles were dissected from ovaries and incubated in tissue culture plates in L15 at 10 follicles per well with steroid precursors. Damselfish have multiple oocyte clutches in the ovary but only a single clutch is ovulated - further batches mature if a brood is lost at a young age. At greater brood ages, vitellogenic oocytes are resorbed and only previtellogenic oocytes remain. Vitellogenic fish were characterized by elevated plasma levels of testosterone (T) and 17ß-estradiol (E2) relative to previtellogenic fish. T and E2 levels were highest in fish that were undergoing FOM or that had ovulated. *In vitro*, vitellogenic follicles produced T and E2 in response to treatment with 17-hydroxyprogesterone (17P) and androstenedione (A), and E2 in response to treatment with T. Previtellogenic follicles produced E2 from T but not T from 17P or A. None of the conversions were strongly GtH-dependent. This suggests that a critical step in the transition from the previtellogenic to the vitellogenic condition is the activation of the P450C17 enzyme complex.

PP-18

SPAWNING FREQUENCY AND BATCH FECUNDITY OF THE WHITEMOUTH CROAKER (*MICROPOGONIAS FURNIERI*) OF THE RÍO DE LA PLATA ESTUARY, ARGENTINA-URUGUAY.

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The reproductive biology of whitemouth croaker, *Micropogonias furnieri*, inhabiting the estuarine waters of the Río de la Plata (Southwest Atlantic, 35° S), was studied by using histological analysis of 878 ovaries. The samples were collected during the main spawning peak (November) and near the end of the breeding season (February-March), during 1995-96 and 1997-98. The spawning area was characterized in terms of temperature and salinity. Whitemouth croaker spawns in the inner part of the Río de la Plata estuary, in coincidence with a bottom salinity front, in a thermohaline range of 18-22 °C and 10-28 salinity units. This species is a multiple spawner with indeterminate annual fecundity. Daily spawning activity occurs mostly at the dusk, based on the incidence of females with new postovulatory follicles. Spawning frequency, determined by using the percentage of females with postovulatory follicles, was about 11% during November 1995 and 7% during February 1996. At these frequencies, each female on average spawned a new batch of eggs every 9 or 14 days, respectively. Batch fecundity ranged from 38,000 (31cm total length) to 850,000 (65 cm total length) hydrated oocytes. The range in relative fecundity was 100 to 300 hydrated oocytes per gram of female (ovary free). Differences in the size-fecundity relationships estimated during the main peak and the end of the spawning season were observed. Fecundity values were significantly lower during February-March, coinciding with an increase in the rates of atresia.

PP-19

PRELIMINARY RESULTS ON THE REPRODUCTIVE CYCLE OF *PAGELLUS ACARNE* IN THE GREEK WATERS

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Pagellus acarne, a member of the family Sparidae, is a species of high commercial value. No information on the reproductive cycle of the species is available for the Greek waters. The present work, considered as preliminary, aims to contribute to the knowledge of this subject. Samples were collected monthly for two years, in the North Evoikos Gulf (eastern Central Greece), using trammel nets. Histological sections of the gonads were used for the determination of the maturity stages. Seven stages were identified for both males and females. The reproduction period was found to extend from June to November. However, this was not the case for all individuals. The statistical analysis of age-maturity stage relationship showed a statistically significant relation, for both sexes. In effect, the examination of the monthly variation of the frequency of each maturity stage with age indicated that the opening of the reproductive activity proceeded successively from the older individuals to the younger ones. The former started their reproductive activity mostly in June, whereas the latter mostly in September. More investigation is needed for the advantages offered by this procedure to the reproductive strategy of the species.

PP-20

SEXUAL MATURATION IN THE BLUEFIN TUNA (*THUNNUS THYNNUS*) FROM THE CENTRAL MEDITERRANEAN SEA

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In order to clarify the spawning patterns of bluefin tuna (*Thunnus thynnus*) during the fishing season in the central Mediterranean Sea (Italy), histological characteristics of gonads and plasma levels of 17ß-estradiol (E2), testosterone (T) and 11-ketotestosterone (11-KT) were examined. Histological observations of the ovary together with measurements of E2 and T in female fish indicate a period of gonadal maturity at the end of May and the beginning of June. E2 and T concentrations in females reached maximum levels of 46 ng/ml. Plasma 11-KT concentrations of mature females do not exceed the 11-KT levels of immature fishes of 3 ng/ml. In some mature female individuals the concentration of 11-KT increased to 10 ng/ml but the testosterone concentration was much higher. In mature males a value of 52 ng/ml testosterone and 54 ng/ml for 11-ketotestosterone was reached. In the plasma of young fish caught in October , that had not reached sexual maturity, the 17ß-estradiol concentration reaches 19 ng/ml compared with 3 ng/ml for testosterone and 11-ketotestosterone. In mature fish with increasing of testosterone concentration it is possible to determine sex from the ratio of [T] / [11-KT]. Histological patterns of the gonads show that all mature female fishes had not released eggs. Our study makes it evident that all bluefin tuna caught in the Mediterranean Sea in May and at the beginning of June have not contributed to the recruitment of the fish population. We thus strongly recommend a closed period to protect the fish stocks at this time.

PP-21

THE ROLE OF OVARIAN ATRESIA / APOPTOSIS IN THE SOLE (*SOLEA SOLEA* L.) WITH RESPECT TO THE REALISED FECUNDITY

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The sole is a multiple batch spawning fish that is heavily exploited in European shelf edge waters. To verify the age based population assessment of these wild stocks a method was adopted that involves dividing the population's annual egg production by the average female's realised fecundity. In order to carry out this work the population was sampled extensively throughout the spawning season and the egg production process within the ovary investigated using a quantitative histological method in order to gain the necessary biological information about realised fecundity and the spawning process. This analysis revealed that the potential fecundity measured at the start of spawning is not all realised as spawned eggs but a significant proportion of developing follicles become atretic. The intensity (number of atretic follicles) and prevalence (proportion of fish with atretic follicles) were greatest just prior to spawning, the latter being estimated to last on an individual basis about 60 days. Smaller follicles in the left hand side of the follicle size distribution comprising the annual fecundity were most likely to be atretic but not exclusively. Preliminary results are presented on work to estimate the persistence of atretic follicles in the ovary using morphological criteria in order to estimate the total number of follicles effected during spawning. These results are compared with a method to measure apoptosis based on 3'end labeling of short chain DNA fragments described previously.

PP-22

REPRODUCTIVE SEASONALITY OF THE FLORIDA GAR, *LEPISOSTEUS PLATYRHINCUS*, COLLECTED FROM ORANGE LAKE, FLORIDA (USA)

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Exogenous environmental factors affect the timing of reproduction in fish. Interpretation of these environmental signals and control of the timing of reproduction is mediated by the nervous and endocrine systems. We are interested in how exogenous signals (photoperiod, water temperature, and xenobiotics) are transduced into endogenous signals (hormones and neurotransmitters) to regulate reproduction. Certain xenobiotic compounds have been shown to behave as exogenous signals and alter the development and functioning of the endocrine system. For us to recognize the abnormal condition of an altered endocrine system, we must first describe the normal reproductive endocrinology of our model. To that end, we carried out a reproductive seasonality study of the Florida gar. Once each month for a year, we collected 24 fish from Orange Lake located in North-Central Florida. Somatic measurements and blood samples were obtained. We collected climactic parameters during the study period as well as historical climate data. Endpoints include gonadosomatic and hepatosomatic indices, follicular diameters, seminiferous tubule areas, and plasma concentrations of estradiol, testosterone, and vitellogenin. We have observed a seasonal pattern in the size of the gonad in both sexes, particularly in the females. Currently in our lab, histology of all gonads is being completed and radioimmunoassays for hormone concentrations are in progress. We are also examining the *in vitro* steroid hormone production and metabolism during the months immediately preceding and following ovulation. In conclusion, this study represents the first report on the reproductive seasonality of the Florida gar.

PP-23

INTERRUPTIONS IN THE SPAWNING CYCLE OF ATLANTIC COD, *GADUS MORHUA*, FROM A FJORD-LIKE INLET IN TRINITY BAY, NEWFOUNDLAND

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Female Atlantic cod, *Gadus morhua* (n=151), were collected from Smith Sound, Newfoundland during an acoustic survey of the area in January of 1999. Visual examination of ovaries revealed three maturity classes: fish that were maturing for the present spawning season as indicated by the presence of opaque oocytes, those that were immature and those that appeared to have spawned in the previous year but were not showing obvious signs of maturing for the upcoming spawning season. Histological examination of the ovaries revealed that the majority of fish in the latter category were undergoing mass resorption of oocytes, most of which had reached the endogenous yolk stage. Fish in this category were in the size range of 43-79 cm and included females that were aborting their first attempt at maturation as well as fish that had spawned previously but would not in the upcoming year. Gonadosomatic indices were significantly lower ($p<0.001$) than maturing females but higher ($p<0.001$) than immature fish. Hepatosomatic indices were significantly lower ($p<0.05$) than maturing fish and did not differ ($p>0.05$) from that of immature fish which may indicate that the interruption in the maturation cycle was due to insufficient nutrient storage required to allow normal ovarian maturation. Instances of non-annual spawning should be considered when predicting spawning biomass and recruitment.

PP-24

CHANGES IN HEPATIC ESTROGEN RECEPTOR CONCENTRATIONS AND THE CORRELATION WITH PLASMA VITELLOGENIN DURING THE ANNUAL REPRODUCTIVE CYCLE OF THE VIVIPAROUS EELPOUT (*ZOARCES VIVIPARUS*)

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The synthesis of vitellogenin (the yolk-protein precursor) is an important part of the reproductive cycle of teleosts. The process of vitellogenesis is under endocrine regulation by the endogenous estrogen 17β-estradiol. Estrogens exert their effect by interacting with the estrogen receptor (ER). We have formerly characterised the hepatic cytosolic ER in eelpout with respect to binding properties and shown that estrogen treatment induces hepatic ER in addition to plasma vitellogenin. In order to better understand the endocrine regulation of vitellogenesis it is important to examine the relations between the different parameters in the estrogenic response. In this study we have examined a natural population of male and female eelpouts. The concentrations of 17β-estradiol, hepatic ER and plasma vitellogenin during the annual reproductive cycle have been measured and will be presented.

PP-25

SEASONAL DYNAMICS OF FEEDING AND ACCUMULATION OF FAT IN FISHES WITH INTERRUPTED AND CONTINUOUS OOGENESIS

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Our research concentrated on comparison of annual dynamics of fat accumulation and feeding in fishes with interrupted oogenesis (sensu Gotting and Oven) and "determined" fecundity and fishes with continuous oogenesis and"non-determined" fecundity. As an example of fishes with continuous oogenesis we used some of the Black Sea species (anchovy, Mediterranean scad and goatfish), and some northern species (cod, herring etc.) as species with interrupted oogenesis. Highest level of body fat content in anchovy was observed just before the winter season (December-early January), lowest level - at the beginning of spawning season (May). The body fat remained low during the whole period of most active feeding and spawning (June - August). Since the middle of August spawning intensity decreased and the fat content increased sharply. The same trends were also observed for Mediterranean scad and goatfish. The body fat content in northern fishes increased gradually from May to October and also gradually decreased during winter time. Vitellogenesis and the building of total fecundity took place during winter season using the reserved body fat. During the vitellogenesis occurs redistribution of body fat - it gradually moves from body to gonads. Thus, our data suggest that the type of oogenesis is directly connected to the energy transformation paths in fishes. As we have shown, the processes of fat accumulation and vitellogenesis never match each other in time. That probably defines the type of oogenesis and different ways by which the fecundity of a particular group of species develops. When the maximum fat content is observed just before the vitellogenesis, the growth and maturation of oocytes occur using available energy resources stored in fat. This results in interrupted oogenesis and "determinated" fecundity. However, in case when the lowest fat content is observed just before the oogenesis, then the gonad maturation occurs using whatever energy is obtained during feeding. In this case the energy obtained from food is used directly for gonad maturation without using it to further increase body fat content. This process is the basis for constant oocyte growth and depends only on actual feeding conditions.

PP-26

CHUM SALMON (*ONCORHYNCHUS KETA*) GONADS CYTOMORPHOLOGY IN LIFE CYCLE

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The Far Eastern *Oncorhynchus chum* salmon ovary cytomophology during estuary, ocean and river life periods was investigated. When juveniles terminated their catadromous migration in estuaries the testis consists of mitotic spermatogonia (I-st maturity stage) and ovaries - cytoplasmatic oocytes at 1 and 2 steps development (II-nd stage). In ocean, when the gonads was in III maturity stage, females weight varied between 1,6-4,0 kg, ovary 10-300 g, gonadosomatic index (GSI) 0,7-9,5 %, oocytes diameter 1,9±0,1 - 6,7±0,2 mm. In the IV gonads maturity stage ovary weight is 300 g and more, GSI 7,5-13%, oocytes diameter is about 5,3±0,2 - 7,4±0,2 mm, eggs envelop thickness 24-26 μm. Oocytes morphology in the part of females shows the begining of last oogenesis period – completing of maturation, and V ovary maturity stage. At Sakhalin spawn rivers in female (2,0-6,6 kg) the ovaries (570-1040 g) have IV-V stage, oocytes diameter 7,0-9,5 mm, GSI 15,6-43,8%, fecundity 1482-4420. Testis morphology in 12-35% males was abnormal: they had many constrictions, haemorragies and (or) additional lobes.

PP-27

THE PROCESSES OF DEVELOPING OOCYTE RESORPTION AND ITS CONSEQUENCE FOR REPRODUCTIVE CAPACITY OF WALLEYE POLLOCK POPULATIONS

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The resorption of developing oocytes is described for many species of fishes. The forms and stages of passing through resorption are well studied, however qualitative and quantitative assessments of this phenomena are absent. The materials for this study were collected during 1987-1998 in Bering, Okhotsk and Japan seas, in miscellaneous periods of annual cycle. The material was treated by histological method and by scanning electron microscopy. Identification of normally and pathologically maturing ovaries was realized in fresh and fixed conditions. The size-morphological oocyte groups and their state were determined *in vitro* analysis. Walleye pollock (*Theragra chalcogramma*) was characterized by synchronous vitellogenesis type, but oocyte maturation, i.e. process of oocyte hydration and its ovulation follows asynchronous pattern. Ovulated eggs accumulate in the ovarian cavity. Walleye pollock was characterized both by high level of oocyte resorption in individual gonad and by considerable number of females with total resorption of oocytes in gonads. Number of females with total oocyte resorption was varied during the spawning season. They constituted at the beginning, in the middle and at the end of spawning period in the Bering Sea 7.1 %, 11.2 % and 3.5 %, respectively; in the Sea of Japan, correspondingly, 3.8 %, 25.1 %, 7.1 %; in the Okhotsk Sea - 0.6 %, 25.3 %, 2.6 %. Such females in summer and autumn can be erroneously taken for spawners. Over 60 % of females had pathologically developing oocytes and their number in some individuals amounted to 80 %. Estimation of the actual individual fecundity resulted in creating a possibility for population fecundity calculation. It is necessary to allow a quantitative assessment of developing oocyte resorption at calculations of number and biomass of spawning stock.

PP-28

LIPID AND FATTY ACIDS CONTENTS IN THE GONAD OF FEMALE AND MALE CATFISH, *PIMELODUS BLOCHII*, DURING GONADAL DEVELOPMENT

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As in higher vertebrates, lipids play a very important role in metabolism and reproduction in fishes. The FA composition of temperate and sub-tropical fish is well documented in the literature; however, few data are available on tropical fish. The present study reports on the content of total lipids and fatty acids in gonad of female and male catfish, *Pimelodus blochii* during gonadal development. Fish samples were collected monthly from February to July from Caño Iguez, Portuguesa State in Venezuelan Plains. Total lipid concentrations in the ovary showed seasonal fluctuations with the highest levels during the preovulatory period. Total lipid concentrations in the gonads of females were significantly higher than those of the males during the two month preceding spawning when plasma levels of oestradiol were at their highest. The FA composition varied with season. In both testis and ovary, the analysis of FA of individual lipid classes indicates different FA compositions for total lipids and phospholipids. During February and March, FA in phospholipids are mostly saturated or monounsaturated. Polyunsaturated FA were found only in small amounts, however, an increased from February to July was observed. In both testis and ovary a decrease in the content of arachidonic acid during the spawning period was observed.

PP-29

GENETIC VARIABILITY IN FIVE NATURAL POPULATIONS OF *ODONTESTHES BONARIENSIS* (PISCES, ATHERINIDAE)

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Odontesthes bonariensis is a silverside freshwater fish. Recent studies indicate that hybrids between two species of silversides produced by artificial insemination show promising performance for intensive aquaculture. With the aim of increasing the number of genetic markers for population and species characterization, isoenzymatic analyses were started. This work describes a genetic study in five populations from differents regions of Buenos Aires Province, Argentina: Madariaga (MA), Coronel Pringles (CP), San Miguel del Monte (SMM), Chasico (CH) and Rio de La Plata (RP). Homogenates were obtained from three differentes tissues. Electrophoretic assays of 10 isoenzymatic system allowed to detect 20 loci, two of them polymorphic. The percent of polymorphic loci and the average heterozygosity estimated were P= 10 %, H= 0.05 for MA and P= 5%, H= 0.02 for CP, SMM, CH and RP. Populations may be differenciated by allelic frequencies and the presence of private alleles. Accordingly, genic and genotypic differentiation were highly significant and the estimated gene flow very low. The low genetic variability in this species is similar to others atherinids and could be a characteristic for this group.

PP-30

ENERGY ASPECTS IN FISH EARLY LIFE HISTORY

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The peculiarities of energy distribution have strong importance in early ontogenesis of Teleostei when the nutrition is only based on endogenous resources. Long-term experimental studies of the patterns of growth, yolk resorption, and oxygen consumption during embryogenesis in fish from different ecological groups served as the basis for estimating the efficiency of utilization of the endogenous resources for growth, as well as the level of energy expenditure at different values of temperature. It is shown the differences between dynamic of embryo growth and yolk resorption. Oxygen consumption is determined by intensivity syntetic processes, from one side, and degree of development of embryo respiratory mechanisms, from another. There is also an estimation of energy balance of embryos and larvae of fish of different ecological groups.

PP-31

SPAWNING DYNAMICS OF CAPE HAKES, *MERLUCCIUS CAPENSIS* AND *M. PARADOXUS*

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The biological data from the hake biomass trawl surveys of the South African West and South coasts, for the period 1986-1997, were analysed. The main aims were: 1) to determine the spatial distribution of spawning hakes; 2) to relate the occurrence of spawning to bottom-depth, -oxygen and -temperature levels; and 3) to compare the gonadosomatic indices (GSI) for this period. Considering that the survey data spans for 11 years, there is a surprisingly low incidence of spawning fish in the trawl catches. However, histological data has shown spawning hake to be prevalent throughout the year. Hake tend to spawn all along the South African coast, within depth strata ranging from 53 to 338 m for *M. capensis* and 162 to 495 m for *M. paradoxus*. The GSI of active, spawning and spent females were compared both seasonally and annually.

PP-32

MOBILIZATION OF LIPIDS IN VARIOUS TISSUES OF *CHANNA GACHUA* DURING OVARIAN CYCLE

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Specimens of *Channa gachua* were collected from local water resources. The fish were sacrified, tissues were taken out and weighed for lipid extraction during different stages of the ovarian cycle: immature, maturing, mature and spent. Lipids play a vital role in proper gonadal development and spawning as well as it is important to know the nutritional value of the fish as such. The lipid content varied depending on the environmental conditions and physiological state. The building up of gonads (ovary) is usually at the expense of body reserves as dietary materials seem inadequate to meet the huge demands made by the sex organs. In maturing fish greater demand resulted in more depletion of reserves. The lipids occurred in fish in order of liver, ovary and muscle. It was observed that lipids were at the highest in the ovary when the fish were fully mature and ready to spawn. At the end of spawning/ovarian cycle the hepatic lipids were recorded to be at a significant maximum.

PP-33

GONADOSOMATIC AND HEPATOSOMATIC INDEX FLUCTUATIONS OF THREE DEEP-WATER SPECIES IN THE WEST COAST OF GREECE (IONIAN SEA)

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The yearly gonadal cycle of three species of deep-sea fish from the Ionian Sea, *Peristedion cataphractum*, *Helicolenus dactylopterus* and *Hoplostethus mediterraneus* were assessed by measuring the gonadosomatic index (GSI) as well as the hepatosomatic index (HSI) in both sexes. The three species were caught during twelve monthly sampling cruises (December 1996-November 1997) in depths between 300 and 750 m. In total, 853 specimens of *P. cataphractum* (422 males and 431 females), 325 specimens of *H. dactylopterus* (157 males and 168 females) and 426 specimens of *H. mediterraneus* (171 males and 255 females) were examined. *P. cataphractum* had highest GSI during April for males (0.59) and during September for females (3.78) and highest HSI during June for males and females (1.80 and 1.90 respectively). *H. dactylopterus* had highest GSI in April for males (0.17) and in February for females (1.72) and highest HSI during June for males (1.02) and during October for females (1.51). The Highest GSI of *H. mediterraneus* was recorded during May for males (0.48) and during March for females (2.30) whereas the HSI was higher in March for males (1.15) and in April for females (1.47). The yearly fluctuation of the GSI showed that the period of breeding activity of *P. cataphractum* ranged from May to September, of *H. dactylopterus* from December to April and of *H. mediterraneus* form March to September. The three species showed an extended reproductive period that could be attributed to the lack of pronounced cyclic environmental factors in the deep-sea.

Reproductive Behaviour

OP-15

SEXUAL PLASTICITY OF BEHAVIOR AND GONADOTROPIN SECRETION IN GOLDFISH AND CRUCIAN CARP

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The gonochoristic goldfish (*Carassius auratus*) exhibits gender-typical behavior and gonadotropin (GTH) secretion. Female sex behavior (oviposition), which is not influenced by ovarian steroids, is elicited at ovulation by hormonal prostaglandin $F_{2\alpha}$ (PG) produced in the reproductive tract in response to ovulated eggs. In contrast, male sex behavior (chasing and ejaculation) is primed by androgen (likely 11-ketotestosterone, KT) and triggered by prostaglandin sex pheromones from ovulatory females. Heterotypical (opposite sex) behaviors do not normally occur in the goldfish but can be induced in adult fish by hormone treatment. Thus, PG injection induces female behavior in males, and KT implantation induces male behavior in females in response to prostaglandin pheromone. Goldfish also exhibit gender-typical GTH surges (a preovulatory GTH surge in females and a pheromone-induced GTH surge in males). Prior to spawning, a male GTH surge is triggered by the steroidal pheromone (17,20β-dihydroxy-4-pregnen-3-one, 17,20β-P) from preovulatory females. KT implant induces female goldfish to display male-typical GTH surge in response to 17,20β-P. KT implant also induces the gynogenetic Japanese crucian carp (*Carassius auratus langsdorfii*) to exhibit male-typical behavior and GTH secretion. Although female-typical GTH surge has not yet been induced in male goldfish, the results from gonochoristic goldfish and gynogenetic crucian carp suggest that mature fish retain a bisexual brain which regulates sex behavior and GTH secretion simply in response to gender-typical hormone production.

OP-16

NATURAL MODULATION OF HORMONAL PHEROMONE ACTION IN THE GOLDFISH

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Hormonal derivatives function as important components of sex pheromones in teleost fish. This conclusion is based on the fact that the olfactory systems of many species of fish detect hormonal derivatives with high sensitivity, and that a few species (goldfish and Atlantic salmon) have been shown to exhibit behavioral and/or endocrinological responses to these olfactory stimuli in the laboratory. However, little is understood either of the complete identity of any sex pheromone (i.e. the natural chemical signal which elicits 'complete' responses in the wild), or of possible modulatory effects of environmental and behavioral factors on these signals. In particular, the specificity of hormonal pheromones has been a puzzle because studies-to-date suggest that fish release large quantities of a variety of hormonal metabolites, many of which function as olfactory stimulants in other species. Here, we address three sets of experiments which demonstrate that the natural actions of hormonal stimuli released by goldfish are modulated by many factors. First, we describe several experiments showing that some hormonal products (odorants) are released exclusively in the urine and that goldfish control urinary release, thereby restricting pheromonal active space. Second, we describe how certain steroidal odorants (androstenedione) and even non-hormonal odorants (body odor) can modulate responsiveness to hormonal cues, thereby providing evidence that natural pheromones are likely rather specific mixtures. Lastly, we present evidence that hormonal components are short-lived in natural waters, presumably because of bacterial breakdown. Together, these experiments suggests that the actions and identities of natural hormonal pheromones are more specific than initial laboratory experiments have indicated.

OP-17

EFFECTS OF TWO FORMS OF GONADOTROPIN RELEASING HORMONE AND A GnRH ANTAGONIST ON THE SPAWNING BEHAVIOR OF THE FEMALE GOLDFISH, *CARASSIUS AURATUS*

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The central effects of two native forms of gonadotropin-releasing hormone (GnRH), salmon GnRH (sGnRH) and chicken GnRH-II (cGnRH-II), and a GnRH antagonist, [ac-delta 3-pro1, 4fd-phe2, d-trp3,6]-mGnRH (analog E) on the spawning behavior of sexually recrudescent female goldfish were investigated. spawning behavior was induced by intramuscular injection of females with prostaglandin $F_{2\alpha}$ and placing them in the presence of mature males. Behavioral responses were quantified by recording the numbers of spawning acts performed by each pair of fish during two hours following brain intracerebroventricular (ICV) injection of different dosages of peptide or saline as control. Each pair of fish was pre-tested to determine their level of spawning behavior, for comparison to spawning behavior following treatment. ICV injections of analog E caused a significant dose-dependent decrease in the number of spawning acts performed by females, suggesting a role of endogenous GnRH in modulating spawning behavior. Injections of sGnRH or cGnRh-II resulted in a biphasic dose-dependent response. Doses of 0.05-0.5 ng/g significantly enhanced female spawning behavior, whereas doses of 1ng/g and higher resulted in an almost complete inhibition of spawning. Analog E suppressed the actions of exogenous sGnRH and cGnRH-ii on spawning behavior, as both sGnRH and cGnRH-II-induced increases in number of spawning acts were inhibited by concomitant treatment with analog E. These results indicate that GnRH plays a major role in the control of female reproductive behavior in goldfish.

OP-18

SPAWNING TACTICS AND REPRODUCTIVE SUCCESS IN TWO EUROPEAN CYPRINIDS: THE BREAM (*ABRAMIS BRAMA*) AND THE BARBEL (*BARBUS MERIDIONALIS*)

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The reproductive behaviour of European barbels species (e.g. *Barbus meridionalis* and *Barbus barbus*) and bream (*Abramis brama*) have already been described. This study presents detailed results on the mating tactics of males and females in both species. Reproductive behaviours were studied in aquaria and data were recorded with a video camera system. The reproductive behaviour of *Barbus meridionalis* is polyandrous. Courting and sneaking strategies were described. The mating system of *Abramis brama* is polygamous, i.e. each male can mate successively with several females and each female can mate simultaneously and successively with several males. Old males *A. brama* defended territories which included spawning substratum. Youngest males developed a sneaking tactic. In both species, some males seemed to be more efficient than others. The spawning success of the different kinds of males was analysed in the two species accordingly to the males spawning tactics and their reproductive status. Are the mating tactics of males well correlated with reproductive success? In order to address this issue we have used a microsatellite-based DNA fingerprinting system for *B. meridionalis*. Results on 41 and 15 larvae allowed the discrimination among putative fathers. The genetic analysis confirmed the behavioural approach in that some individuals seemed more efficient. Similar analysis should be developed in the bream.

OP-19

ACOUSTIC BEHAVIOUR OF RUSSIAN STURGEON DURING PRESPAWNING PERIOD

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Russian sturgeon *Acipenser guldenstadti* likewise the other sturgeon species usually do not produce any acoustic signals. But during the prespawning period acoustic activity of sturgeons exceedingly increases. Russian sturgeon produced during induced maturation impulse signals, various sounds like whistle and some other signals. Males produced acoustic signals tenfold more than females. The number of whistle signals produced by fishes is in correlation with the state of fish readiness to spawn and increases after injection of sturgeon pituitary preparation. The number of signals of the other types did not change noticeably. Observations on the response of Russian sturgeon to emitting sounds by the transducer showed that the fish movement activity of both sexes increases. During two first emitting each of fifteen minutes long some fishes went towards the transducer while one of whistle signals was emitted.

PP-34

EXTRA-RETINAL PHOTORECEPTORS ARE MORE IMPORTANT THAN RETINAL PHOTORECEPTORS FOR SEXUAL MATURATION IN THE THREE-SPINED STICKLEBACK (*GASTEROSTEUS ACULEATUS*)

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In addition to the retina, non-mammalian vertebrates also have extra-retinal photoreceptors in the pineal gland and elsewhere in the brain. In birds extra-retinal photoreceptors can mediate photoperiodic stimulation of reproduction, whereas the retinae can not do this. Long, but not short, photoperiod stimulates sexual maturation in blinded sticklebacks. Thus, extraretinal photoreceptors can mediate this response in this fish, but whether the retinal photoreceptors also are able to do this has not yet been established. To examine this, the top of the skulls of adult male sticklebacks were covered with black plastic foil to shield extraretinal photoreceptors, but leaving the eyes uncovered. Controls were covered with transparent plastic. The fish were kept at c.17° C and a photoperiod of 16 hours light: 8 hours dark under three different light intensities and under darkness. One hour per day all fish had full light so that they could feed. After one month the experiment was terminated. Maturation was assessed by the development of breeding colours and kidney hypertrophy, an androgen-dependent secondary sexual character in sticklebacks. Fish matured to a higher extent under high light intensities than under low ones. Control fish matured more than covered fish. This suggests that retinal photoreceptors are ineffective, or at least less effective than extraretinal photoreceptors, in mediating photoperiodic stimulation of reproduction in sticklebacks.

PP-35

EFFECTS OF SEX STEROIDS ON UPSTREAM MIGRATORY BEHAVIOR OF MASU SALMON

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In a previous study we found that testosterone (T) can stimulate the upstream migratory behavior of immature salmonids. In the present study, the effects of T, 11-ketotestosterone (11-KT), estradiol-17β (E2) and 17α,20β-dihydroxy-4-pregnene-3-one (DHP) on the upstream behavior of yearling masu salmon were investigated using artificial raceways. In October, Silastic capsules containing 500 μg of T, 11-KT, E2, DHP or vehicle were implanted into 30 each precocious males that were castrated in August and 30 each immature females. Thirty sham-operated precocious males were also implanted with capsules which contained vehicle. Both precocious males and immature females were separately transferred to lower ponds of the raceways, and upstream behavior was observed for 2 months. Blood samples were taken from fish which moved upward and remained in order to measure plasma levels of hormones by RIAs. In precocious males, the frequency of upstream behavior was 72, 56, 44, 32, 20 and 16 % in sham, 11-KT, T, DHP, E2 and castrated groups, respectively. In immature females, frequency of the behavior was 61, 52, 45, 36 and 18 %, in E2, 11-KT, T, DHP and control groups, respectively. Plasma levels of T and DHP in sham-operated precocious males were high, while levels of T, 11 KT, E2 and DHP in castrated precocious males and immature females were low. Each injected steroid caused high plasma levels. These results indicate that sex steroids such as 11-KT, T and E2 have strong stimulatory effects on the upstream behavior. Upstream behavior occurred during night, suggesting photoperiod is a timing factor for the upstream behavior.

PP-36

A SEX PHEROMONE OF MALE JAPANESE DACE, *TRIBOLODON HAKONENSES*, INVOLVED IN ATTRACTING FEMALES TO THE SPAWNING GROUND

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Japanese dace, *Triborodon hakonensis*, congregate at the spawning ground (rapids of the middle reaches of rivers) in late spring and spawn in a promiscuous mating system. Under experimental conditions, ovulation and spawning do not occur in the absence of appropriate environmental stimuli, even if both mature males and females are reared in the same pond. Thus, appropriate environmental conditions are prerequisite for the physiological processes associated with final maturation. Moreover, it is anticipated that efficient communication systems are in place to guide the animals to a distinct spawning ground. In the present study, we carried out a preference test with females using a Y-maze like concrete waterway (1x5m) to confirm the presence of an attractant from the males. Experiments were carried out in June, during the natural spawning period of Japanese dace. The upper two compartments, which were partitioned with a screen, held either both sexes or males only as a stimulus source. One side of the stimulus compartments was set up as a simulated spawning site by covering the bottom with gravel. In the absence of any fish in the stimulus compartments, test females randomly chose both sides. However, when fish of both sexes were held together in the compartments, test females showed a preference for compartment with a spawning environment without actually sighting the males. Furthermore, when only males were housed in the compartments, similar selectivity was observed. These results suggest that a sex pheromone, released from males in the spawning environment, attracts females of the Japanese dace.

PP-37

PHYSIOLOGICAL ACTION OF THE SUITABLE ENVIRONMENT FOR SPAWNING ON OVULATION AND SPAWNING IN JAPANESE DACE, *TRIBOLODON HAKOKENSIS*

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Japanese dace, *Tribolodon hakokensis*, migrate to the spawning ground just prior to spawning, and lay its eggs to the surface of gravel. It is considered that the suitable environment for spawning has not only the ecological significance but also the physiological roles on reproduction. In the present study, we investigated the physiological action of the suitable environment for spawning on final maturation in Japanese dace. Ovulation and spawning tests were carried out in 4 different rearing conditions as following. Mature females were placed in the ponds with (group 1) or without (group 2) the suitable environment for spawning. In addition, both mature females and males were placed in the ponds with (group 3) or without (group 4) the suitable environment for spawning. The ovulation and spawning were checked every morning after transfer to the experimental conditions. In the group 1 (with the environment, female alone), ovulation and release of ovulated eggs were induced in 23 and 12 out of 35 females respectively. In the group 3 (with the environment, both sexes), ovulation and release of ovulated eggs were induced in 34 out of 40 females. In contrast, ovulation and spawning were not induced under the condition without the suitable environment (group 2 and 4). The serum concentrations of 17α, 20β-dihydroxy-4-pregnen-3-one (DHP) increased after transfer to the suitable environment for spawning. These results suggest that the existence of the suitable physical environment is necessary for ovulation and spawning in Japanese dace.

PP-38

CHANGES IN THE OLFACTORY EPITHELIUM DURING THE GONADAL DEVELOPMENT AND SPAWNING IN JAPANESE DACE.

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The existence of sex pheromones for synchronous maturation and spawning has been known in some teleost. Japanese dace, *Tribolodon hakonensis*, is one of the fish that require chemical communications between female and male for spawning. Male fish secreted the pheromone to attract females into the spawning ground. At this time, it is possible that the olfactory organ undergo physiological changes. In our previous study, 22 kDa proteins in the olfactory epithelium increased during gonadal development in female fish. In the present study, we investigated the structure of the olfactory epithelium and localization of the protein using a special antibody against 22 kDa protein. The immunoreaction with the antibody was observed in the goblet cell of the olfactory epithelium by immunofluorescent analysis. However, some of the goblet cells show no reactions with the antibody against 22 kDa proteins. These result indicate the different type of the goblet cell existed in the olfactory epithelium. Moreover, the secretion of this protein to the surface of epithelium from the immunoreacted cells was observed in the mature females that congregated at the spawning ground. These results suggest that the protein may be related with the sensitivity of sex pheromone or the reproductive phenomena.

PP-39

LACK OF SPECIES SPECIFIC PRIMER EFFECTS OF ODOURS FROM FEMALE ATLANTIC SALMON (*SALMO SALAR* L.) AND BROWN TROUT (*SALMO TRUTTA* L.)

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We exposed, in two successive spawning seasons, individually placed precocious male Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.) parr to odour stimuli (ovarian fluid and urine mix) from ovulated conspecific or heterospecific anadromous females. Atlantic salmon parr had significantly higher plasma concentrations of the hormones 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P), 11-ketotestosterone (11-KT) and testosterone (T) after exposure to odours from conspecific females or from brown trout females compared to parr exposed to a control solution (0.9 % NaCl). We did not observe any significant differences between the hormone levels in salmon parr exposed to the two female odours. The salmon parr exposed to conspecific odours had significantly higher volumes of strippable milt compared to the controls, but we did not find any significant differences when comparing the effect of the two female odours. Brown trout parr had significantly higher plasma 17,20β-P levels following exposure to heterospecific female odours compared to control males, but there was no significant difference between males exposed to the different female odours. We did not observe any significant differences in plasma levels of T and 11-KT and in milt volumes between exposed and control trout. Taken together, the results from both tested species indicate that the potency of heterospecific stimuli in stimulating increased plasma sex steroid hormone levels in male parr was as strong as stimuli from conspecific females.

PP-40

AUDITORY REPRODUCTIVE PRIMING IN MATURE MALE ATLANTIC SALMON (*SALMO SALAR* L.) PARR

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Salmonids are known to use a variety of sensory signals during reproduction. Chemical (pheromones), vibrational and visual cues have all been shown to have a significant role in synchronising the reproductive status of males and females prior to spawning. Here we report that the sound produced by wild female Atlantic salmon during redd cutting has a significant priming effect on the reproductive physiology of mature male Atlantic salmon parr. Underwater recordings of redd cutting obtained using an acoustic hydrophone were played back to mature male parr in the laboratory. The results indicate that there were significant increases in both the levels of plasma 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) and the levels of expressible milt compared to the control groups. However, there were no significant differences in plasma levels of testosterone and 11-ketotestosterone between the groups. In addition, the levels of 17,20βP and expressible milt produced in response to the sound of redd cutting were not significantly different in parr, which had been exposed to the priming pheromone, Prostaglandin $F_{2\alpha}$. The data suggests that auditory cues may have a significant role in synchronising reproductive physiology in the Atlantic salmon.

PP-41

11-KETOTESTOSTERONE LEVELS AT DIFFERENT REPRODUCTIVE PHASES AND ITS EFFECT ON BEHAVIOUR IN THE THREE-SPINED STICKLEBACK (*GASTEROSTEUS ACULEATUS*)

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Plasma levels of the androgen 11-ketotestosterone (11KT) were studied in breeding male three-spined sticklebacks. This was performed at the following reproductive phases; the initial nestbuilding and sexual (NS) phase, i.e. courting males with nests but not allowed to spawn; parental (P) phase, i.e. spawned males, brooding eggs in the nest and fanning; and the 2nd cycle nestbuilding and sexual (2ndNS) phase, i.e. spawned males, which had already gone through the first fanning and brooding phase, that have built a new nest and are courting for a second time, but again are not allowed to spawn. Plasma levels of 11KT were measured by means of radioimmunoassay. 11KT levels in individual plasma samples were significantly higher during the NS and the 2ndNS phases than during the P phase. In order to find out the function of the decline in 11KT levels in the P phase, mature males were treated with Silastic capsule implants of the androgen 11-ketoandrostenedione (11KA), which is known to be readily converted to 11KT extratesticularily in fish. This treatment prevented the natural decline in 11KT, the consequence of which was studied regarding the behavioural response of the males. So far, the natural increase in fanning behaviour and decline in courtship behaviour seen during the P phase was also observed in the 11KA-treated males.

PP-42

SPAWNING SUCCESS AND OSMOREGULATORY ABILITY OF ATLANTIC SALMON (*SALMO SALAR*) BROODSTOCK MAINTAINED IN SEA WATER OR TRANSFERRED TO FRESH WATER DURING MATURATION

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Maturing farmed Atlantic salmon (*Salmo salar*) broodstock are usually transferred from sea water to fresh water prior to spawning. This is to avoid the poor gamete quality and losses of broodstock which have been reported to occur in fish maintained in sea water. Surprisingly there has been little work to identify the optimum times for transfer of broodstock salmon to fresh water or on the effects of this transfer on the blood chemistry and reproductive performance of the broodstock. However, the timing of such a transfer may have a significant impact on gamete quality and broodstock survival, which is very important for the success of a farm producing eggs. Aspects of these questions were addressed in the current work. Groups of female grilse, with a natural spawning time of October-November, were transferred to fresh water in July, September and October or maintained in sea water until spawning. Median spawning times were significantly advanced for females transferred to fresh water in July and September compared to females maintained in sea water. Egg viability to the eyed stage of development was not significantly different in the four groups. At ovulation, female grilse held in fresh water had significantly lower plasma and coelomic fluid osmolalities and Na^+ and K^+ levels than females maintained in sea water. The results presented suggest that the timing of transfer of broodstock from sea water to fresh water, significantly influences blood chemistry and spawning success in farmed Atlantic salmon.

PP-43

INTERMALE COMPETITION IN SEXUALLY MATURE ARCTIC CHARR: EFFECTS ON STRESS RESPONSES, ENDOCRINE LEVELS AND BEHAVIOUR

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Sexually mature Arctic charr (*Salvelinus alpinus*) males were allowed to interact in pairs for three days in the absence of females. Agonistic behaviour was quantified, and at the end of the experiment, levels of glucose, cortisol, testosterone (T), 11-ketotestosterone and 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-P) were analysed in plasma, along with brain concentrations of serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA, the major 5-HT metabolite), dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC, a major DA metabolite). Plasma concentrations of T, 11-KT and 17α,20β-P were significantly higher in dominant than in subordinate males. Further, plasma levels of these gonadal steroids were correlated with the number of aggressive acts performed but not with the number of aggressive acts received. By contrast, circulating plasma cortisol and glucose were significantly elevated in subordinate fish, and the number of aggressive acts received showed positive correlations with plasma levels of both cortisol and glucose. In the present study, plasma cortisol concentrations did not correlate with either 5-HIAA/5-HT or DOPAC/DA ratios in any of the brain parts analysed. In contrast, plasma glucose levels showed positive correlations with brain 5-HIAA/5-HT ratios. Negative correlations were observed between brain 5-HIAA/5-HT ratios and plasma levels of T, 11-KT and 17α,20β-P. Telencephalic DOPAC/DA ratios displayed a negative correlation with circulating plasma levels of T, 11-KT and 17α,20β-P, but only in dominant males. In subordinate males there was no significant relationship between brain DOPAC/DA ratios and plasma levels of gonadal steroids. These results show that intra sexual competition in sexually mature Arctic charr males affects endocrine levels even in the absence of females. Further, the results suggest that stress responses and monoaminergic neurotransmission is altered by sexual maturation.

Gonadal Physiology

OP-20

NUCLEAR AND MEMBRANE STEROID RECEPTORS AND THEIR FUNCTIONS IN TELEOST GONADS

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Gonadal steroids activate a variety of steroid receptors to regulate many of the morphological and physiological changes occurring in the gonads during the reproductive cycle. Typically the mechanism of steroid action is genomic, mediated by binding and activation of nuclear steroid receptors located inside the target cell which in turn bind to hormone response elements on genes resulting in alterations in their transcription rates. However, evidence has gradually accumulated that steroids can also exert fast, nongenomic actions by binding to specific receptors on the plasma membranes of target cells resulting in activation of signal transduction pathways. Both classes of hormone receptors have been identified in teleost gonads and gametes. Progress in our understanding of the binding characteristics, structure, multiplicity and physiological roles of nuclear and membrane estrogen, androgen and progestogen receptors in teleost gonads will be reviewed. The identification of nuclear androgen receptors in ovarian as well as testicular tissues and nuclear estrogen receptors in the testis and ovary suggests that both sex steroids are involved in the regulation of ovarian and testicular functions in teleosts. Moreover, recent studies demonstrate that steroid membrane receptors are involved in the maturation of both male and female gametes and in the regulation of testicular androgen production. The contribution of these recent finding in teleosts to our understanding of steroid actions in vertebrate gonads will be discussed. In addition some practical implications of the results will be considered, especially endocrine disruption mediated by binding of xenobiotic chemicals to gonadal steroid receptors.

OP-21

11β-HYDROXYLASE AND ANDROGEN RECEPTOR mRNA EXPRESSION IN THE OVARY, TESTIS AND BRAIN OF THE PROTOGYNOUS HERMAPHRODITE *THALASSOMA DUPERREY*

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In *Thalassoma duperrey*, distinct changes in steroidogenic enzyme expression correlate with sex change. As the gonad redifferentiates from an ovary to a testis, expression of ovarian aromatase is down-regulated while 11β-hydroxylase (11β-OH) is up-regulated indicating the importance of the respective hormones, estradiol (E2) and 11-ketotestosterone 11-KT, to phenotypic sex. Whereas aromatase is absent in the testis, 11β-OH is present at substantial levels in the ovary suggesting a potential role in normal ovarian physiology as well as testicular differentiation.To determine whether the ovary can respond directly to 11-KT, we cloned and characterized the expression of an androgen receptor (AR) from *T.duperrey*. Specific RT-PCR for *T.duperrey* AR amplified a 403 bp product from Initial Phase testes, ovaries, and brains as well as Terminal Phase testes and brains. Interestingly, expression appear to be higher in ovaries and brains relative to either testis phenotype. *In situ* hybridization studies confirmed the presence of AR mRNA in follicular cells indicating the ovary may be directly responsive to 11-KT via the AR. Nevertheless, the physiological function of 11-KT in the ovary remains unresolved. In contrast, 11-KT is generally assumed to act in the brain to control male-specific behavior; therefore, we also characterized expression of 11β-OH and AR mRNA in the brain. Surprisingly, 11β-OH mRNA appears to be widely distributed in discrete nuclei throughout the brain of both sexes. Although 11-KT has long been thought to act on the brain, these results clearly indicate 11-KT conversion occurs within the brain. Consequently, mechanisms of 11-KT action in the brain may parallel the E2 mechanisms described for mammalian sexual behavior. Co-localization of AR mRNA in some of the same nuclei further supports the hypothesis that 11-KT plays a role in sexual behavior of *T.duperrey*. Investigations to determine sexual dimorphisms of 11β-OH and AR expression in the brain as well as changes in the gonad throughout sex change are currently underway.

OP-22

CHARACTERIZATION OF OVARIAN- AND OVULATION-SPECIFIC PROTEINS IN THE BROOK TROUT (*SALVELINUS FONTINALIS*)

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Using stage-specific subtractive cDNA cloning, a family of cDNAs (0.8, 1.1, 1.4, 1.7 kb) that was highly upregulated at the time of ovulation was obtained and named TOPs (Trout Ovulatory Proteins). Northern blots revealed that TOP mRNA levels increase up to the time of ovulation and peak at 12 hours postovulation. Following ovulation in salmonids, the eggs are held in the peritoneal cavity and bathed in coelomic fluid. Western blots of ovarian tissue and coelomic fluid confirmed that TOP proteins increase up to the time of ovulation, peak at 24-48 hours postovulation, then decrease by 4-8 days postovulation. *In situ* hybridization and immunocytochemistry localized TOP RNA and protein, respectively, to the granulosa layer. The sequence of the 0.8 kb (TOP-1) and the 1.4 kb (TOP-2) cDNAs were homologous to mammalian antileukoproteinase, a heat- and acid-stable serine protease inhibitor. Using a chromogenic peptide substrate, the anti-protease activity of postovulatory brook trout coelomic fluid was measured. Trypsin, chymotrypsin, and pancreatic elastase activity were significantly inhibited by coelomic fluid containing 5.0, 10.0, and 25.0 μg of total protein, respectively. Coelomic fluid could be heated to 50 °C or treated at a pH less than 5.2 without significantly decreasing the inhibitory activity. Further, coelomic fluid from which TOPs were immunoprecipitated had significantly less anti-trypsin activity than nonimmunoprecipitated controls. *In vitro* incubations of intact brook trout follicles with secondary messenger agonists, hormones, and other compounds were performed to measure their effects on TOP levels.

OP-23

FISH PITUITARY GLYCOPROTEIN HORMONE RECEPTORS: cDNA CLONING AND CHARACTERIZATION OF TWO DIFFERENT GONADOTROPIN RECEPTORS FROM GONADS AND TWO THYROTROPIN-LIKE RECEPTORS FROM THYROID OF AMAGO SALMON (*ONCORHYNCHUS RHODURUS*)

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Two biologically and functionally distinct gonadotropins (sGTH I and II) are produced by salmonid pituitaries and two classes of GTH receptors have been identified through ligand binding studies. In order to study changes in these receptors during sexual differentiation and gametogenesis, we cloned two different types of gonadotropin-like receptor (sGTHR) cDNAs from amago salmon ovary, and two different types of thyrotropin-like receptor (sTSHR) cDNAs from amago salmon thyroid. The two sGTHR cDNAs encode proteins of 658 and 725 amino acids, and the two sTSHR cDNAs encode proteins of 814 and 793 amino acids, respectively. The transmembrane domains of these four proteins are highly conserved. Northern blot analyses revealed different patterns of expression of the two sGTHR mRNAs in the developing ovary. One sGTHR mRNA is largely expressed in the full-grown ovarian follicle in which final oocyte maturation can be induced by 17α,20β dihydroxy-4-pregnen-3-one. mRNAs for the two sTSHRs were detected in the thyroid but not in ovary or testis. COS-7 cells were transfected with each receptor cDNA and then exposed to purified salmon GTH I, II and mammalian TSH, followed by measurement of cAMP production. Results were consistent with the existence of two types of gonadotropin receptors originally proposed by Swanson and co-workers.

OP-24

DISCREPANCY BETWEEN STRUCTURAL CHARACTERISTICS AND LIGAND SPECIFICITY OF AN AFRICAN CATFISH GONADOTROPIN RECEPTOR

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We have cloned a cDNA encoding a putative gonadotropin receptor from the testis of the African catfish, *Clarias gariepinus*. Conceptual translation predicted a protein that showed the characteristics of vertebrate glycoprotein hormone receptors. Sequence analysis of the putative catfish gonadotropin receptor gene (i.e. the region preceeding the last exon) indicated that the cloned receptor is structurally more related to FSH, than to LH or TSH receptors. The isolated cDNA encoded a functional receptor since its transient expression in HEK-T 293 cells resulted in ligand-dependent reporter gene expression. Challenging the transfected HEK-T 293 cells with the LH-like gonadotropin from African catfish (cfLH; a FSH-like gonadotropin has not been found in catfish) or with ligands for human glycoprotein hormone receptors (human recombinant LH [hrLH] and hrFSH, as well as purified hCG and hTSH) revealed that cfLH has the highest potency (EC50=30ng/ml) to activate the catfish gonadotropin receptor-mediated reporter gene expression. From the other ligands tested, only hrFSH was active, however, showing a ~4-fold lower potency (EC50=130ng/ml) than cfLH but the same maximal stimulation (~8-fold over basal level). We demonstrated that hCG (as well as cfLH, hrFSH, but not hTSH) was able to stimulate testicular androgen secretion *in vitro*, and it has been shown that hCG is biologically active in catfish (e.g. induction of ovulation). The above data indicate that we cloned a catfish gonadotropin receptor (structurally related to vertebrate FSH receptors), and that a second type of gonadotropin receptor (structurally most likely related to vertebrate LH receptors) is expressed in African catfish testis.

OP-25

SEASONAL CHANGES IN OVARIAN EXPRESSION OF STEROIDOGENIC ENZYMES AND GONADOTROPIN RECEPTORS IN THE CHANNEL CATFISH, *ICTALURUS PUNCTATUS*

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In all vertebrates, appropriate and timely alterations of gonadal synthesis of sex steroids are required for growth and maturation of the gametes. These changes in gonadal steroidogenesis are a result of altered expression of the genes encoding the steroidogenic enzymes which are primarily regulated by the plasma gonadotropins (GtH I and GtH II in fish) activating their membrane-bound receptor. In this study, the seasonal changes in the follicular expression of four key steroidogenic enzymes and 2 types of GtH receptors were examined in ovaries of a northern and a southern population of channel catfish. Significant titers of the sex steroids were detectable in the plasma throughout the year although the titers varied by a factor of five. It was apparent that the expression of each of the steroidogenic genes (3 β-hydroxysteroid dehydrogenase, 17-hydroxylase, side chain cleavage, and aromatase) was under different regulatory influences. Expression of 3 β-HSD was relatively unchanged throughout the year whereas the expression of aromatase and 17-hydroxylase peaked soon after recrudescence and during the mid-vitellogenic growth phase of the ovary. The expression of the GtH receptors were differentially regulated throughout the season, and like the steroidogenic enzymes, their expression was closely correlated with the *in vitro* production of ovarian steroids (including the abundant and novel hydroxylated-pregnenolones that were recently identified), steroid plasma titers, and ovarian growth.

OP-26

TWO PATHWAYS FOR C21 STEROID BIOSYNTHESIS IN NORTH SEA PLAICE *PLEURONECTES PLATESSA*: WHAT CONTROLS THEM AND WHAT IS THEIR FUNCTION?

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There are two pathways of C21 steroid biosynthesis in plaice gonads, one in which the steroids are 21-hydroxylated and the other in which they are 20β-hydroxylated. The precursor for both pathways is believed to be 17-hydroxyprogesterone, which is converted to either 11-deoxycortisol (17,21-P) or 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P). Both steroids (presumably acted on by the same enzymes) then undergo 5β-reduction, 3α-OH or 3β-OH reduction and sulphation. There are abundant amounts of 5β-pregnane-3x,17,20β-triol 20-sulphate and 3x,17,21-trihydroxy-5β-pregnan-20-one 21-sulphate in the plasma of spawning male and female plaice. Although 17,20β-P is possibly the maturation-inducing steroid (as it is in other fish), the role of its metabolites and of 17,21-P remains unknown. We have adopted several approaches to determine the function of these steroids. Firstly, we have investigated whether the two pathways are under differential control - by fractionating plaice pituitary glands and testing the ability of the fractions to stimulate *in vitro* "17,20β" and "17,21" steroid production by plaice testes. Secondly, we have investigated the presence of steroid receptors in brain, testis and ovary. Thirdly, we have been testing their effects *in vivo*. We have so far found that the two pathways appear to be controlled by the same pituitary hormones, and that there is binding of radioactive 17,20β-P by the gonads. The *in vivo* experiments are in progress.

PP-44

INHIBITION OF LH ACTION BY INSULIN AND INSULIN-LIKE GROWTH FACTOR I IN TROUT THECA LAYERS

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It is well known that gonadotropins (LH and FSH) are the major regulators of ovarian function in all gnatostome vertebrates. The action of FSH and LH in the vertebrate ovary is also known to be modulated by other endocrine/paracrine factors, such as insulin-like growth factor I (IGF-I). We recently reported that IGF-I inhibits the stimulatory effects of LH on steroid production by salmon theca layers. The purpose of the present study is to investigate the *in vitro* effects of insulin on steroid production by preovulatory theca layers of brown trout (*Salmo trutta*), compare them to those of IGF-I and to investigate the mechanisms by which insulin and IGF-I modulate the steroidogenic effects of LH. In theca layers of the brown trout ovary, insulin and IGF-I inhibit the effects of LH on testosterone (T) production in a concentration-dependent manner. Insulin and IGF-I also inhibit the stimulatory effects of dibutyryl cAMP, an analog of cAMP, and IBMX, a phosphodiesterase inhibitor, on T production. Furthermore, results from preliminary experiments suggest that insulin and IGF-I may not modulate the stimulation of the production of cAMP by LH in theca layers. Wortmannin, an inhibitor of PI-3 kinase, and PD98059, and inhibitor of MAP kinase, block the inhibitory effects of insulin and IGF-I on LH-stimulated T production. These results suggest that in trout theca layers, insulin and IGF-I may activate the PI-3 and MAP kinase pathways and that the inhibition of LH action occurs at a site distal to the production of cAMP.

PP-45

RELATIVE BINDING AFFINITIES OF STEROIDS FOR SEX STEROID BINDING PROTEIN ARE SIMILAR DESPITE DIFFERENCES BETWEEN SPECIES IN AFFINITY AND CAPACITY FOR ESTRADIOL

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Sex steroid binding protein (SBP) was investigated in four fish species; a salmonid (rainbow trout, *Oncorhynchus mykiss*), a pleuronectid (greenback flounder *Rhombosolea tapirina*) and two sparids (black bream *Acanthopagrus butcheri*, and snapper (=red sea bream) *Pagrus auratus*). The affinity of SBP for a range of steroids including estrogens (estradiol, estrone, estriol), androgens (testosterone, 11-ketotestosterone, androstenedione), progestogens and cortisol, was measured by incubation with a constant amount of ^{3}H-estradiol and increasing concentrations of unlabelled competing steroid. In trout, bream and snapper, SBP bound estradiol with the highest affinity, followed by testosterone. Estrone and androstenedione were also bound with moderate affinity to snapper SBP. All other steroids had relative affinities of less than 10% of that of estradiol in trout, bream and snapper. In contrast, testosterone was bound more than twice as strongly as estradiol to flounder SBP. Binding of estrone to flounder SBP was about one third that of estradiol, and the relative affinities of all other steroids were less than 20% of that of estradiol. Despite differences in binding affinities and capacities between the species, the binding site on the SBP is still essentially an estradiol and testosterone binding site in all these species. SBP in trout and snapper binds mainly estradiol, with some binding of testosterone, while very little testosterone would be expected to be bound to bream SBP, and in flounder, testosterone would be bound in preference to estradiol.

PP-46

MODE OF EMBRYONIC NUTRITION IN FOUR SPECIES OF *JENYNSIA* (TELEOSTEI: ATHERINOMORPHA: ANABLEPIDAE)

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The atherinomorph genus, *Jenynsia*, comprises nine species distributed in the southern region of South America. One aspect of the reproductive physiology of these fishes is that they are internally fertilized and viviparous. Despite this interesting reproductive biology, information on embryonic and maternal modifications is restricted to the study of a single species completed over 50 years ago. In order to describe modifications associated with vivparity and to explore possible modes of maternal embryonic nutrient transfer in species of *Jenynsia*, four speices, *J. maculata*, *J. lineata*, *J. alternimaculata*, and *J. multidentata* were examined histologically. A close association between the extensive ovarian folds and the gill tissue of the embryos was found in all species examined. Additionally, evidence supporting the proposal that embryos are ingesting cells sloughed off from the ovarian epithelium was found. Embryonic and maternal modifications are described and possible modes of embryonic nutrition are discussed.

PP-47

GONADAL DEVELOPMENT AND SERUM PROFILES OF VITELLOGENIN AND STEROID HORMONES IN CAPTIVE PACIFIC HERRING, *CLUPEA PALLASII*, DURING THE FIRST AND SECOND REPRODUCTIVE CYCLES

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The present study examined the relationship between gonadal development and serum profiles of steroid hormones and vitellgenin during the first (1+) and second (2+) reproductive cycles in caprive Pacific herring. The maturity of ovary during the first reproductive cycle was histologically divided into six periods, i.e., immature (April to September), early-vitellogenesis (August to October), mid-vitellogenesis (October to December), late-vitellogenesis (December to March), maturation and spawning (March to April), and spent (late April). The serum vitellogenin pattern in females well reflected the ovarian maturity, increasing from September to a March peak. Serum estradiol-17β (E2) levels in female increased from September to a December peak, then decreased slowly until early April. Serum 17α,20β-dihydroxy-4-pregnen-3-one (DHP) in females showed a single sharp peak in the functional maturation period. Testis maturity during the first reproduction was histologically divided into five periods, i.e., early-spermatogenesis (April to June), mid-spermatogenesis (July to September), late-spermatogenesis (October to March), functional maturation (early April), and spent (late April). Serum 11-ketotestosterone (KT) in males increased from September, and maintained high levels from January to March. Serum DHP in male showed a single sharp peak in the functional maturation period as in females. Similar patterns of the changes in serum levels of vitellogenin, E2, KT and DHP were shown during the second reproduction. These results suggest that vitellogenesis is controlled by E2, final oocyte maturation is induced by DHP in females, spermatogenesis is promoted by KT, and milt production is induced by DHP in male Pacific herring.

PP-48

LUNAR SYNCHRONIZATION OF GONADAL DEVELOPMENT IN THE GOLDEN RABBITFISH, *SIGANUS GATTATUS*

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Characteristics of the lunar reproductive cycle in the golden rabbitfish, *Siganus gattatus*, were determined by histological observation of gonadal development, immunological measurements of plasma vitellogenin (VTG) and steroid hormones, estradiol-17β (E2), testosterone (T), 11-ketotestosterone (11-KT), 17α,20β-dihydroxy-4-pregnen-3-one (DHP) and 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S). Gonadal and plasma samples were collected every week according to the lunar periodicity from May through July. Weekly changes of gonadosomatic index (GSI) had two peaks at the young moon in female fish and the new moon in male fish in June and July, the reproductive season. Gonads developed synchronously within the specific lunar phase during the reproductive season. In the female fish, the plasma steroids (E2, T, DHP and 20β-S) and VTG levels changed in correlation with changes in GSI. Those changes coincided with vitellogenic activity, and the first and second peaks of plasma steroids occurred one day prior to the peak of spawning. In the male fish, the plasma steroids (T, 11-KT and DHP) reached first and second peaks one week before the spawning and coincided with the change in testicular activity. These cyclic changes support the hypothesis that lunar periodicity is the major factor in stimulating the reproductive activity of *S. gattatus*.

PP-49

COMPARISON OF mRNA LEVELS OF OVARIAN STEROIDOGENIC ENZYMES BETWEEN ARTIFICIALLY MATURING JAPANESE AND NATURALLY MATURING NEW ZEALAND EELS

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Japanese eels (*Anguilla japonica*) captured from the wild have immature ovaries and do not undergo further ovarian development under captive conditions. Therefore, artificial induction of gonadal maturation has been carried out using injections of salmon pituitary homogenate (SPH). However, artificial maturation protocols have never led to the production of elvers. In order to contribute to the solution of this problem, we have been investigating the mechanisms of eel ovarian steroidogenesis. We have already cloned full - length cDNA encoding P450 17 α - hydroxylase/C17-20 lyase, 17 β - hydroxysteroid dehydrogenase type I, and aromatase from Japanese eel ovary. Furthermore, the changes in the levels of these mRNAs during ovarian development have been investigated. However, it is unknown whether the changes in mRNA levels of these steroidogenic enzymes during artificially induced ovarian development reflect those under natural conditions. The New Zealand longfinned eel (*Anguilla dieffenbachii*) captured from the wild has ovaries containing mid-vitellogenic oocytes. Therefore, mRNA levels of ovarian steroidogenic enzymes during pre and mid-vitellogenesis were compared between artificially maturing Japanese eels and wild New Zealand longfinned eels. Target mRNA species were detected in mid-vitellogenic ovaries after SPH injections, but not in previtellogenic ovaries of saline - injected Japanese eels. In contrast, specific transcripts were not very abundant in pre and mid-vitellogenic ovaries of wild New Zealand eels. These findings suggest that steroidogenic enzyme genes are over - expressed during artificial maturation of the Japanese eel due to the large quantities of hormone injected.

PP-50

SHIFTS IN GONADAL STEROIDOGENESIS REGULATE GROUP-SYNCHRONOUS OVARIAN DEVELOPMENT, SPAWNING AND SPERMIATION IN THE EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*)

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In salmonids Gonadotropin-1 mediates gonadal growth and stimulates the follicular production of testosterone (T) and 17β-estradiol (E2) while Gonadotropin-2 regulates final maturation and ovulation/spermiation and stimulates a shift in steroidogenesis from the synthesis of T and E2 to that of maturation-inducing steroids (MIS). The European sea bass has group-synchronous ovarian development which results in several consecutive spawnings during each reproductive season. A specific process is therefore required which enables maturation to occur in the first clutches of oocytes without arresting vitellogenesis in the following ones. A possible mechanism based on shifts in gonadal steroidogenesis, which may be responsible for regulating group-synchronous ovarian development, spawning and spermiation, is proposed. Shifts in steroidogenesis resulted in a sequential elevation of the plasma levels of T and E2 or MIS. Each surge of MIS coinciding with the ovulation of one clutch of oocytes. Following each MIS surge, there was a new shift in the elevation of the T and E2 plasma levels enabling vitellogenesis of other clutches to occur. A similar pattern was observed in males.

PP-51

STEROIDOGENIC PROPERTIES OF COMMON CARP OVARY INTERSTITIAL TISSUE

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In order to determine the involvement of interstitial tissue cells in the stimulation of final stages of carp oocyte maturation *in vitro*, two experiments were performed: a) a comparison of steroidogenic activity of carp ovarian follicular and interstitial cells was performed using a monolayer culture of isolated cells. In this experiment progesterone (P), estradiol (E2) and androgens (A) were measured after 2, 4, and 6 hours of incubation using specific radioimmunoassays. Tissues were collected in the periovulatory period (May) as well as during gonadal recrudescence (December). b) the steroidogenic activity of interstitial tissue fragments was compared to the activity of ovarian fragments containing the interstitial tissue and oocytes. The experiment was carried out during the periovulatory period. Interstitial tissue or ovarian fragments were incubated alone or in the presence of carp hypophyseal homogenate (chh, 100 μg/ml). 17,20αP and 17,20βP concentrations were measured after 1, 3, 7, 10 and 24 hour incubation using a specific ELISA. The results of experiment A showed that follicular as well as interstitial cells produce P, E2 and A, the interstitial cells being the main source of androgens in both periods of gonadal development. In the experiment B, it was shown that both tissues produce 17,20αP (from 1 to 3 pg/ml) as well as 17,20βP (from 3 to 8 pg/ml).There was no statistical differences between the concentrations of steroids in the absence or presence of chh in the culture medium. This could indicate that the steroid production by interstitial cells is not directly affected by gonadotropin.

PP-52

DIFFERENT GONADAL ESTROGEN RECEPTOR mRNAS IN THE RAINBOW TROUT: MOLECULAR CHARACTERIZATION AND MEASUREMENT DURING REPRODUCTIVE DEVELOPMENT

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Estrogen receptors (ERs) are intracellular proteins, that when bound to the sex steroid estradiol, function as transcriptional regulators of estrogen responsive genes. In fishes, most of the work on ERs and their transcripts have been confined to the liver. This study sought to establish the presence of ER mRNAs in the gonads of the rainbow trout (*Oncorhynchus mykiss*) in an effort to understand the intra-ovarian role of estradiol. Using a reverse transcription-polymerase chain reaction (RT-PCR) approach, the mRNAs for two different ERs have been discovered in the ovaries and testes of the rainbow trout. The first transcript, termed ER-1, is identical to a previously described ER mRNA from the liver of this species. Northern blotting analysis shows a single, similar sized 4.2 kilobase (kb) transcript in liver, ovary, and testes. A quantitative RT-PCR method has been developed utilizing an internal standard to measure tissue levels of ER-1 mRNA. Analysis of different ovarian samples shows that ER-1 message is present throughout reproductive development. A second, different ER mRNA discovered in the trout ovary, termed ER-2, is also present in liver, ovary, and testes. There are two ER-2 transcripts identified by Northern blotting in the ovary and liver (4.2 and 2.1 kb), but only one (2.1 kb) in the testes. A comparison of the deduced amino acid sequence of ER-2, with that of ER-1, show similarities of 75% and 82% within the DNA binding and steroid binding domains, respectively.

PP-53

EFFECTS OF 17α,20β-DIHYDROXY-4-PREGNEN-3-ONE ON FINAL MATURATION OF OOCYTES AND OVULATION IN ARTIFICIALLY MATURED JAPANESE EEL, *ANGUILLA JAPONICA*

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Effects of 17α,20β-dihydroxy-4-pregner-3-one (DHP) on germinal vesicle breakdown (GVBD) of oocytes and ovulation in artificially matured Japanese eel were examined. Injection of carp pituitary extract (CPE) and human chorionic gonadotropin (HCG) induced the production of 17α, 20β-DHP in artificially matured Japanese eel and resulted in ovulation. Oocytes at migratory nucleus stage ranging from 700 to 900 μm in diameter incubated with CPE, HCG and eel pituitary extract (EPE) *in vitro*, only EPE induced the production of 17α,20β-DHP significantly. The production of 17α,20β-DHP also increased significantly when 17-hydroxyprogesterone was added substrate incubated with oocytes ranging from 700 to 900 μm in diameter. 5 and 10 ng/ml of in17α,20β-DHP induced 49.4±4.3% and 84.3±5.4% GVBD in oocytes ranging from 700 to 900 μm in diameter, respectively; the percentage of oocytes underwent GVBD reached 90.6% in response to 17α,20β-DHP at concentration of 50 ng/ml. Oocytes ranging from 600-700 μm in diameter did not undergo GVBD in response to 17α,20β-DHP at concentrations of 5 to 100 ng/ml. During induced ovulation of the artificially matured Japanese eel by LHRH-A + DOM or CPE + HCG injection, the serum GtH and levels 17α,20β-DHP increased dramatically and formed a sustained peak. These results indicated that the 17α,20β-DHP, an oocyte final maturation inducing hormone which synthesis and release under the regulation of GtH, play key role during oocyte GVBD and ovulation in artificially matured Japanese eel.

PP-54

OSMOTIC ASPECTS OF FINAL OOCYTE MATURATION IN ATLANTIC COD

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The hyposmotic condition of the body fluids of marine teleosts is a challenge for the developing embryos since they lack the organs responsible for osmoregulation in the adult fish. The realised solution is that the mother fish, prior to spawning, supplies the egg with a water reservoir during final oocyte maturation when the oocyte swells 4-5 times. A relative water content of 90-93 % is typical of pelagic eggs of marine teleosts. The mechanism for this water uptake into the swelling oocyte of marine teleosts is the focus of the present study. Atlantic cod (*Gadus morhua*) is used as the model fish. Ovary biopsies and samples of the ovary fluid have been taken repetitively from a mature female cod during several oocyte cycles. Single oocytes in different stages of maturity were isolated and analysed for osmolality (Clifton nanoliter osmometer), water content, ionic content (Na, K, Cl, PO_4), free amino acids (FAA), and protein content. The yolk protein profile was determined by gel electrophoresis (SDS-PAGE). The data will be discussed in osmotic terms in relation to the water flow into the swelling oocyte of marine teleosts with pelagic eggs.

PP-55

EVOLUTIONARY PERSPECTIVES OF FREE AMINO ACIDS AND PROTEIN DYNAMICS DURING EGG AND YOLK-SAC LARVAL DEVELOPMENT IN SALMONID FISHES

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This study concerns the changes in free amino acids (FAA) and protein content in developing embryos and yolk-sac larvae of some salmonid fishes (salmon, brown trout, rainbow trout, grayling, whitefish). Salmonids are regarded as phylogenetically primitive teleosts that spawn in fresh water but often are capable of migrating to the ocean at later stages to benefit from the rich feeding grounds there. In this trait, salmonids presumably resemble the teleost forerunners as they evolved in fresh water during the Silurian and Devonian periods. Without a key adaptation for the egg, the hyperosmotic condition of seawater undoubtedly prohibited the earliest teleosts from spawning in the oceans. Our previous studies have shown that the pelagic eggs of extant marine teleosts contain a large pool of FAA and, moreover, that this pool is remarkably similar for fishes of different taxonomic groups. Gel electrophoresis (SDS-PAGE) has shown a yolk protein of about 100 kDa to disappear concurrently with oocyte swelling and the generation of the FAA pool. In the salmonids, the egg FAA pool is low at spawning but increases with development and seems to reach a maximum at the end of yolk-sac stage. SDS-PAGE shows a 100 kDa protein to be present also in salmonids. Further analyses are underway. The data will be discussed in relation to teleost evolution and the adaptations which were necessary to overcome the osmotic problems involved in the change of living habitat of teleosts from freshwater to the ocean millions of years ago.

PP-56

SYNTHESIS OF PROGESTINS BY TESTES OF THE WHITE CROAKER, *MICROPOGONIAS FURNIERI,* DURING SPERMIATION

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As scarce information is available on gonadal steroids of testes of male sciaenid, we developed studies on steroidogenesis in *Micropogonias furnieri* male gonads. Testes from spermiating fish were incubated for 6 h, with 10 µCi of ^{3}H-17-hydroxy-4-pregnen-3,20-dione, at 22° C. Ethanolic and dichloromethane extractions were applied to culture media and tissues and metabolites were purified and analysed by thin layer chromatography in a benzene:acetone 80:20, v:v solvent system and by reverse phase HPLC in acetonitrile:methanol:water 26:33:41, v:v:v and tetrahydrofuran:water 35:65, v:v. Metabolites were characterised by enzymatic oxidation in the presence of the true steroid and by ligation with specific anti-serum. The main metabolites identified were the 17,20ß-dihydroxy-4-pregnen-3-one (17,20ßP) and 11ß,17,21-trihydroxy-4-pregnen-3,20-dione (cortisol). As in salmonids, the 17,20ßOHP appear as an important metabolite synthesised by testes of *M. furnieri* during the time of spermiation suggesting a role in the regulation of the end of spermatogenetic cycle. Nevertheless, in a close species (*M. undulatus*) it was shown the presence of specific binding sites for the 17,20ß,21-trihydroxy-4-pregnen-3-one (20ßS) in spermatozoa and an effect of the progestin on sperm activation, suggesting a role of the 20ßS in the regulation of gamete maturation of sciaenid fish. However, to our knowledge no data are available in male *M. undulatus* showing the synthesis of 20ßS by testes of the species. Then, the role of these two progestins (20ßS and 17,20ßOHP) at the end of the spermatogenetic cycle of sciaenid fish must be clarified.

PP-57

TEMPERATURE IS IMPORTANT FOR THE ONSET OF SPERMIATION IN GILTHEAD SEABREAM (*SPARUS AURATA*)

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The two major environmental factors controlling gilthead seabream reproduction are water temperature and photoperiod. Although the effect of photoperiod is well documented the effect of temperature is not well established. In this work two experiments were performed (1996-97 and 1997-98) to study the effect of water temperature during summer on seabream male reproduction. In each experiment two groups of 15 fish (one year-old, approx. 300g average body weight) were kept in tanks with running water and natural photoperiod. The water temperature of the experimental tank was kept 2.5+/-1.1°C (1996-97) and 1.7+/-1.5°C (1997-98) bellow natural water temperature. Blood was collected and sperm production was checked monthly until sperm production started and then fortnightly afterwards in the first experiment, and every three weeks in the second experiment. At the end of each experiment gonads were collected for histology and the gonadosomatic index (GSI) was determined. In the first experiment sperm production started one month earlier in the control group and lasted a month longer. In the control group all fish had fluent sperm production for over a month while in the 'treated' lasted no longer than two weeks. In the second experiment sperm production started three weeks earlier in the 'treated' group and lasted three weeks longer. GSI values at the end of both experiments were low in both groups indicating that the reproductive season had ended. Results suggest that the optimal temperature for the onset of spermiation in August-September is 19-22°C.

PP-58

CHARACTERIZATION OF THE GONADAL GnRH SYSTEM IN A MARINE HERMAPHRODITE, THE GILTHEAD SEABREAM (*SPARUS AURATA*)

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The presence of gonadotropin-releasing hormone and its receptor in the ovary and testis of vertebrate species has been firmly established, although the relative expression and functionality of multiple forms of GnRH within the gonads is as yet unclear. The present study describes the detection and localization of seabream (sb)GnRH, salmon (s)GnRH and chicken (c)GnRH-II gene expression within the gonadal tissues of the gilthead seabream during several characteristic phases of the reproductive cycle. RNase protection assays and *in situ* hybridization analysis of ovarian and testicular tissues at the end of the sex reversal period in September demonstrated low levels of mRNA for all three GnRH forms in recrudescent tissues. The gonadal component undergoing regression at this time demonstrated the presence of sGnRH and cGnRH-II mRNAs, and relatively lower levels of sbGnRH gene expression. During the breeding season, sbGnRH and sGnRH gene expression were evident in the functional ovarian and testicular tissues, while levels of cGnRH-II mRNA were barely detectable. Dormant ovarian tissues of breeding males demonstrated significant levels of cGnRH-II mRNA, and levels of sbGnRH and sGnRH mRNAs were relatively low or undetectable. For all three forms of GnRH, gene expression was localized to early primary-growth oocytes of ovarian tissues and Sertoli and spermatogenic cells of the testis. Immunocytochemical localization of the corresponding peptides and localization of the gene expression for the GnRH receptor were also utilized in order to gain initial insight into the relative contributions each of these GnRH forms might have on the local control of gonadal functioning.

PP-59

EXPRESSION OF PROLACTIN AND PROLACTIN RECEPTOR (PRL-R) mRNA IN GILTHEAD SEABREAM (*SPARUS AURATA*) AFTER TREATMENT WITH ESTRADIOL-17β

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Prolactin (PRL) is unequivocally involved in the physiology of reproduction in mammals. In fish it is considered to be an osmoregulatory hormone, although some studies suggest PRL may be associated with production of steroid hormones, the onset of gonadal development and reproductive behaviour. The gilthead sea bream (*Sparus aurata*) is a protandrous hermaphrodite; the mechanisms underlying sex reversal in this species are poorly understood. The present study was carried out to determine if PRL and its receptor are involved in this process. Sea bream were treatead with estradiol-17β (E2; 10 mg/kg body weight) implants during the breeding season. One week after implantation, plasma was collected for quantification of E2 levels by means of radioimunoassay. Fish were sacrificed and pituitaries and gonads were removed and frozen in liquid nitrogen for subsequent analysis. mRNA was extracted from these tissues and the expression of sea bream PRL and sea bream prolactin receptor (PRL-R) was analysed by northern blot and reverse transcriptase polymerase chain reaction (RT-PCR). The results will be discussed.

PP-60

PURIFICATION OF MATURATION-INDUCING HORMONE, 17α, 20β-DIHYDROXY-4-PREGNEN-3-ONE, RECEPTOR SOLUBILIZED FROM ISOLATED CORTICES OF GOLDFISH (*CARASSIUS AURATUS*) OOCYTES

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Fish oocyte maturation is induced by a maturation-inducing hormone (MIH), 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP) or 17α, 20β, 21-trihydroxy-4-pregnen-3-one (20β-S), acting upon an oocyte surface receptor. We demonstrated that a pertussis toxin-sensitive inhibitory GTP-binding protein (Gi) was involved in the signal transduction pathway of 17α, 20β-DP, the salmonid MIH, in rainbow trout oocytes. We have further determined that two Giα subunits, Giα1 and Giα2, are expressed and possibly couple to the 17α, 20β-DP receptor in medaka (*Oryzias latipes*) oocytes*. Adenylyl cyclase was identified as an effector molecule of the G-protein system in rainbow trout oocytes. However, the details of the signal transduction pathway are still unclear due to the ambiguity of 17α, 20β-DP receptor molecule. We have been trying to purify the 17α, 20β-DP receptor from solubilized goldfish oocyte cortices which preferentially bind 17α,20β-DP over 20β-S. Several proteinaceous bands remained after gel-filtration, anion-exchange, hydrophobic interaction, and lectin affinity chromatography, however, to increase recovery after purification we are currently testing a non-radioactive method to quantify the 17α, 20β-DP receptor activity. Using BIACORE, a surface plasmon resonance biosensor, 17α, 20β-DP is biotinylated at the C3-position and immobilized on a streptavidin-coated sensor chip. We will present the results of this methodology.

*Oba et al. (1997), Inhibitory guanine-nucleotide-binding-regulatory protein alpha subunits in medaka (*Oryzias latipes*) oocytes - cDNA cloning and decreased expression of proteins during oocyte maturation. Eur. J. Biochem., 249, 846-853.

PP-61

CORTISOL EFFECTS ON PLASMA 11KT AND ON *IN VITRO* STEROID SECRETION IN MALE COMMON CARP, *CYPRINUS CARPIO*

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Cortisol plays a key role in the restoration of homeostasis during or after stress. The response to chronic and severe stress may require much energy and therefore may force the organism to make adaptive choices. Energy which is normally available for processes like growth, immune response or reproduction is now channeled in restoration of the disturbed homeostasis, resulting in maladaptation which may affect reproduction or growth. Indeed, stress has been shown to interfere with reproduction and the functioning of the brain-pituitary-gonad (BPG) axis. Our previous experiments to study the effect of cortisol on pubertal development in carp have shown that repeated temperature stress, but especially prolonged feeding with cortisol containing food pellets caused a retardation of the first waves of spermatogenesis, and a decrease in 11-ketotestosterone (11KT) and LH plasma levels. The objective of the present study was to investigate if the decrease in plasma 11KT is caused by a direct effect of cortisol on the steroid producing capacity of the testis or by an indirect effect such as a decrease in plasma LH. To that end, adolescent and pubertal isogenic male common carp were fed with either cortisol containing food pellets or control food pellets over a prolonged period. At several time intervals body growth, testicular growth and plasma 11KT was measured. Testis tissue was incubated *in vitro* and both the basal and the LH stimulated steroid secretion was quantified by means of radioimmunoassay. The first results allow the hypothesis that the effect of cortisol depends on the time window of cortisol treatment during the period of pubertal development.

PP-62

CDNA CLONING AND EXPRESSION OF BRAIN AND OVARY AROMATASE IN TILAPIA, *OREOCHROMIS MOSSAMBICUS*

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P450 aromatase is essential for estrogen biosynthesis in the ovary. In the brain, this enzyme has been suggested to have a crucial role during the development of sexual differentiation in early life stages, while in adults it may control the activation of sexual behaviour. The aim of this work was to clone the gene encoding for P450 aromatase in the ovary and brain of *Oreochromis mossambicus*, in order to study sexual differentiation in this species. A 300 bp (base pair) probe was initially obtained by RT PCR using primers designed from known fish sequences. This probe was used to screen an ovary cDNA library. A 1872 bp clone of P450 aromatase was isolated and sequenced. A Apa I restriction fragment encompassing a highly conserved region was then used to screen a brain cDNA library yielding a second clone ca. 2000 bp in length. On the basis of sequence similarity the two clones correspond respectively to ovarian and brain aromatase. Northern blot analysis was used to confirm expression of both mRNA.

PP-63

EVIDENCE OF THE INFLUENCE OF POLYUNSATURATED FATTY ACIDS *IN VIVO* AND *IN VITRO* IN THE REPRODUCTION OF THE EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*)

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Polyunsaturated fatty acids (PUFAs) of the n-3 series, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been shown to influence female reproductive performance in the European sea bass. However, only a few studies describe the influence of series n-6 PUFAs (i.e.: arachidonic acid, AA). At the cellular level, PUFAs modulate gonadal steroidogenesis and they are the metabolic precursors of prostaglandins (PGs) which are involved in steroidogenesis and oocyte maturation. Studies using freshwater fish have shown that AA, through conversion to PGs, stimulates testosterone (T) production while EPA and DHA, attenuate steroid production. However, the role of PUFAs and eicosanoids in gonadal physiology of the marine teleosts is still unclear. A series of experiments were designed to elucidate the role of PUFAs and PGs on the processes of spermatogenesis, maturation, ovulation and spermiation in the sea bass. The results suggest that PUFAs and PGs modulate oocyte maturation *in vitro*. In the testis *in vitro*, PUFAs regulated the synthesis of PGE2, androgens and progestagens. Males fed PUFA-enriched diets exhibited a better reproductive performance as compared to males fed natural diet. Oocytes obtained from females fed a PUFA-enriched diet had higher rates of maturation, less atresia and higher production of PGE2.

PP-64

ANNUAL VARIATIONS IN PLASMA LEVELS OF ESTRADIOL-17β, TESTOSTERONE AND GONADOTROPIN OF THE CATFISH, *HETEROPNEUSTES FOSSILIS*

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In the present investigation adult female catfish *Heteropneustes fossilis*, weighing 40-55g, were procured from the local fish market in and around Varanasi in order to study the changes in the gonadosomatic index (GSI) and the plasma levels of various hormones such as estradiol-17β (E2), testosterone (T) and gonadotropin (GtH) during the annual reproductive cycle of the catfish *H. fossilis*. The fish were weighed and blood was collected in heparinized tubes by caudal puncture between 9 and 11 a.m. taking into account of circadian changes in hormonal profiles. The ovaries were dissected out and weighed to calculate the changes in the GSI during the annual reproductive cycle. Plasma was separated from the blood by centrifuging at 800Xg for 20 min at 40°C and stored at 200°C until further analysis. E2 and T were extracted from the plasma sample by diluting with distilled water and then with the addition of diethylether. The radioimmuno assay of E2 and T were carried out by following the procedure of Abraham (1974). Plasma GtH levels were measured using a heterologous RIA system following the method of Goos et al. (1986). A significant annual variation in plasma levels of E2, T and GtH was noticed in correlation with GSI. E2, T and GtH levels were undetectable in resting phase (October-January) and appeared in preparatory phase (February). In the recrudescent phase, the plasma GtH and E2 levels elicited two secretion peaks: a minor GtH peak in late preparatory phase (April) followed by a major E2 peak in early prespawning phase (June), followed by a minor but inconsistent (E2) peak in early spawning phase (July). After the spawning, the hormone levels decreased concomitantly with GSI.

PP-65

ISOLATION AND CHARACTERIZATION OF 2 DIFFERENTIALLY EXPRESSED cDNAS IN THE OVULATORY AND POST-OVULATORY TROUT OVARY

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Using differential display PCR and PCR subtraction, 2 differentially expressed cDNAs were isolated from ovulatory and post-ovulatory ovaries of the rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). A 1784 nucleotide cDNA, coding for a 383 amino acid polypeptide, was isolated from the brook trout ovary using differential display PCR. Both nucleotide and amino acid sequences showed a very high similarity with members of the aspartyl proteinase gene family. Two active catalytic domains, largely conserved among species, were identified in the amino acid sequence. This suggest a proteolytic activity in the ovary for this protein that is common to all known aspartyl proteinases. On Northern blots, the mRNA levels of this transcript are undetectable at the time of ovulation, increase continuously after 48 hours post ovulation, and reach very high levels 8 to 10 days after ovulation. A novel 1621 nucleotide cDNA, coding for a 347 amino acid polypeptide, was isolated from the rainbow trout ovary using subtractive PCR. On Northern blots, the mRNA levels of this transcript are low at the begining of ovulation, increase dramatically at the end of ovulation and stay elevated 24 to 48 hours post-ovulation. Levels then decrease and are undetectable after 4 to 5 days post-ovulation. Using *in situ* hybridization, this transcript was localized to granulosa cells at ovulation. Neither the amino acid nor nucleotide sequence show any similarity to known cDNAs and proteins. However, an iterative search of a protein database indicated some similarity with the trans-activation domain of several p53 proteins.

PP-66

THE REGULATION OF BROOK TROUT KALLIKREIN/COMPLEMENT FACTOR D IN PREOVULATORY FOLLICLES

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The fish homologue of mammalian kallikrein/complement factor D (KT) was recently cloned from the ovary of the brook trout, *Salvelinus fontinalis*. The expression of KT mRNA is strongly upregulated during the periovulatory period. KT protein is highly expressed in both ovarian tissue and fluid, where it may play a role in ovulation and/or postovulatory events. While the upregulation of KT suggests that it may play an important role in ovarian physiology around the time of ovulation, the specific factors responsible for its upregulation are unknown. Therefore, the purpose of this study was to investigate KT expression in brook trout follicles stimulated with various agonists and antagonists prior to maturation and ovulation. Ovarian follicle-oocyte complexes were isolated from adult female brook trout during the spawning season and were incubated in Cortlands medium in the presence of a variety of factors. The incubations were terminated after 1, 3, 6, or 12 h and the follicle cells were isolated. Protein and total RNA were isolated and assayed by dot blot hybridization for expression of KT. All treatments were carried out on 6 fish. The results indicate that kallikrein, PMA/A23187, TGFa, L-NAME, and orthovanadate downregulate KT RNA expression at 6 and/or 12 h after stimulation. PMA decreased expression of KT protein at 6 and 12 h. The regulation of KT protein expression by the other factors may occur after 12 h. In conclusion, ovarian KT expression is decreased by several factors in brook trout follicles.

PP-67

ISOLATION BY CLONING OF FISH PROSTAGLANDIN ENDOPEROXIDE SYNTHASE (CYCLOOXYGENASE)

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Various studies have investigated the role of cyclooxygenase metabolites in fish reproduction. They have been implicated in ovulation, steroidogenesis and reproductive behaviour. Further, how the synthesis of eicosanoids is controlled has also been investigated. The key enzyme for the production of endoperoxides, prostaglandins, thromboxanes and prostacyclin is prostaglandin endoperoxide synthase (PGS; cyclooxygenase (COX)). Two forms of this enzyme, COX1 and COX2, have been cloned and well characterized in mammals. However, while prostaglandins are certainly produced in lower vertebrates, PGS has never been cloned from these vertebrates. Past attempts we have made to clone fish PGS using RT-PCR and heterologous library screening with mammalian cDNA probes and antibodies, were unsuccessful. However, using newly designed degenerate primers, we have now obtained several fragments of the fish PGS mRNA. RT-PCR was performed with two different primer combinations using cDNA obtained from the ovaries of ovulatory brook trout. The two primer combinations each produced one band of approximately 280 and 450 bp. When cloned and sequenced, the smaller band translated to a protein that was most homologous (approx. 65% identical) with mammalian COX1, while the larger band translated to a protein most homologous (approx. 80% identical) with COX2. These PCR fragments are now being used to obtain full-length cDNAs by library screening.

PP-68

RELEASE OF SEX STEROIDS INTO THE WATER BY ROACH (*RUTILUS RUTILUS*)

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Radioimmunoassays were used to determine the profile of sex steroids (both free and conjugated) released into the water by male and female roach. Fish were placed, individually, in 10 litre plastic buckets each containing 5 litres of water. They were either injected with saline as a control, or Carp Pituitary Extract (CPE;1 to 20 mg/100 g). They were then transferred to fresh buckets at regular intervals over a period of 24 hours, after which time blood samples were taken and the gonads removed to determine the stage of maturity. Water samples (500ml) were pumped through activated C18 cartridges (Sep-Pak Plus) and the steroids eluted with methanol. It was found that, in response to CPE, both male and female roach released very high levels of free and glucuronidated 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P) and 17,20β,21-trihydroxy-4-pregnen-3-one. Substantial amounts of free 11-deoxycortisol, androstenedione, 17-hydroxyprogesterone and glucuronidated 11-ketotestosterone were also released by some of the fish. The amounts of sulphated steroids appeared to be low. Intriguingly, in two experiments, high release of 17,20β-P was found in apparently immature fish (i.e. with thread-like gonads). It is hoped to repeat these observations (with a more detailed examination of the gonads) during the spring of 1999.

PP-69

OOCYTE DEVELOPMENT AND ENDOCRINE CHANGES IN PLASMA OF MATURING ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS L.*)

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Ovarian development in captive Atlantic halibut is described from previtellogenic oocytes through final maturation and ovulation. Levels of estradiol-17β and testosterone were measured in plasma of mature female Atlantic halibut. The ovary of three-year old halibut, four years before maturation, had two size classes of previtellogenic oocytes. Two main size classes of previtellogenic oocytes were also found in six-year old fish, assumed to mature the following year, but here an additional small group of oocytes was in the early cortical alveoli stage with Balbiani bodies visible in the outer area. Balbiani bodies were not found five months prior to spawning when the cortical alveoli stage was more pronounced. The diameter of oocytes and yolk granules increased towards spawning. During final maturation, i.e. when the nucleus migrates from the centre to the animal pool of the oocyte, coalescence of yolk granules was observed at the vegetal pool. Spawning individuals had ovaries with oocytes in several developmental stages; previtellogenic, vitellogenic, final maturation and hyaline oocytes, as well as post ovulatory follicles. Plasma levels of estradiol-17β and testosterone were highest close to release of the first egg batch, then fluctuated throughout the spawning period in accordance with gonadal development stages. Steroid levels decreased toward the end of spawning. The results indicate that the halibut ovary is vitellogenically active both before and during spawning.

PP-70

HISTOLOGICAL STUDIES ON THE GONADS OF DIPLOID AND TRIPLOID ATLANTIC SALMON (*SALMO SALAR* L.)

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A histological study was carried out to compare the gonadal development of triploid and diploid Atlantic salmon as part of a comprehensive study to assess the comparative performance of triploid and diploid salmon as cultured stocks, and evaluate the environmental impact of triploid salmon through tagging and release studies. Triploid and diploid smolts were microtagged in January 1996. A group was transferred to a sea cage and sampled in November 1996, February, March and finally in May 1997 prior to harvest. A second group was released to the wild and gonads were sampled from ranched fish that returned as grilse from July to September 1997. The ovaries of the triploid females sampled from November 1996 to March 1997 remained in the primary growth phase and in May 1997 the majority of oocytes were at the chromatin nucleolus stage. In contrast, the majority of the oocytes in the ovaries of diploid fish at this time had developed to the cortical aveolus and oil drop stages of secondary growth phase. The ovaries of triploid females ranched returns remained in the primary growth phase. However, diploid females ranched returns were in advanced oogenesis by August 1997 with the majority of oocytes being either at the oil drop or primary yolk sac stage. Gonadal development in male triploid and diploid fish was similar over the period monitored (primary spermatogonia). Testes of triploid and diploid male ranched had developed to the secondary spermatogonia phase by September 1997.

PP-71

GONADOTROPIN-RELEASING HORMONE STRUCTURE, FUNCTION AND REGULATION IN THE GOLDFISH OVARY

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There is clear and unambagious evidence for the presence of GnRH and GnRH receptors (GnRH-R) in the ovary of goldfish. The goldfish brain and ovary contains two forms of GnRH, [Trp7, Leu8]-GnRH (sGnRH) and [His5, Trp7, Tyr8]-GnRH (cGnRH-II), although their concentrations are significantly lower in the ovary than in the brain. The general structure of GnRH gene is conserved to a large extent among various species, and it has been demonstrated for the first time in goldfish that ovarian sGnRH is transcribed, translated and processed in the same way as hypothalamic sGnRH. There is also clear evidence for the presence of GnRH-R in the goldfish ovary with similar binding characteristics to those in the pituitary. The regulation of ovarian GnRH production was only studied in terms of the steady state mRNA levels because of their low abundance. Treatment of goldfish ovarian follicles with gonadotropin was found to stimulate both sGnRH and cGnRH-II transcript levels with differential sensitivity. Estradiol treatment was also found to stimulate sGnRH transcript in the goldfish follicles, indicating that GnRH production are regulated by both pituitary and ovarian hormones. In goldfish, GnRH was found to exert both stimulatory and inhibitory actions on oocyte meiosis and follicular steroidogenesis, depending upon the presence or absence of gonadotropins. Administration of a number of GnRH variants including sGnRH and cGnRH-II, were found to significantly stimulate resumption of meiosis in the follicle-enclosed goldfish oocytes. In the presence of gonadotropin hormone, sGnRH treatment significantly inhibited gonadotropin-induced response, whereas cGnRH-II treatment was without an inhibitory effect. This suggests that goldfish ovary might contain GnRH receptor subtypes or mechanisms through which sGnRH and cGnRH-II could interact with the same receptor molecule in different ways leading to activation of alternate post-receptor mechanisms. Overall, the findings provide a strong support for the hypothesis that GnRH plays a physiological role in the control of ovarian function in goldfish.

PP-72

PUTATIVE GONADOTROPIN RECEPTORS IN TILAPIA (*OREOCHROMIS NILOTICUS*) GONADS: cDNA CLONING AND EXPRESSION DURING OOGENESIS

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Two different types of gonadotropins (GTH-I and GTH-II) are found in fish. These two gonadotropins are thought to act via specific membrane receptors present in their target cells in testis and ovary. A two receptor model has been proposed for salmon gonadotropins (Yan et al., 1992). In the present study, poly(A)+ RNA prepared from tilapia testes was subjected to RT-PCR using degenerate primers derived from conserved regions of the pituitary glycoprotein hormone receptors. Two distinct cDNA clones with high sequence similarity to mammalian gonadotropin receptors were isolated and denoted as tilapia gonadotropin receptors, tGTHRA and tGTHRB. These two tilapia gonadotropin receptor cDNAs encode proteins of 677 and 693 amino acids, respectively, with highly conserved transmembrane domains. Northern blot analyses using tilapia ovarian follicles at different stages of development revealed that tGTHRA mRNA was largely expressed in early-vitellogenic follicles. In contrast, tGTHRB mRNA was predominantly expressed in late-vitellogenic and postvitellogenic follicles. These results suggest that tGTHRA may be involved in oocyte growth whereas tGTHRB is likely to play a role in oocyte maturation and ovulation.

PP-73

3ß-HYDROXYSTEROID DEHYDROGENASE GENE: SITES OF EXPRESSION IN TROUT GONADS AND cAMP-DEPENDENT REGULATION

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A cDNA encoding rainbow trout ovarian 3ß-hydroxysteroid dehydrogenase (3ß-HSD) was used to generate digoxigenin-labelled sense and antisense cRNAs for use in *in situ* hybridization studies on cellular sites of expression in rainbow trout gonads and to assess the effects of salmon gonadotropin II (GTH II) and forskolin *in vitro* on mRNA levels. Immunohistochemistry employing a specific antiserum was used to visualize rainbow trout 3ß-HSD enzyme. In vitellogenic ovarian follicles, moderate hybridization signals and immunoreactivity were confined to scarce, flattened, elongated cells in the thecal layer corresponding to special thecal cells. Hybridization signals, immunoreactivity and numbers of positive thecal cells markedly increased in postovulatory follicles. Granulosa cells of vitellogenic and post-vitellogenic follicles occasionally showed very weak hybridization signals and immunoreactivity with evidence for a transient increase in expression at the time of ovulation. In the testis, 3ß-HSD gene expression appeared to be confined to Leydig (interstitial) cells. Treatment of early vitellogenic follicles with GTH II or forskolin for 18-36 h *in vitro* resulted in strong increases in 3ß-HSD mRNA and enzyme levels in cells of both the thecal and granulosa layers. Treatment of pre-vitellogenic follicles with forskolin similarly led to very strong hybridization signals and immunoreactivity in granulosa cells, but GTH II appeared to be ineffective. Using testes from juvenile trout, *in vitro* studies revealed that 3ß-HSD gene expression in Leydig cells is also enhanced through a cAMP-dependent mechanism.

PP-74

cDNA CLONING AND FUNCTIONAL CHARACTERIZATION OF A NOVEL ANDROGEN RECEPTOR SUBTYPE IN THE JAPANESE EEL

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There has been general acceptance that only one type of androgen receptor (AR) exists in an individual. This contrasts with other members of the nuclear receptor superfamily where multiple forms have been reported (e.g. ER alpha/beta, TR alpha/beta etc.). We have previously identified 11-ketotestosterone (11KT, a potent androgen in teleosts) as the spermatogenesis-inducing hormone of the Japanese eel and have cloned its receptor (eAR1) cDNA from eel testis. Here we report on the cloning of a cDNA encoding a second type of AR (eAR2) from the eel testis and the functional characterization of the encoded protein. This cDNA contains a complete open reading frame encoding 798 amino acid residues. The amino acid sequence of eAR2 shows high homology with other ARs, including eAR1, in the DNA-binding (92-88%) and ligand-binding (59-78%) domains,whereas the other domains show low homology (<35%). In transient transfection assays of mammalian cells, eAR2 protein showed androgen-dependent activation of transcription through the androgen-responsive MMTV promoter. Of the endogenous androgens found in the Japanese eel, 11KT was the most effective activator of transcription. Synthetic androgens were significantly more effective than 5α-dihydrotestosterone in activating transcription by eAR2, but not BY eAR1. Androstenedione induced weak transcription by eAR2, whereas transcription by eAR1 was not significantly stimulated. We conclude that eAR2 is a novel AR in the eel, and we suggest it should be named eel AR beta to distinguish it from eAR1 (eAR alpha).

PP-75

CARBONYL REDUCTASE-LIKE 20β-HYDROXYSTEROID DEHYDROGENASES IN THE OVARIAN FOLLICLE OF A TELEOST FISH, THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*): THEIR STRUCTURES AND REPRODUCTIVE FUNCTIONS IN OOCYTE MATURATION

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17α,20β-dihydroxy-4-pregnen-3-one (17a,20β-DP) has been identified as the maturation-inducing hormone (MIH) of several teleost fishes. In salmonids, 17α,20β-DP is produced by conversion of 17a-hydroxyprogesterone (17α-HP) in granulosa cells by the action of 20β-hydroxysteroid dehydrogenase (20β-HSD). Two closely related 20β-HSD cDNAs were cloned from rainbow trout (*Oncorhynchus mykiss*) ovarian follicles and the proteins they encode share 60% homology to mammalian carbonyl reductases (Crs). These were named rainbow trout CR/20β-HSD cDNA type A and type B. Genomic DNA analysis showed that the two CR/20β-HSD cDNAs are derived from two different genes. Recombinant CR/20β-HSD proteins produced in *E. coli* were used to demonstrate that CR/20β-HSD type A encoded a protein with 20β-HSD as well as carbonyl reductase enzyme activity, while type B is devoid of either activity. These proteins differ by only three amino acids. Site-directed mutagenesis showed that Ile15, present in type A protein but substituted by Thr15 in type B protein, plays an important role in the formation of enzyme-coenzyme complex as determined by site-directed mutagenesis. Northern blot and RT PCR analysis revealed that trout CR/20β-HSDs are expressed in various tissues, with greatest abundance in liver and gill. The expression of CR/20β-HSD type A is inducible in granulosa cells by GTH stimulation, which results in the increase of the conversion of 17α-HP to 17α,20β-DP at stage just before oocyte maturation. The effects of GTH on the promoter regions of CR/20β-HSD genes will increase understanding of hormone modulation of steroidogenic enzymes.

PP-76

TESTOSTERONE AND ESTRADIOL CYTOSOL BINDING IN FOREBRAIN OF SALMONID FISHES (*SALMO SALAR* L., *SALMO TRUTTA* L.)

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The study was performed by the radioligand assay. We revealed that average values of cytosol testosterone specific binding (TB) in forebrain (bulbus olfactorius + corpora striata) were significantly higher as compared with the same in other regions of salmon and trout brain, with the exception of pituitary gland. TB in forebrain of mature fishes exceeded the same of immature ones 3-5 times. On the other hand, average values of cytosol estradiol specific binding (E2B) in forebrain of mature fishes were similar or decreased as compared with the same of immature ones. The differences in TB and E2B values between sexes and species were found also. The increase of forebrain (olfactory region of brain) sensitivity to testosterone during reproductive cycle may be important in control of maturation, homing and/or reproductive behaviour: it is known that testosterone and its metabolites, but not estradiol, may act as pheromones in salmon and trout.

PP-77

SEX-RELATED DIFFERENCES OF TESTOSTERONE BINDING BY ADIPOSE FIN AND NASAL CARTILAGINOUS TISSUE IN THE ATLANTIC SALMON (*SALMO SALAR* L.)

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The increase of adipose fin size and growth of nasal cartilaginous tissue in maturing males are the components of sexual dimorphism development that are important for realization of reproductive behaviour in salmon. Testosterone is, probably, involved in control of these changes. That is why a comparative study of testosterone cytosol specific binding (TB) values in above tissues of immature and mature males and females was carried out. The radioligand assay was applied. We revealed no reliable differences between average values of TB in adipose fin of immature males and females. On the other hand, TB in adipose fin of mature fishes of both sexes exceeded the same of immature ones 3 - 4 times. Values of TB in adipose fin of mature females were significantly higher as compared with the same of mature males. In general, TB in nasal cartilaginous tissue was higher than TB in adipose fin. The average values of TB in nasal cartilaginous tissue of mature females exceeded the same of mature males approximately 2,5 times, but there were no reliable differences between immature males and females. The positive correlation between TB in adipose fin and degree of gonad maturity in males and females, as well as between TB in this tissue and fish body length was found (r = 0,86-0,93). On the contrary, there was no significant correlation between TB in nasal cartilaginous tissue and body length of males and females. The possible connection between TB values, blood testosterone levels and/or relative shares of free fraction of this hormone receptors in tissues of males and females is discussed

PP-78

CONFIRMATION OF MATURATION-INDUCING STEROID IN A FRESHWATER CATFISH, *CLARIAS BATRACHUS*

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17α,20β-dihydroxy-4-pregnen-3-one (20β-P) has been identified as a maturation-inducing steroid (MIS) in many fishes. In addition, 17α,20β,21-trihydroxy-4-pregnen-3-one (21-P) also has been found in few fishes. Earlier studies in an Indian catfish, *Clarias batrachus* showed that 20β-P is the MIS. The present study was made to confirm whether 21-P appears alongwith 20β-P in the catfish or not. *In vitro* and *in vivo* methods were carried out to analyse the appearance of 20β-P and 21-P in the culture medium and in the blood plasma using HPLC and RIA assays. Salmon gonadotropin (SG-G100) injected fishes showed the 20β-P elevation in the plasma which is in good agreement with the oocytes cultured with SG-G100. However, the level of 20β-P was very low (4.2ng/ml) but the sulfated 20β-P (20β-P-S) showed ten times higher than the free steroid. It is well understood that the free 20β-P is rapidly converted into 20β-P-S in the blood stream. When the oocytes were incubated with SG-G100, 20β-P was highly synthesized by the follicles and not the 21-P. The non-appearance of 21-P in the medium confirms that the 20β-P is the MIS in catfish. The less amount of 20β-P-S recorded in the oocyte cultured medium indicates that predominant conjugation does not take place in follicles. In the SG-G100 injected animal, 21-P as well as 21-P-S were very low when compared with control. This might be due to the 20β-P synthetic pathway which dominates over the 21-P synthetic pathway. Hence the present experiment concludes that 20β-P is the MIS in *Clarias batrachus*.

PP-79

MEDAKA (*ORYZIAS LATIPES*) FTZ-F1 POTENTIALLY REGULATES THE TRANSCRIPTION OF P-450 AROMATASE IN OVARIAN FOLLICLES: cDNA CLONING AND FUNCTIONAL CHARACTERIZATION

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In nonmammalian vertebrates, estradiol-17β, a major estrogen converted from testosterone by cytochrome P-450 aromatase (P-450arom), is responsible for the growth of oocytes, i.e. vitellogenesis. In contrast to the well-known physiological functions of estradiol-17β, very little is known about the molecular mechanisms of P-450arom activation in ovarian follicles during oocyte growth. Our previous findings suggest that the activity of P-450arom in the ovarian follicle of medaka (*Oryzias latipes*) is regulated at the transcriptional level. Promoter analysis of the medaka P-450arom gene identified putative orphan nuclear receptor binding sites (Ad4-1 and Ad4-2 site). By RT-PCR, seven distinct fragments encoding putative orphan nuclear receptors were amplified from total RNA of medaka ovarian follicles. One of these fragments exhibiting high homology with Ftz-F1s of various species, fragment B, showed a clear expression profile which correlated well with P-450arom expression. Subsequently, we cloned a full length cDNA for medaka Ftz-F1 (mdFtz-F1). *In vitro* translated mdFTZ-F1 and nuclear extract from medaka ovarian follicles formed complexes with Ad4-1 and Ad4-2 oligonucleotides. Furthermore, the binding capacity of mdFTZ-F1 shows a good correlation with the expression of medaka P-450arom in ovarian follicle cells during oogenesis. Transfection assays further indicate that mdFTZ-F1 acts as a positive transcription factor on the medaka P-450arom promoter through the Ad4-1 and Ad4-2 sites. Taken together, these results suggest that a change in the binding capacity of mdFTZ-F1 is the predominant mechanism regulating P-450arom in the ovarian follicle of medaka.

PP-80

ZEBRAFISH MUTAGENESIS: ISOLATION AND CHARACTERIZATION OF REPRODUCTIVE MUTANTS

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The zebrafish, *Danio rerio*, is a popular model for the study of developmental genetics. Several large-scale chemical mutagenesis screens have been performed on zebrafish, identifying over 1200 mutations in 350 genes found to be necessary for embryogenesis. We have used a chemical mutagenesis scheme to recover mutations affecting ovarian and testicular maturation and develop-ment. Point mutations were induced in adult male zebrafish using N-ethyl-N-nitrosourea (ENU). A classical three-generation (F2) crossing scheme was used to recover recessive mutations in the F3 generation. At sexual maturity, whole body cross-sections of the F3 generation are screened histologically for alterations in gonad morphology and stage of development. To date, several gonadal mutations have been observed in both sexes. Male mutations include testes with no sperm in which spermatogenesis is arrested at either the spermatogonia or spermatocyte stage of deve-lopment. A testicular mutation in which spermatogenesis does not progress beyond the spermato-gonia stage of development has already been confirmed by outcrossing to be present in the germ-line. Female mutations include ovaries containing degenerating oocytes surrounded by hypertro-phied follicle walls and/or stroma, ovaries with oogenesis arrested at the early perinucleolus, yolk vesicle, or oil drop stage of development, and ovaries with large numbers of resorbing oocytes. Mutations that are verified to be present in the germline are being genetically mapped using simple sequence length polymorphism (SSLP) markers and bulked segregant analysis. These mutations will also be further characterized using light microscopy on semi-thin plastic sections.

PP-80A*

PERIPHERAL-TYPE BENZODIAZEPINE RECEPTOR(PBR) IS A TARGET OF ENVIRONMENTAL HAZARDS AFFECTING THE HORMONE REGULATION OF TESTICULAR STEROIDOGENESIS.

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We have shown that PBR is a key element in the acute hormone regulation of steroidogenesis in mammals. Furthermore, in nonmammalian vertebrates, PBR has been immunolocalized in adrenocortical steroidogenic cells. The objective of this study was to determine the *in vitro* anti-androgenic effects of the respective xenobiotics, the perfluorodecanoic acid (PFDA) and the bezafibrate, two peroxisome proliferators, on the mouse tumor Leydig cells MA-10 steroidogenesis. The first part of this study was performed to evidence the *in vitro* effect of these endocrine disruptors on the MA-10 progesterone biosynthesis. Our results show that PFDA and bezafibrate suppress in time-and dose-dependent manner the hCG-stimulated progesterone biosynthesis by MA-10. In parallel, both PFDA and bezafibrate cause a significant decrease of PBR ligand binding. The purpose of the second part of this study was to determine the molecular mechanisms involving PBR in this anti-androgenic effect of the PFDA and bezafibrate on MA-10 cells. Our results show that PFDA affected the transfer of cholesterol to the inner mitochondria membrane rather than the P450-scc activity. Image analysis of Western and Northern blot indicates that PFDA and bezafibrate induce a significant reduction of PBR mRNA and 18 kDa PBR protein. However, image analysis of StAR immunoblot shows that PFDA did not affect hCG-induced 31 kDa StAR protein, known for its involvement in hormone regulation of steroidogenesis. These data show that the inhibitory effect of PFDA on steroidogenesis is localized at the level of the cholesterol transport to the inner mitochondria membrane and is mediated by PBR.

* The Scientic Committee apologizes for including this contribution too late for a full, separate number.

Special Plenary Lecture

OP-27

GONADAL STEROID HORMONES: MAJOR REGULATORS OF GONADAL SEX DIFFERENTIATION AND GAMETOGENESIS IN FISH

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In fish, as in other vertebrates, gonadal steroid hormones play important roles in regulating gonadal sex differentiation and gametogenesis. These hormones are synthesized by specialized somatic cells in gonads under the influence of pituitary gonadotropins. This review briefly summarizes our findings on the hormonal regulation of ovarian and testicular differentiation, spermatogenesis, sperm maturation, oocyte growth, and oocyte maturation. In tilapia, steroid-producing cells in gonads of genetic females (XX) immunohistochemically express all of the steroidogenic enzymes required for estradiol-17β biosynthesis two weeks prior to the first sign of histological ovarian differentiation. In contrast, positive immunostaining can not be confirmed in gonads of genetic males (XY) during testicular differentiation. Furthermore, an aromatase inhibitor induces the development of phenotypic males from genetic females. These results strongly suggest that endogenous estrogens act as natural inducers of ovarian differentiation in tilapia.

Using an organ culture system for eel testes, we have shown that the hormonal regulation of spermatogenesis in eel testes involves gonadotropin stimulating of Leydig cells to produce 11-ketotestosterone. In turn, 11-ketotestosterone acts on Sertoli cells through an androgen receptor to produce activin B. Activin B then acts on spermatogonia to induce *de novo* synthesis of G1/S cyclins and CDKs leading to the initiation of mitosis. A distinct shift in testicular steroidogenesis from 11-ketotestosterone to 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP) occurs around the time of spermiation in several fishes. Our *in vivo* and *in vitro* data suggest that 17α,20β-DP is involved in inducing spermiation and the acquisition of sperm motility.

Estradiol-17β and 17α, 20β-DP or 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S) are the important regulators of oocyte growth and maturation, respectively. Estradiol-17β and 17α,20β-DP production occurs via the interaction of two cell types, thecal and granulosa (two-cell type model) in the ovarian follicle. Immediately prior to oocyte maturation, there is a distinct shift in the steroidogenic pathway from estradiol-17β to 17α,20β-DP. The biochemical changes associated with this steroidogenic shift include marked induction of 20β-hydroxysteroid dehydrogenase mRNA expression as well as an abrupt decrease in aromatase mRNA transcripts in granulosa cells. FTZ-F1, an orphan nuclear receptor, may play a role in transcriptional regulation of these two enzymes. 17α,20β-DP binds to a novel, unknown membrane receptor coupled to a signal transduction cascade associated with inhibitory G-proteins. The early steps following 17α,20β-DP action involve the formation of the major mediator of this steroid, maturation-promoting factor (MPF) which consists of cdc2 kinase (34 kDa) and cyclin B. Immature oocytes contain only 35 kDa cdc2. Cyclin B mRNA is already present in immature oocytes. 17α,20β-DP induces oocytes to synthesize cyclin B, which in turn activates preexisting 35 kDa cdc2 kinase through threonine 161 phosphorylation, producing 34 kDa cdc2 kinase. A 54 kDa Y box protein and polyadenylation of cyclin B mRNA may be involved in 17α,20β-DP-induced cyclin B mRNA translation.

Gametogenesis and Sex Differentiation:
Spermatogenesis and Sex Differentiation

OP-28

SPERMATOGENESIS AND ITS ENDOCRINE REGULATION

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Spermatogenesis provides a large number of highly differentiated spermatozoa which develop in three phases. First, stem cells initiate a fixed, species-specific number of mitotic cell cycles to produce spermatogonia. They then enter two meiotic cell cycles, resulting in haploid spermatids which carry a recombined, unique set of genes. Finally, spermatids differentiate into flagellated spermatozoa. Throughout spermatogenesis Sertoli cells support the germ cells structurally and physiologically. Spermatogenesis strictly depends on androgens produced by the interstitial Leydig cells under the influence of luteinizing hormone (LH). However, androgen receptors have not been detected in germ cells. Hence somatic (mainly Sertoli) cells are considered to mediate the androgen effects. Still, the first androgen target gene expressed by Sertoli cells and with a regulatory function in spermatogenesis, has to be identified yet. Next to androgens, follicle-stimulating hormone (FSH) is a major factor in the endocrine regulation of spermatogenesis. In mammals Sertoli cells express FSH receptors; they do not bind LH. Gene knock out experiments have shown that FSH signaling greatly enhances the efficiency of spermatogenesis, although FSH signaling is not a prerequisite for the process *per se*. In fish, however, the FSH receptor-like protein may not yet have evolved to discriminate clearly between FSH- and LH-type gonadotropins, while a high ligand binding specificity is shared by the LH receptors in mammals and fish. In general, it appears that the endocrine system mainly targets the infrastructure needed for spermatogenesis, while the timing of the developmental steps depends on autonomous germ cell systems.

OP-29

THE GERMINAL EPITHELIUM IN FISH GONADS

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Germinal epithelia border a body surface or lumen and have a tripartite morphology composed of a supporting basement membrane and two cell types: germ cells and somatic cells. In male fish, and depending upon testis type, the lumen is found in anastomosing tubules or lobules. In the ovary, the germinal epithelium borders the ovarian lumen. In testes, germ cells consist of spermatogonia, spermatocytes, spermatids, and sperm. The somatic cells are the Sertoli cells. Epithelial cells, which transform into follicle cells, represent the somatic cell component of the ovarian germinal epithelium. Oogonia and oocytes, through diplotene of the first meiotic prophase, represent the germ cell component. Accurate information regarding the germinal epithelium in teleosts is practically nonexistent. Changes taking place in the testicular germinal epithelium of fish between breeding seasons are used to define continuous and discontinuous germinal epithelia in testes and to divide maturation into five classes: regressed, early maturation, mid maturation, and late maturation, and regression. In the ovary, the germinal epithelium is discontinuous. The recruitment of oocytes from the germinal epithelium into the ovarian lamellae is a continuous process throughout the year, even in regressed fish. It has been observed that oogonia give rise to oocytes, which remain within or attached to the germinal epithelium at least through early diplotene, the beginning of the primary growth phase. This also coincides with the completion of folliculogenesis.

OP-30

EXPRESSION OF TWO ESTROGEN RECEPTORS DURING INDUCED SEX REVERSAL IN SEABREAM, *SPARUS AURATA*

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The seabream, *Sparus aurata*, is a protandrous hermaphrodite developing a testis during their first year of life and undergoing sex reversal to female between the second and the third years. Estradiol-17β (E2) has been identified as a mediator of gonadal feminization in fish. In order to study the mechanism of E2 action in seabream, particularly during sex reversal, cDNAs encoding two different forms of seabream estrogen receptor (sbER) were cloned. On the basis of sequence similarity, one form was homologous to the classical ER type alpha while the other was more related to the newly discovered ER type beta. Tissue expression of each sbER revealed some important differences with ER type alpha being expressed mainly in liver and ER type beta as the major form present in ovary. In an experiment to study the expression of the two receptors, sex reversal was induced by E2 administrated with the food (15mg E2/kg food) during 10 weeks. The results of northern blot analysis will be used to discuss the differential role of the two receptors in sex reversal in seabream.

OP-31

STEROIDS AND GONADAL SEX DIFFERENTIATION IN THE RAINBOW TROUT, *ONCORHYNCHUS MYKISS*

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Steroids have been used since Yamamoto's studies in the seventies to control phenotypic sex differentiation in fish. However, their physiological implication in gonadal differentiation and their mode of action during steroid-induced differentiation needs to be elucidated further. For this purpose several methodological approaches have been used including *in vivo* treatment experiments and gene expression analysis performed only on genetic males and genetic female populations of rainbow trout. Steroid metabolism analysis shows some sex-specific syntheses: estrogens for ovarian differentiation and 11-oxygenated androgens for testicular differentiation. *In vivo* treatments with a natural 11-oxygenated androgen, 11β-hydroxyandrostenedione, lead to complete masculinization, but metopyrone, a specific inhibitor of 11β-hydroxylase (11βH), did not show any effect. On the other hand, estrogens induce feminization while an aromatase inhibitor (androstatrienedione) brings about complete masculinization. However, steroid receptors antagonists have no effect. We also investigated gonadal gene expression of some steroid enzymes (aromatase = P450ar and 11BH = P450c11) and steroid receptors (androgen receptor α and β = ARα and ARβ, estrogen receptor = ER) during sexual differentiation. P450ar is specifically expressed during ovarian differentiation and P450c11 during testicular differentiation, and these expressions are detected very early, i.e., 2 weeks before the first histological features of gonadal differentiation. Expression of ARα, ARβ and ER are also detected very early but with no sex specific pattern. Altogether these results agree with a physiological involvement of sex steroids in fish gonadal differentiation, and indicate that steroid enzyme potentialities are probably the main key steps in that process.

OP-32

AROMATASE GENE EXPRESSION IN TEMPERATURE-INDUCED GONADAL SEX DIFFERENTIATION OF TILAPIA *OREOCHROMIS NILOTICUS*

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In tilapia *Oreochromis niloticus* sex is determined by genetic factors (GSD), temperature (TSD) and temperature/genotype interactions. During gonadal sex differentiation progenies can be functionally masculinized by high rearing temperatures of 35°C when applied throughout the thermosensitive period. In several reptile species, temperature is the main factor determining the phenotypic sex: high or low incubating temperatures produce either female or male offsprings. Recent investigations in turtles have demonstrated that temperature influences some major genes involved in the sex determination cascade such as the steroid enzyme aromatase P450ar. In teleost fish, aromatase seems to be a key enzyme for ovarian differentiation. Aromatase gene expression was assessed in the current study to determine its implication in the gonadal sex differentiation induced by temperature in tilapia. Three types of progenies were produced: genetically all-female population sired from XX males (progenies showing different degree of thermosensitivity) and genetically all-male populations sired from YY males (negative control). Populations were reared at both 27°C (control) and 35°C (masculinizing treatment). Dissection of gonads was performed in both groups before histological gonadal sex differentiation. Sex ratio was determined by microscopic analysis of gonadal squashes. A tilapia aromatase cDNA of 1000 bp was obtained by screening an ovary cDNA library. Gene expression was analyzed by virtual Northern blots using this probe and by quantitative RT-PCR. Higher levels of aromatase gene expression were revealed in all-female populations when compared to males. Progenies in which temperature caused total sex inversion of gonads, showed a decrease in aromatase expression in treated groups.

OP-33

ROLE OF ENDOGENOUS STEROID HORMONES ON GONADAL SEX DIFFERENTIATION IN FISH

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The mechanism of gonadal sex differentiation in fish is still unknown. The initial differentiation and development of steroid-producing cells accompanying gonadal sex differentiation was observed at ultrastructural level and immunocytochemically using polyclonal antibodies. Antibodies against four steroid-metabolizing enzymes (cholesterol side chain cleavage cytochrome P450 (P450scc), 3β-hydroxysteroid dehydrogenase (3β-HSD), cytochrome 17α-hydroxylase/17,20 lyase (P450c17), and cytochrome P450 aromatase (P450arom)) essential for the biosynthesis of all major sex steroid hormones, including androgens and estrogens were used. In addition to male and female mixed group, we used genetically controlled all-female group, obtained by mating artificially induced pseudo-XX males with normal females. Using the group of all female fish, the expressions of the above four enzymes were examined immuno-histochemically, during the process of sex reversal following the administration of androgen and an aromatase inhibitor. In this paper, we will discuss about the endocrine control of gonadal sex differentiation in fish.

OP-34

MASCULINIZATION OF NILE TILAPIA WITH STEROIDS: ALTERNATE TREATMENTS AND ENVIRONMENTAL EFFECTS

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Steroid-treated food is widely used to masculinize tilapia. We previously showed that short-term immersion in steroids can also masculinize Nile tilapia. For the following study, we determined 1) the sensitive period for masculinization via immersion, 2) if multiple immersions during critical days produces all-male populations, and 3) if 17α-methyltestosterone (MT) persists in the environment. Tilapia fry were immersed in 500 g/L of Trenbolone Acetate (TA) for 3 hr on 12, 13, or 14 days post-fertilization (dpf; experiment 1) or in combinations of days between 12 and 15 dpf (experiment 2). In experiment 3, fry were fed a masculinizing dose of MT (60 mg/kg) for four weeks beginning at the initiation of feeding in model ponds which consisted of jars or aquaria that contained soil. In experiment 1, significant masculinization in comparison to controls occurred in fish immersed on 12 dpf, and even greater masculinization occurred in fish immersed on 13 dpf or on 14 dpf. In experiment 2, repeated immersions produced significantly higher proportion of males than controls with fry immersed on 12, 13, 14, and/or 15 dpf having greatest effect. In experiment 3, concentrations of MT in water peaked at 2-to-3 weeks after the onset of feeding, before decreasing to background levels by 5 weeks (1 week after the end of treatment with MT-impregnated food). In contrast, MT levels in the soil were high at 4 weeks after the onset of feeding and remained detectable in the soil through 7 weeks (3 weeks after ending treatment with MT-impregnated food).

PP-81

HISTOLOGICAL EVIDENCE SUPPORTING THE TWO CRITICAL PERIOD MODEL OF GONADAL DEVELOPMENT AND SEXUAL MATURITY IN MALE SALMONIDS

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Groups of Arctic charr, *Salvelinus alpinus*, were subjected to six week intervals of continuous starvation from September to April. Histological observations of the spermatogenic cycle made during this time lends support to the theory that there are two critical decision periods in the maturation cycle of salmonids. The first critical period occurs in autumn, approximately one year before spawning. Fish starved from mid-September to the end of October showed reduced proportions of fish moving from a prespermatogonial state of development into a stage where spermatogonia were organized within testicular lobules. This effect, however, was temporary, as subsequent re-feeding permitted resumption of gonadal development. A second critical period occurs in spring when fish make the decision to either continue with gonadal development or to postpone maturation for another year. Histologically, this critical period is realized by the fish either entering into cyst development and spermatocyte production or remaining immature. Although starvation did not have an obvious effect on gametogenesis at this time, it is considered a critical point in the gametogenic cycle, as once spermatocyte production has begun, spermatozoa formation ensues. This study revealed that the process of spermatogenesis in Arctic charr is highly asynchrononus and flexible both in individuals and within the population making food restriction alone an ineffective method of preventing maturation in this species.

PP-82

ANNUAL CYCLE OF THE TESTIS IN THE SWAMP EEL, *SYNBRANCHUS MARMORATUS* (TELEOSTEI, SYNBRANCHIDAE), A PROTOGYNIC, DIANDRIC FISH

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To characterize the annual reproductive cycle in the lobular testis of *S. marmoratus*, histological criteria were used to reveal the presence of both continuous and discontinuous germinal epithelia. Five reproductive classes were observed: regressed, early maturation, mid maturation,late maturation, and regression. *S. Marmoratus* were caught in lagoons in the Corrientes Province, Argentina. Testes were removed, fixed in Bouin's solution or buffered 2% glutaraldehyde, and processed for light and for electron microscopy. The germinal compartment, delineated by a basement membrane, is organized into lobules which terminate at the testis periphery and form anastomosing networks near the ducts. Sperm developed within spermatocysts whose borders are comprised of Sertoli cell processes. Spermatogenesis began during autumn (March-July), continued throughout the winter (July-September), and peaked during the summer (December-March). At the beginning of spermatogenesis, a continuous germinal epithelium was observed near the ducts. The tripartite germinal epithelium consisted of the basement membrane supporting germinal cells and associated Sertoli cells. Beginning in the spring (September-December), spermatocysts became separated, forming a discontinuous germinal epithelium, as sperm matured and were released into the lobules. The discontinuous epithelium was first noted near the testis ducts. As maturation progressed, it extended to the testis periphery. The interstitial compartment contained prominent Leydig cells with abundant smooth endoplasmic reticulum, myoid cells, and collagen. Melanomacrophage centers were observed throughout the year, but they were particularly abundant after the breeding season and during sex reversal in this protogynic eel.

PP-83

SPERM MODIFICATIONS IN INSEMINATING OSTARIOPHYSAN FISHES, WITH NEW REPORTS OF INSEMINATING SPECIES

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Prior to this report, only three ostariophysan fish groups were known to be inseminating, where the male introduces sperm into the female's ovary. These groups are the characiform subfamily *Glandulocaudinae*, the tribe Compsurini within the characiform subfamily *Cheirodontinae*, and the siluriform family *Auchenipteridae*. From our ongoing survey of gonad morphology in two of the ostariophysan orders, *Characiformes* and *Siluriformes*, we can now report insemination in seven additional species. Insemination is based on the microscopic demonstration of spermatozoa within the ovaries of mature females. The inseminating species of *Characiformes* are: *Brittanichthys axelrodi*, *Bryconamericus pectinatus*, *Cheirodon ortegi*, *Monotocheirodon pearsoni*, and two new genera and species of the characid subfamily *Tetragonopteridae*. Since the phylogenetic relationships of these species to other characiforms have not yet been determined, at least some of these species may represent independent acquisitions of this reproductive habit. Additionally, we found that the siluriform species, *Scoloplax dicra*, family *Scoloplacidae*, is also inseminating, this clearly representing a second independent occurrence of this habit within this order. To date, all externally fertilizing ostariophysan species that we have analyzed produce spermatozoa with spherical nuclei (aquasperm). The spermatozoa of the above inseminating species, as well as those of two inseminating species of the siluriform family, *Auchenipteridae*, that we also analyzed, *Trachelyopterus lucenai* and *T. galeatus*, produce spermatozoa with nuclei that range from slightly deformed to highly elongate. The possible selective advantages of modified sperm nuclei for the transfer of sperm to the female will be discussed.

PP-84

EFFECT OF CALCIUM AND IONIC STRENGTH ON THE MOTILITY OF TURBOT (*PSETTA MAXIMA*) AND SEA BASS (*DICENTRARCHUS LABRAX*) SPERMATOZOA

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Flagellar motility of spermatozoa is regulated both by intrinsic components and by external factors. The flagellar beating of sea bass and turbot spermatozoa which is triggered by contact with seawater, is described with modeling during the swimming period. The effect of ionic composition, osmolality and ionic strength on motility properties are studied *in vivo* (native spermatozoa) and *in vitro* (demembranated/ATP-reactivated spermatozoa). As a function of time post-activation, an asymmetry appears in the flagellar beat pattern of sea bass leading to a decrease of the trajectories diameter. Using demembraned flagella, it was shown that such asymmetry resulted from the increase of the intracellular calcium concentration. In turbot, no effect of calcium (1 nM to 5 mM) was observed. The reactivation of the beating of turbot axonemes observed from 150 up to 500 mM of K-Acetate showed a significant decrease of the wave number (compared to the control at 100 mM) of the portion of the flagellum presenting waves and the velocity. However, the flagellar beat frequency was not modified, except at 400 mM K-Acetate or above. For sea bass axonemes, the curvature, the amplitude and length of the wave were also significantly affected when increasing the K-Acetate concentration, as well as that of other ions. However, no change of the flagellar beating was observed by addition of glucose. As a consequence, the evolution of the wave pattern observed *in vivo* can be mimicked by only an increase of the ionic strength of the reactivation medium *in vitro*. The arrest of the flagellar beating *in vivo* is a result of the increase of the internal ionic strength which occurs during the swimming phase of turbot or sea bass spermatozoa.

PP-85

DYNAMICS OF CYCLINS AFTER THE INITIATION OF SPERMATOGENESIS IN A TELEOST, THE JAPANESE EEL (*ANGUILLA JAPONICA*)

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In cultivated eel testis, seminiferous tubules are filled with type A- and early B-spermatogonia. These spermatogonia begin to proliferate and enter meiosis by GTH via 11-ketotestosterone *in vivo* and in an *in vitro* organ culture system. These hormonal treatments eventually induce the formation of spermatozoa. After hormonal induction of spermatogenesis, the number of type A- and B-spermatogonia incorporating BrDU increases markedly within 1 day after hCG treatment. To clarify the molecular mechanism regulating the initiation of spermatogenesis, we isolated cyclin cDNAs from eel testis cDNA library. First, changes in expression pattern of B-type cyclins were examined in testes during the earlier phases of hCG-induced spermatogenesis. Northern blot analysis showed that cyclin B1 and B2 mRNAs appeared and increased after the initiation of spermatogenesis and further increased during spermatogonial proliferation. *In situ* hybridization showed that a few spermatogonia expressing cyclin B1 and B2 mRNAs are present in testes in the first few days after hCG treatment, with most spermatogonia exhibiting cyclin B1 and B2 mRNAs after day 3. Western analysis indicates that protein levels increased during spermatogonial proliferation and reached a plateau when the most advanced spermatogenic cells differentiated into spermatocytes. Immunoblot analysis after immunoprecipitation with anti-cyclin B1 and B2 antibodies indicated that the partner of both B-type cyclins was a cdc2 kinase, but not a cdk2 kinase. Analysis of H1 kinase activity of cyclin B1-cdc2 and cyclin B2-cdc2 during spermatogenesis showed that both displayed activity after the initiation of spermatogonial proliferation.

PP-86

GONADAL FORMATION AND DIFFERENTIATION IN A TELEOST, *TILAPIA NILOTICUS*

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In *Tilapia niloticus*, fertilized eggs grew and hatched out after 4 days at 26 °C. At 0 day after hatching (dah), primordial germ cells (PGCs), which are distinguishable from somatic cells morphologically, are located in the outer layer of lateral plate mesoderm around the hind gut. At 3 dah, PGCs are located in the gonadal anlage through the formation of the coelomic cavity in lateral plate mesoderm rather than through active migration of PGCs. To clarify the characteristics of germ cells during differentiation and gametogenesis, we cloned tilapia vasa homologue (vas) cDNA. Vasa is recognised as one of the germ cell determinants in *Drosophila*. Using this cDNA, we examined the expression of vas mRNA in germ cell stages from PGCs to gametogenesis. RT-PCR analysis showed that tilapia vas mRNA is expressed only in gonads but not in other tissues, e.g., brain, heart, liver, spleen and kidney. *In situ* hybridization for vas mRNA indicated that signals are present in migrated PGCs at 0 dah. PGCs continued to express vas mRNA after incorporation in the gonadal anlage. During oogenesis, vas mRNA was expressed strongly in stages from oogonia to diplotene oocytes. Thereafter, hybridization signals decreased in stages from vitellogenic oocytes to full grown oocytes, though vas mRNA was detected in these oocytes by Northern analysis. These results suggest that in tilapia, vas mRNA is expressed continually in stages from PGCs to full grown oocytes, though the degree and location of expression changes dramatically during oogenesis.

PP-87

ISOLATION AND CHARACTERIZATION OF RAINBOW TROUT VASA GENE AND ITS APPLICATION TO PRIMORDIAL GERM CELL'S MARKER

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In teleost, little is known about the molecular mechanisms of germ cells determination. To facilitate such studies on primordial germ cells (PGCs), techniques to purify PGCs will be required. Recently, Vasa has been detected in germ cells of a wide range of animals. Although the function of Vasa is still unclear, its distribution is restricted to germ cells, and can therefore, serve as a good marker molecule for PGCs. In this study, we have characterized trout vasa cDNA and gene, and produced transgenic trout carrying a green fluorescent protein (GFP) gene driven by rainbow trout vasa flanking sequences in order to identify PGCs *in vivo*. cDNA cloning was performed by degenerated- and RACE-PCR. The predicted amino acid sequence contained eight consensus sequences and five arginine-glycine-glycine repeats, the common character for known Vasa homologues. Overall amino acid similarity to *Drosophila* Vasa was 79.2%. Whole mount *in situ* hybridization for trout embryos revealed that signals were localized to the putative PGCs. Northern blot hybridization for RNA extracted from adult trout tissue demonstrated that Vasa transcript was abundant in gonad and was also detected in heart and brain. The vectorette-PCR was applied to amplify 5'- and 3'-flanking regions of vasa gene which is expected to contain cis-regulatory elements. These fragments were ligated to GFP gene (vasa-GFP) and were introduced into trout embryos by microinjection. These transgenic trouts showed green fluorescence in putative PGCs of early embryos. In conclusion, vasa is expressed abundantly in germ cells, making it a useful marker to distinguish PGCs in trout embryos. In addition, vasa-GFP is a powerful tool to identify and isolate live PGCs from trout embryos.

PP-88

ULTRASTRUCTURE OF THE GERM CELLS IN THE TESTIS OF MATRINXÃ, *BRYCON CEPHALUS* (GUNTHER, 1869) (TELEOSTEI, CHARACIDAE)

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Matrinxã is a native teleost of economical importance in Amazonic basin (Brazil). This is a very popular fish for sport fishing, because it fights hard when hooked, what is very attractive for the anglers, bringing more people to the practice of this activity. For this study we stocked matrinxã from March 1994 to February 1996 as 1 fish/m^2 in four 200 m^2 tanks, at the "Centro de Pesquisa em Aquicultura do Vale do Ribeira" -CEPAR (Ribeira Valley Acquaculture Research Center), from Fisheries Institute in Pariquera-Açu, São Paulo, Brazil. Fragments of the testes were fixed in 3% glutaraldehyde in 0,1M phosphate buffer (pH 7,2) for 18 hours, at 4°C, and postfixed in 1,0% osmium tetroxide for two hours. Samples were processed according to routine techniques for electron microscopy. The testis of matrinxã is composed of 2 compartments: (1) the seminiferous compartment, containing the cystic cells (Sertoli) and the germ cells, and (2) the interstitial compartment. Four stages of germ cells can be distinguished: spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa. Those cells are located inside the tubules. The cystic cells are located in the intratubular compartment forming the wall of the cysts. They are always associated to the germ cells, but with distinct characteristics, suffering modifications during all the reproductive cycle. The interstitial compartment is located outside the tubules, containing elements from the connective tissue (fibrocytes, collagenous fibers, myoid cells), the basal membrane, the insterstitial cells or Leydig cells, and blood vessels.

PP-89

SPATIAL AND TEMPORAL EXPRESSION OF SF-1 (STEROIDOGENIC FACTOR-1) DURING EMBRYOGENESIS IN ZEBRAFISH, *DANIO RERIO*

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The orphan nuclear receptor, steroidogenic factor-1 (SF-1), plays an important role in steroidogenesis and is also an essential factor in the development of adrenals and gonads. SF-1 regulates the expression of Anti Müllerian Hormone, which initiates the regression of the Müllerian ducts. So far research on function of SF-1 has been concentrated on mammals. The mammalian FTZ-F1 gene codes for SF-1 and a truncated splicing variant, embryonal long terminal repeat-binding protein (ELP). While the distribution and function of ELP is still uncertain, much more is known concerning SF-1. The regulation and function of SF-1 in fish is however more unclear. We have investigated the temporal and spatial expression of the zebrafish homolog to SF-1 (zFF1A) and its splicing variant (zFF1B) during embryogenesis. Several developmental stages between fertilisation and hatching have been examined and mapped using RT-PCR and whole mount *in situ* hybridisation. The SF-1 homologs were expressed in tissues corresponding to those of the developing somites, pronephric duct, pituitary, mandibular arch, hind-brain and liver. These results indicate a wider tissue distribution of the SF-1 homologes, during zebrafish embryogenesis than previously observed.

PP-90

CHANGES IN LEVELS OF GROWTH-RELATED HORMONES AND TESTICULAR IGFS mRNA DURING THE FIRST GONADAL RECRUDESCENCE IN RAINBOW TROUT

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We analyzed changes in levels of growth-related hormones in relation to the precise gametogenic stage and steroid hormone levels in an autumn spawning strain of trout. At the beginning of gonadal development we observed significant and transitory increases in T_3 and T_4 levels, coincident with a transitory increase in estradiol in both sexes. At later stages, T_3 levels decreased during vitellogenesis and rapid testicular growth. Plasma concentrations of growth hormone generally remained low (<1 ng/ml); the highest levels were found in the initial stages of vitellogenesis and in spermiating males. IGF I was measured only in male plasma, and significantly increased from January to July and between stages I and IV of spermatogenesis. During this same period, GH concentration, liver GH receptors and liver IGF I mRNA levels were stable or tended to decline. Testicular IGF I and II expressions were detectable in the «immature» gonad and remained at the same level during the initial stages of spermatogonial mitosis up to the initiation of meïosis (stages II and III). Both transcripts increased in stage IV of testis development (all meïosis steps and first appearance of spermatozoa), then remained stable or slowly increased until spermiation. Relative amounts of IGF I and IGF II mRNAs were positively correlated ($r=0.87$; $p<0.001$) and the levels of these transcripts were analysed with reference to other endocrine parameters. Changes in testicular IGF mRNAs appeared related to GtH1 and androgen plasma levels rather than to circulating GH changes or GH receptor concentration in the testis. Possible involvment of these hormones in gonadal physiology will be discussed.

PP-91

CHARACTERIZATION OF ANDROSTENEDIONE AND 17β-ESTRADIOL METABOLISM DURING THE CRITICAL PERIOD OF SEX DIFFERENTIATION IN THE TILAPIA *OREOCHROMIS NILOTICUS*

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Previous studies have shown that *Oreochromis niloticus* has endogenous sex steroids prior to gonadal development, and that the sex differentiation process of *O. niloticus* can be influenced by the exogenous application of steroid agonists. To better understand the role of sex steroids in gonadal development we have examined steroid metabolism of whole animal preparations of *O. niloticus* during a proposed critical period of development, encompassing the earliest stages of gonadal differentiation. Homogenates from mono-sex populations, including XX, XY and YY genotypes, of 8-14 day post-fertilization (dpf) fish were incubated with ^{3}H-androstenedione or ^{3}H-17β-estradiol. Metabolites extracted from the organic phase were identified by thin-layer chromatography, microchemical reactions and recrystallization. Androstenedione was metabolized into at least seven readily identifiable compounds including 5β-androstane-3α,17β-diol and testosterone. There was no production of 17β-estradiol from androstenedione during this time period. 17β-Estradiol was metabolized into at least five metabolites including estrone and several unknowns. Based on time-course accumulation patterns some of these unknowns are believed to be metabolites of estrone. Both the type of metabolites produced as well as the quantity of specific metabolites changed from 8 to 14 dpf. However, under these incubation conditions there is no discernible difference in the steroid metabolism capabilities based on genotype.

PP-92

THE IGF SYSTEM IN GONADS OF AN HERMAPHRODITIC SPECIES, *SPARUS AURATA*

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Intragonadal IGF system exists in fish, as in higher vertebrates. The present study analyzes the expression of IGF-I, IGF-II and IGF-1R in gonads of a protandrous hermaphroditic fish: *Sparus aurata*. IGF-I and IGF-II expression was studied in gonads of different developmental stages by RT-PCR and Northern blot. mRNA levels were highest in bisexual gonads and decreased during gonadal development. IGF-II mRNA levels were higher than IGF-I, regardless of the stage of gametogenesis. IGF-1R RNA analyzed by RT-PCR and by Northern blot was expressed in gonads at all stages studied. Several transcripts were detected when gonadal RNA was hybridized with a cDNA clone coding for the N-terminal of *Sparus* IGF-1R. A major transcript of 11 kb was found in gonads and also in gill arch and brain but not in liver and muscle. Cellular localization of IGF-I, using a homologous antibody, revealed irIGF-I in chromatin-nucleolus stage oocytes and in granulosa cells of vitellogenic and late vitellogenic follicles. In testis, irIGF-I was found in somatic cells surrounding the cysts, interstitial cells and spermatogonia A. Use of homologous antibodies to two types of IGF-1R demonstrated positive reaction in theca cells of primary follicles and in theca and granulosa cells of vitellogenic and late vitellogenic follicles. In the testis, a positive reaction was identified in spermatogonia A, spermatocytes II and in spermatids for the germ cells and also in somatic cells surrounding the cysts as well as in interstitial cells. The local expression and production of IGFs and their receptors in fish gonads supports a role for the IGF system in gonadal physiology.

PP-93

HISTOLOGICAL DETERMINATION OF SEXUAL DIFFERENTIATION IN ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS* L.)

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Knowledge of the timing of sexual differentiation in fish is very important in the application of hormones for production of monosex populations in aquaculture. This is determined by histological examination of the primordial gonads and their development toward testes and/or ovaries. Samples of Atlantic halibut (*Hippoglossus hippoglossus* L.), ranging from hatch to post-settlement, were histologically analysed for gonadal formation and development. This occurs in two stages: cytological and anatomical differentiation. The former involves the differentiation of oogonia and oocytes or spermatogonia and spermatocytes, while the latter involves structural changes of the undifferentiated gonadal primordia into testes or ovaries, including formation of an ovarian cavity. Such characteristics are discussed with respect to body size, which is a more accurate indication of development than age. Once this is determined, the application of hormones prior to sexual differentiation (sexually labile period of development) can produce monosex populations, which is of great interest in aquaculture of halibut, since females have greater growth capacity and later maturation than males.

PP-94

INHIBITION OF AROMATASE ACTIVITY INHIBITS HIGH-TEMPERATURE FEMINISATION OF GENETIC MALE NILE TILAPIA, *OREOCHROMIS NILOTICUS*

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Temperature-dependent sex determination (TSD) has been observed in several fish species. However, how temperature acts on the process of sex determination in fish is obscure. Recently, it has become apparent that the Nile tilapia (*Orechromis niloticus*), which has an XX/YY chromosomal genetic sex determination mechanism, also exhibits TSD at high temperature (approximately 34-37°C). The relationship between temperature and sex determination in this species appears to be complex, with some genetic males developing as phenotypic females and some genetic females developing as phenotypic males at high temperature. Aromatase activity has been demonstrated to be important in the development of genetic females as phenotypic females at lower temperatures. It has been demonstrated in certain reptiles that aromatase, which converts estrogens into androgens, is active in the developing gonads at female-determining temperature but not male-determining temperatures. A series of experiments was designed to study the role of aromatase activity in high temperature TSD in this species. Separate single sex batches of YY, XY or XX fry were exposed to two different rearing temperature (28 or 36°C) during the period of sexual differentiation with or without a chemical aromatase inhibitor. YY groups showed a significant percentage of feminisation at the higher temperature, which was suppressed by the aromatase inhibitor. These results imply that aromatisation is mechanistically associated with TSD in this species.

PP-95

HISTOLOGICAL OBSERVATIONS ON THE GONAD OF HONEYCOMB GROUPER, *EPINEPHELUS MERRA*

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Grouper is generally known as a protogynous teleost. However, little is known about the process of gametogenesis and sex change. In the present study, we examined the gonadal structure of honeycomb grouper, *Epinephelus merra*, to understand the process of sex change. Moreover, we investigated the morphological change in the gonad of 17α-methyltestosterone (MT) pellet implanted fish. The females ranged from 2 to 6 years old and males from 4 to 8 years old. Some of the fish between 4 and 6 years old had oocytes of perinucleolus stage in the testes. There were small cysts of spermatogonia, spermatocyte and spermatid in the ovary of all females. However, the cysts of spermatozoa were not observed in the ovary. In our experiment, sex change from female to male was induced by MT. One month after the implantation of MT, the cysts of male gametes increased in number. After three months, the cysts of spermatozoa were observed in the gonad, while oocytes disappeared from the gonad. MT induced sex changes in the fish of various ages, including 2 to 3 years old that do not undergo sex change in natural condition. The honeycomb grouper may undergo sex change from female to male between 4 to 6 years old in natural condition already. However, they may have the ability of sex change even at a younger age of 2 to 3 years old and this could be induced by some factors such as hormone treatment.

PP-96

GONADAL STEROIDOGENESIS DURING ESTROGEN-INDUCED SEX REVERSAL IN SEA BREAM, *SPARUS AURATA*

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The seabream is a protandrous hermaphrodite undergoing sex reversal when administered estrogen at the appropriate concentration and length of time. In order to study the changes in steroidogenesis during sex change, male seabream were fed two different concentrations of estradiol-17β and their gonads incubated for different periods of time with 1.2Ci 4-pregnen-17-ol-3-one [1,2,6,7-^{3}H] in 1 ml ringer solution at 1, 6 and 14 weeks of experiment. Free and conjugated fractions of incubation products were isolated and identified by HPLC and TLC (normal and reverse phase) before and after microchemical reactions. Recovery of metabolites from incubates decreased with longer incubation periods, in particular in feminised fish and towards the end of the experiment. Incubations with dissected portions of ovarian and testicular tissue in intersex gonads showed that this could be largely attributed to type of tissue. The results indicate that unidentified metabolites remained in the water phase after solvent extraction and were being progressively synthesised as ovarian tissue developed. Desmolase and 5β-reductase were the most active enzymes, especially in male tissue. 3α-hydroxysteroid dehydrogenase and 11β-hydroxylase showed preferential activity in testicular tissue. A lower amount of 6α/β-hydroxylation was also detected, with a peak at 6 weeks, but did not seem to be related to sex.

Gametogenesis and Sex Differentiation:
Oogenesis

OP-35

UNSCRAMBLING THE EGG: CELLULAR, BIOCHEMICAL, MOLECULAR AND ENDOCRINE ADVANCES IN OOGENESIS

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An oocyte autonomously makes most of the machinery for DNA and protein synthesis, as well as mRNA needed immediately after fertilisation for the developing embryo. In fish, as in other oviparous vertebrates however, specialised egg constituents, such as yolk and some egg coat substances, are synthesised outside the ovary and have to be transported to and sequestered by the oocyte. Many (if not all) of the processes in the co-ordinated assembly of an oocyte involve an interplay of endocrine, paracrine and autocrine control. Knowledge of the processes governing the synthesis of an oocyte is central in our understanding of what makes a viable egg. This keynote paper focuses on the recent advances in the cellular, biochemical, molecular and endocrine biology of oocyte growth in fish. Vitellogenesis, when yolk is provisioned in the oocyte, and zonagenesis, where the 'egg shell' is formed, are topics in oogenesis that have received much of the recent focus. Recently the cDNAs for vitellogenin (VTG), the VTG receptor (VTGR), zona radiata proteins (ZRPs) and various yolk processing enzymes (cathepsins and lipoprotein lipase) have been cloned and sequenced. Recent advances in our knowledge of the endocrine control of oogenesis are limited, but there is now strong evidence for the involvement of follicle stimulating hormone (FSH - GtH I) in oocyte growth. Further evidence has been presented to support roles of insulin-like growth factors and activin/inhibins in oogenesis in fish, but an oocyte-specific growth factor(s) has yet to be identified. Our knowledge on the mechanisms controlling oocyte recruitment in fish, as in all other animals is still minimal. Some features of ovarian physiology, including zonagenesis, vitellogenesis, formation of an ovarian cavity, and the presence of oocytes in the 'testis' of 'male' fish are now key biomarkers for studies on environmental oestrogens.

OP-36

NEW APPROACH TO FISH OOCYTE VITELLOGENESIS

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The oocyte of oviparous vertebrates accumulates the nutritional reserves of the egg during vitellogenesis. These reserves allow the development of the embryo and of the larvae until it can obtain a food supply unaided. These reserves, the yolk, mainly originate in fish from the incorporation of vitellogenin (VTG) and lipids in the oocyte. It is now well established that VTG is a glycolipoprotein precursor of the yolk, synthetized by hepatocytes under estrogenic stimulation. It is secreted in the blood stream and selectively sequestered by growing ovarian follicles via specific oocyte receptors. However, it is not yet demonstrated that lipids have an extra-oocyte origin. Nevertheless, from the data found in the literature we hypothesize that lipids enter the ovarian follicle as fatty acids deriving from VLDL and accumulate in the oocyte as triglycerides in lipid globules. So, the yolk could be the result of exogenous material processed within the ooplasm to provide easily available nutrients for the embryo. Ultrastructural observations on numerous fish species clearly show that endocytosis of the main components of the yolk occurs at the same time. Nevertheless, two successive phases can be distinguished: (i) a type I vitellogenesis when lipid accumulation predominates over incorporation of VTG which is very low and can only be detected by electron microscopy; (ii) a type II vitellogenesis when endocytosis of VTG is largely predominant over lipid uptake. Recent studies underline the importance of cellular tools being set up during previtellogenesis to prepare the endocytotic activity of the oocyte during vitellogenesis.

OP-37

GLUCOCORTICOIDS INHIBIT THE EXPRESSION OF ESTROGEN RECEPTOR (RTER) AND VITELLOGENIN (VG) IN THE LIVER OF RAINBOW TROUT

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In fish, stress is known to affect some reproductive parameters like vitellogenin and GTHs plasmatic levels. In the liver of trout, it is known that estradiol (E_2) increases rtER mRNA and subsequently the rtVg mRNA levels (Flouriot et al, J. Cell Science, 105 : 407-416, 1993). Here, we have studied the effect of cortisol or dexamethasone (dex) on both rtER and rtVg mRNA levels. In previtellogenic trout, we have shown that cortisol implants (5 days) strongly decreased both rtER and rtVg mRNA amounts. Moreover in hepatocyte cultures, dex inhibits both basal and E_2-stimulated rtER mRNA levels. In the same aggregate cultures treated with actinomycin D, the stability of rtER messengers was not affected by dex showing that this hormone likely exerts a transcriptional effect which was confirmed in co-transfected CHO cells with the rtER promoter and the glucocorticoid receptor (rtGR). First, we showed that dex inhibition is specific of the rtER promoter, and is mediated by the rtGR. We then determined the promoter region involved in dex inhibition on the rtER promoter. This region, named "FP3" is protected in footprint experiment by trout liver nuclear extracts, and contains an Oct-1 -like sequence (Lazennec G. et al, Molecular Endocrinology, 10 : 1116-1126, 1996). Gel shift experiments demonstrated that the rtGR inhibits the binding of one or several proteins on the FP3 region. Although Oct-1 was expected to be involved in this complex, the use of specific Oct-1 antibodies invalidate this hypothesis. Although the implicated factors remain to be identified, this study shows that the rtGR interfers with the expression of the rtER in the liver and possibly other organs.

OP-38

OOCYTE HYDRATION AS A KEY FEATURE IN THE ADAPTIVE EVOLUTION OF TELEOST FISHES TO SEAWATER

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The fossil record shows that the ancestors of teleost fishes lived in freshwater for about 250 million years before returning to the sea during the Jurassic period. The hyposmotic blood of extant marine teleosts is assumed to reflect this freshwater origin. About 100 million years ago a sudden differentiation of the teleosts occurred with a burst of new marine species evolving. This transition from freshwater to the sea demanded certain osmotic adaptations, especially for the embryos since they lack the organs responsible for osmoregulation. In extant marine teleosts that spawn pelagic eggs, a large pool of free amino acids (FAA) is generated in the oocytes by hydrolysis of a ~100 kDa yolk protein, and this provides the major part of the osmotic drive for the water uptake into the oocyte. Intriguingly, this pool of FAA is remarkably similar regardless of the taxonomic position of the species, implying that the hydrolysed fraction of the yolk protein is evolutionary conserved. This yolk protein is a fragment of the N-terminal end of a derivative of vitellogenin. A comparative aspect of our research is revealing how widespread this mechanism is among extant marine teleosts, and whether remnants of the mechanism can be found also among fresh water fishes. Our latest findings will be reported. In our opinion, the establishment of the yolk protein hydrolysis at final oocyte maturation with the resulting increase in the FAA pool and oocyte hydration was a key step in teleost evolution that gave rise to their successful differentiation in the oceans about 100 million years ago.

OP-39

ROLE AND REGULATION OF GAP JUNCTIONS DURING OOCYTE MATURATION IN TELEOSTS

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There are two opposing hypotheses concerning the role of gap junctions (GJ) during maturation of the vertebrate ovarian follicle. Simply stated, GJ are necessary either to (1) maintain meiotic arrest or to (2) induce meiotic resumption of the oocyte. Our goal is to resolve this controversy using teleosts as our experimental models. Ultrastructural studies with Atlantic croaker and red seabream revealed that heterologous (granulosa cell-oocyte) and homologous (granulosa cell-granulosa cell) GJ in full-grown follicles increase during the maturation-inducing hormone (MIH)-independent early stage of oocyte maturation. Gonadotropin markedly enhances heterologous and homologous GJ concomitantly with the acquisition of oocyte maturational competence (OMC) in follicles of both species, and insuling-like growth factor-I has the same effects in seabream follicles. In both species, GJ seem to reach their highest levels at the time of germinal vesicle migration and onset of MIH production. Moreover, in croaker follicles the mRNA for the GJ protein, Cx32.2, as well as OMC are stimulated by gonadotropin and protein kinase A (PKA) activators and suppressed by PKC activators. Finally, ultrastructural and functional (dye-transfer) experiments indicated that heterologous GJ in croaker follicles remain operational well beyond the time at which the oocyte irreversibly commits to resume meiosis (during the MIH-dependent late stage of maturation). Our observations are compatible with the hypothesis that heterologous GJ are an integral component of the MIH signaling mechanism that activates the resumption of meiosis in intact ovarian follicles.

OP-40

APOPTOTIC CELL DEATH IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AND GOLDFISH (*CARASSIUS AURATUS*) OVARIAN FOLLICLES *IN VIVO* AND *IN VITRO*

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Apoptosis has been identified as the molecular mechanism responsible for follicular atresia in mammalian and avian species. This cell death process is under the control of a variety of hormonal factors. We have initiated studies to examine the role of apoptosis in follicular atresia of teleost fish, and the role of hormones and growth factors in modulating this process *in vitro*. Ovarian follicles from rainbow trout and goldfish were isolated and either frozen in liquid nitrogen or incubated for 24-72 h in serum-free medium. Total genomic DNA from the follicles was extracted, 3'-end labeled with 32P-ddATP, size-fractionated by agarose gel electrophoresis and examined for evidence of oligonucleosomal fragmentation, indicative of apoptosis. Vitellogenic follicles of goldfish showed higher levels of apoptosis than preovulatory follicles *in vivo*, whereas preovulatory follicles of rainbow trout had significantly greater quantities of fragmented DNA than both vitellogenic and preovulatory goldfish follicles. Visibly atretic goldfish follicles did not display elevated levels of apoptosis as compared to healthy follicles, suggesting that follicular DNA fragmentation is an early event in atresia. Incubation (24-72 h) in serum-free medium did not induce significant increases in fragmented DNA in goldfish follicles at either stage of development, but resulted in a large increase in DNA fragmentation in preovulatory follicles of rainbow trout. *In situ* labeling of fragmented DNA in paraformaldehyde-fixed trout vitellogenic follicles sections localised apoptosis to the thecal cell layer. Results suggest species- and developmental stage-specific differences in apoptotic cell death in teleost ovarian follicles.

PP-97

OESTROGEN REGULATED HEPATIC GENE EXPRESSION

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Several studies have shown effects of oestrogenic substances on endocrine and reproductive systems in wildlife. Measuring plasma vitellogenin (VTG) is a commonly used method for determining exposure to oestrogenic substances in fish. There is, however, a growing need for additional sensitive and accurate methods for determining the oestrogenicity of substances *in vivo*. Besides VTG, other hepatic genes regulated by oestrogens include the vitelline envelope proteins (VEPs) and the oestrogen receptor (ER). The ER and the VEPs might be alternative for VTG when determining the oestrogenicity of a substance. In this study we show that VEP mRNA and ER mRNA exhibit earlier induction than VTG mRNA following injection of juvenile Arctic char (*Salvelinus alpinus*) with a single dose of 17β-estradiol (E2). These results indicate that this group of proteins have a higher sensitivity for E2 than VTG and therefore would be more suitable as markers of oestrogenicity. We also show indications of an early and sex-independent expression of VEP-β in oestrogen unchallenged juvenile Arctic char. The regulatory mechanisms of VEP-β are of great interest due to the high sensitivity of VEP-β to oestrogen induction and also due to the apparently sex-independent expression in juvenile Arctic char.

PP-98

EFFECTS OF LONG TERM 17β-ESTRADIOL TREATMENT ON THE GROWTH AND PHYSIOLOGY OF FEMALE TRIPLOID BROOK TROUT (*SALVELINUS FONTINALIS*)

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Triploid female fish are unlike normal diploids in that they show delayed oocyte growth and are unable to produce viable offspring. In an attempt to accelerate rates of oocyte growth, female triploid brook trout were fed a diet of pelleted food sprayed with 17β-estradiol (E2) (30mg E2/kg feed) for five months prior to ovulation in diploid females. Lengths, weights and blood samples were taken each month and any morphological changes were recorded for triploid treated females (n=11), triploid control females (n=14) and diploid female (n=12) groups. Growth rates of the triploid treated group (1.84 + 1.46 g/day) were not significantly different from diploids (2.30 + 0.80 g/day), but were significantly less than triploid controls (4.25 + 0.74 g/day). The hematocrits of the triploid treated group fluctuated at a significantly lower level (25.5 + 5.0 - 30.4 + 10.0%) than the diploid and triploid controls (39.0 + 4.5 - 48.8 + 2.3%). Blood hemoglobin levels for the triploid treated group remained significantly lower than the diploids, but both showed similar rates of decline over time. In contrast, hemoglobin levels for the triploid controls remained fairly constant over time, and were not significantly different from the diploids. Secondary sex characteristics typically associated with maturing diploid females (skin darkening, gonadal pore enlargement) were apparent in the triploid treated group following three months on treated food. In diploids, plasma estradiol levels peaked four to six weeks prior to ovulation. Plasma estradiol of the triploid treated and control groups were significantly lower than the diploids during this time. Plasma estradiol levels were consistently higher in the triploid treated compared to the control groups, but the difference was not significant. Triploid treated (n=5) and control (n=7) fish sacrificed after the study period showed no differences in number or diameter of developing oocytes. Thus, long term estradiol treatment of triploid females fed 30mg E2/kg feed was not sufficient to raise plasma estradiol levels comparable to maturing diploid females, nor induce accelerated rates of oocyte growth.

PP-99

A SIMPLIFIED *IN VITRO* CULTURE TECHNIQUE FOR ANALYSIS OF VITELLOGENIN GENE EXPRESSION IN LIVER SLICES OF JAPANESE EEL, *ANGUILLA JAPONICA*

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In maturing female teleosts, egg yolk precursor proteins (vitellogenin; VTG) are produced in response to gonadotropin-induced ovarian estrogen. To analyze the details of the regulation of VTG synthesis, *in vitro* hepatic cell culture systems have been applied in many cases. Cell culture, however, requires a lot of time and many processing steps, while protease treatment may cause the decrease or loss of some cell functions. In the present study, a simplified *in vitro* culture system, using liver slices, was used to analyze the endocrine control of VTG gene expression in Japanese eel, *Anguilla japonica*. Slices of 500 μm in thickness and about 50 mg in weight were prepared from livers of immature male eels by a tissue chopper, and incubated for 7 days at 20°H under 100 % air and continuous shaking in L-15 medium containing 0.5% bovine serum albumin in the presence or absence of estradiol-17β (E2) at 10-8 to 10-6 M. Incubated tissues were used for histological observation and for measurement of VTG mRNA levels by RT-PCR using primers that amplified two types of VTG cDNA from Japanese eel liver. Hepatic cells appeared healthy throughout the incubation period which lasted for at least 3 days. By this time, VTG mRNA was detected in the tissues in a dose-dependent manner in response to E2, providing further support for the potential of our incubation system in future studies. Separate measurement of two types of VTG mRNA is currently in progress.

PP-100

A HISTOLOGICAL DESCRIPTION OF THE POST-OVULATORY FOLLICLES IN *LOLIGO VULGARIS REYNAUDII*

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Post-ovulatory follicles (POF) in the ovaries of the chokka squid *Loligo vulgaris* reynaudii were classified into five stages using histological criteria. The main criteria used were the degree of hypertrophy of the granulosa and thecal layers, vacuolisation of the cytoplasm and nuclear pycnosis on the granulosa and vascularity and the presence of fibroblasts and collagenous fibers on the theca. The frequency of occurrence of the post-ovulatory follicles in the ovaries confirmed serial spawning in this species. A future study will investigate the rate of resorption of the POF's, which will hopefully provide us with the number of eggs spawned per batch as well as the total number of eggs deposited. This information will assist in managing the jig fishery for this species, most of which concentrates on actively spawning animals.

PP-101

IMMUNONEUTRALISATION OF FSH CAUSES A SUPPRESSION OF VITELLOGENIC GROWTH OF OOCYTES IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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In salmonids, two gonadotrophins (FSH and LH) are involved in the control of the ovarian growth and maturation and are released by the pituitary into the circulation at specific times during the reproductive cycle. The function of LH in oocyte maturation has been clearly established in fish, but the role(s) of FSH is less certain. In this study passive immunoneutralisation was employed as a technique to investigate the role of FSH in ovarian development in the rainbow trout (*Oncorhynchus mykiss*). Female rainbow trout were injected with antibodies against the β subunit of salmonid FSH at regular intervals over a two month period during early vitellogenesis and the effect(s) on ovary growth and oocyte development were subsequently determined. The immunoneutralisation technique employed resulted in a reduction in the amount of circulating FSH. In fish treated with antibodies against FSH, the ovary grew at a slower rate compared with the controls; the resulting vitellogenic oocytes also appeared to be smaller (by volume) than in the controls. The results obtained support the hypothesis that FSH is involved in vitellogenic growth of oocytes. More studies of this nature are needed to establish more fully the involvement of FSH in oogenesis at the different stages of ovarian development.

PP-102

MOLECULAR CHARACTERISATION OF ENZYMES MEDIATING YOLK PROCESSING IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*): CATHEPSINS AND LIPOPROTEIN LIPASE

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Despite the fundamental importance of yolk storage and mobilisation in the synthesis of a viable embryo, very little is known about these processes in fish. Cathepsins are lysosomal endoproteases that appear to play an important role in both the processing of vitellogenin (VTG; the major precursor of yolk) and also subsequently in the mobilisation of this yolk during embryogenesis. Lipoprotein lipase (LPL) is likely to be important in lipid dynamics in the oocytes. Cathepsin and LPL, therefore, are key enzymes in the vitellogenic growth phase of oocytes and in embryogenesis. Molecular studies have been initiated to characterise cathepsins (B and L; we have already sequenced cathepsin D), and LPL in the rainbow trout, with a view to elucidating their developmental expression during yolk processing. Degenerate primers were designed for cathepsin B and LPL, based on highly conserved regions of the target sequences in various mammals and in the chicken. For cathepsin B, two fragments of the trout sequence (552 bp and 379 bp in length) have been amplified by PCR and they have between a 63% and 73% identity with their mammalian and chicken counterparts. For trout LPL, two fragments of the sequence have also been amplified (799 bp and 475 bp in length) which have between a 68-73% identity with mammalian and chicken LPLs, and 76-78% with the partial sequence for zebra fish LPL. We are now in the process of obtaining the full sequences for cathepsin B and LPL, and for cathepsin L.

PP-103

DEVELOPMENT OF A SURGERY PROTOCOL FOR UNILATERAL OVARIECTOMY (ULO) ON COD (*GADUS MORHUA*)

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Unilateral ovariectomy (ULO) has been applied in experiments on amphibians and fish to study the regulation of fecundity. The application of ULO on cod (*Gadus morhua*) required modifications to a surgical procedure previously used on trout (*Oncorhynchus mykiss*). Electronically-tagged first-time spawners of artificially-reared Arcto-Norwegian cod were used for the experiment. Each fish was inhalation-anaesthetised using benzocain and placed upside-down in a cradle. Throughout the surgical procedure the gills were irrigated with sea water containing anaesthetic. The abdomen was opened, and the right ovary removed. Silk was used for the internal sutures, whilst the external sutures were of monofilament: the placement of the latter was critical. An injection of 10 mg/kg enrofloxacin (Baytril®) was used to prevent infections in the wound. A blood sample was taken from the caudal vein. Recovery from deep anaesthesia was sometimes rather slow, necessitating a combination of forced ventilation of the gills and stimulation of the heart by massaging the cardiac area. All the fish recovered from the anaesthetic. The best time to remove the stitches seemed to be after approximately 4 weeks, when in most cases the wounds had healed satisfactorily. The protocol proved to be a useful experimental tool for investigating oocyte recruitment mechanisms in cod and preliminary data indicate that compensation occurred.

PP-104

INVOLVEMENT OF DUAL-VITELLOGENIN SYSTEM IN THE CONTROL MECHANISM OF EGG BUOYANCY IN BARFIN FLOUNDER AND WALLEYE POLLOCK

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Structural changes of two lipovitellins (Lvs) derived from two forms of vitellogenin (Vg), Vg A and Vg B, were examined during vitellogenesis and oocyte maturation in barfin flounder (*Verasper moseri*). Two Lvs, vLv A and vLv B, were identified electrophoretically and immunologically in post-vitellogenic oocytes. Each appeared to be comprised of distinct heavy-chains (vLvH A and vLvH B) and light-chains (vLvL A and vLvL B) by SDS-PAGE analysis. Results from N-terminal amino acid sequencing and Western blotting using antisera to vLvH A and vLvH B verified the existence of two Vg polypeptides, which give rise to vLvH A-vLvL A and vLvH B-vLvL B, respectively, in serum from estrogen-treated fish. During oocyte maturation in barfin flounder, native dimeric vLv B was cleaved into a native monomer (oLv B). Meanwhile, vLv A was extensively cleaved including complete degradation of vLvH A into free amino acids. Calculated proportional ratio of degraded part of Vg polypeptides were 87% in Vg A and 39% in Vg B. The quantitative ratio, 4 : 6, of vLv A to vLv B was constant among post-vitellogenic oocytes from seven females. The similar pattern of yolk proteolysis during oocyte maturation was also observed in walleye pollock, *Theragra chalcogramma*. From these results, we suggest that the quantitative ratio of incorporated Vg A to Vg B in post-vitellogenic oocytes regulates the buoyancy of the spawned pelagic eggs by controlling availability of free amino acids which function as osmotic effectors during oocyte hydration in some marine teleosts.

PP-105

MORPHOPHYSIOLOGICAL CONDITION OF PREVITELLOGENIC OOCYTES OF SOME SPECIES FISH IN VARIOUS TEMPERATURE

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We investigated morphology (light and electron microscopy) of previtellogenic oocytes of 5 species *Cyprinidae* living in considerable temperature fluctuation, and rainbow trout (*Salmonidae*) constantly living in low temperature. *Cyprinus carpio*, *Hemiculter leucisculus*, *Hypophthalmichthys molitrix*, *Rhodeus ocellatus*, *Pseudorasbora parva* and *Parasalmo mykiss* (= *Salmo gairdneri*) were the objects of investigation.We observed dense or more intensive coloured patches of cytoplasm of oocytes of cyprinid fishes from cold (40) water using light microscope. Electron microscope observation revealed that these patches consisted of groups of cytoplasmic organelles: mitochondria, endoplasmic reticulum, the Golgi complex. The structure of cytoplasm of oocytes of fishes from warm water (250) seemed homogeneous under light microscope because under these conditions cytoplasmic organelles did not form groups. Group localization of organelles, observed in oocytes in low temperature, could be connected with the decrease of metabolic processes both of fishes and of oocytes.The effect of temperature of water on oocytes of Cyprinidae was studied both in natural and in experimental conditions. Analogous structures of trout consisted of fibrogranular material, but not cytoplasmic organelles. Cytoplasmic structures in oocytes of salmon fishes were observed all the year round. Probably, it was connected with their living in cold water.

PP-106

MOLECULAR CHARACTERIZATION OF THE WINTER FLOUNDER OOCYTE VITELLOGENIN RECEPTOR

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In oviparous animals, the major egg storage protein, vitellogenin, is synthesized in the liver, secreted into the bloodstream and taken up by an oocyte-localized receptor for processing and deposition into the egg yolk. To better understand the recognition of vitellogenin by its receptor (oocyte vitellogenin receptor or OVR), we have cloned and sequenced a full-length OVR cDNA. The winter flounder OVR is >70% identical to those of rainbow trout, chicken and *Xenopus* as well as mammalian VLDL receptors. The winter flounder OVR gene appears to be expressed as two different splice variants, with or without a 25 amino acid segment. In mammals and birds, this segment is rich in serine and threonine and is highly O-glycosylated, but the winter flounder sequence has only one serine and no threonines and instead is proline-rich (12 residues). While northern blots detect OVR expression only in the ovary, RT-PCR experiments demonstrate that the short form (without the 25 aa region) is highly expressed in ovary and is the more abundant form in testis and brain. The long form is preferentially expressed in muscle and heart. Immunoprecipitation of *in vitro* translated protein confirmed the identity of the OVR cDNA. The OVR protein was detected in detergent extracts of ovary membranes by western and ligand blots. Antibodies to chicken OVR and mammalian VLDLR both recognize the 95 kDa winter flounder OVR. OVR ligands include vitellogenin and receptor associated protein, but not lipoproteins of the very low, low and high density classes. Further characterization of the interaction of vitellogenin with OVR will be discussed.

PP-107

BOTH OVARY AND LIVER CONTRIBUTE TO THE SYNTHESIS OF THE WINTER FLOUNDER EGGSHELL

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Fish eggshells contain two highly conserved proteins that are homologous to mammalian ZP2 and ZP3 proteins. cDNAs encoding these proteins have been characterized from a limited number of fish species. Interestingly, there seem to be differences in the tissue in which these genes are expressed. In higher vertebrates (mammals, birds, amphibians) and some fish (goldfish, carp) both genes are expressed in the ovary. In medaka, both genes are expressed only in the liver. Immunological studies of eggshell proteins in plasma suggest that this is also the case for a number of other fish species. In winter flounder, the ZP2 gene has been shown to be expressed exclusively in the liver (Lyons et al., J Biol. Chem. 68: 21351-8, 1993). We have characterized a winter flounder cDNA encoding the ZP3 protein and investigated its expression. The ZP3 cDNA was isolated from a winter flounder ovary library during a random screen for expressed sequence tags. The cDNA is 1622 bp in length and encodes a 503 amino acid protein with a calculated molecular mass of 55.7 kD. The winter flounder ZP3 differs from other fish ZP3 sequences in that it is 40 - 70 amino acids shorter at the N-terminal end, but has a 110 amino acid extension at the C-terminal end. Northern blots of winter flounder ZP3 show that it is expressed exclusive in the ovary. We have confirmed that the ZP2 gene is expressed exclusively in liver and thus winter flounder represents an unusual instance of coordinated expression of eggshell proteins in two different tissues.

PP-108

KINETICS OF THE FOLLICULAR GROWTH DURING VITELLOGENESIS IN TWO STRAINS OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) CHARACTERIZED BY ESTIVAL OR SPRING OVULATION TIME, IN RELATION TO THE PLASMATIC CONCENTRATIONS OF ESTRADIOL-17ß AND VITELLOGENIN

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Female rainbow trouts from two strains characterized respectively either by estival or spring ovulation times were individually tagged immediately after the first ovulation. They were thereafter periodically submitted to anesthesia, followed by blood sampling and surgical removal of a small piece of ovary containing 10 to 25 follicles of the largest cohort. This handling was performed every month, alternatively on half of the fish of each strain. For each female, successive ovarian samples were taken alternatively either from the right or the left ovary. Approximately fifty percent of the fish subjected to these samplings were kept until the next ovulation, and most of them normally ovulated and gave fertilizable eggs. The blood samples were assayed to determine the concentrations of vitellogenin and estradiol 17-ß in plasma, and the ovarian samples were processed to determine the diameters of follicles containing vitellogenic oocytes. The results show a great variability within and between the two strains, concerning the individual kinetics of follicular growth. This appears however to start slightly earlier and to proceed much more quickly in the estival strain than in the spring one. The analysis of the relations between the individual profiles of evolution of the plasmatic concentrations of estradiol and vitellogenin, and the kinetics of follicular development show a rather good correspondence, but does not allow to establish a relationship precise enough to characterize individuals by using only one sampling.

PP-109

IDENTIFICATION AND CHARACTERIZATION OF SIBERIAN STURGEON, *ACIPENSER BAERI*, OOCYTE RECEPTOR FOR VITELLOGENIN

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Oocyte growth in oviparous animals is dependant upon the uptake of exogenous nutritional material stored as yolk in the oocyte, until to be used by the embryo during early stages of its development. In fish, yolk is mainly made of vitellogenin (VTG), delivered to the oocyte by the bloodstream and selectively internalized into the oocyte via specific oocyte membrane receptors. In the present study, we describe the characterization of oocyte VTG receptors from vitellogenic female Siberian sturgeon, *Acipenser baeri*. The receptors were solubilized from the ovarian membrane homogenates with the non-ionic detergent octyl-b-D-glucoside, and vizualized by ligand-blotting, with ^{125}I-VTG, as a 97 kDa protein. Characterization of the VTG receptor was performed using a solid phase filtration assay after incubation of ^{125}I-VTG with solubilized ovarian membranes preparation. The VTG receptor appeared to be saturable by increasing amounts of Siberian sturgeon ^{125}I-VTG. Scatchard analysis of the saturation indicated a single class of binding sites with an apparent K_D of $7.49 \cdot 10^{-7}$ M. Competition assays, performed by ligand-blotting and by filtration assay, confirmed the specificity of this receptor for VTG : the binding of iodinated homologous VTG was abolished by both homologous (Siberian sturgeon) and heterologous (trout) cold VTG. Total inhibition was obtained with suramin.

PP-110

SPONTANEOUS AND INDUCED OOGENESIS IN THE EEL - DO MORPHOLOGICAL DIFFERENCES IN OOCYTE CYTOLOGY REFLECT ABERRANT YOLKING?

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Artificial propagation of the Japanese eel (*Anguilla japonica*) has been much hindered by the arrest of gametogenesis in early stages of development if fish are held captive and by the lack of maturing wild animals for reference. Treatments with pituitary suspensions may override gametogenic arrest, but, despite recent advances, the eel life cycle has still not been completed in captivity. It has been suggested that induced vitellogenesis may not mimic that occurring naturally. Thus, the build-up of intra-oocytic nutrient stores, required to sustain early development and ontogeny, may be aberrant during artificial maturation. To satisfy our need for a reference source, we used the New Zealand longfinned eel (*Anguilla dieffenbachii*), which reaches midvitellogenic stages while still in fresh water, as a model for comparison of serum vitellogenin and oocyte cytology with artificially maturing Japanese eels. Serum vitellogenin levels in longfinned eels were substantially lower than those in Japanese eels of comparable developmental stage. This was reflected by lower lipovitellin immunoreactivity in the oocytes of longfinned eels and a corresponding higher abundance of lipid vesicles. A striking difference in eggshell thinkness was observed, being nearly twofold greater in oocytes from artificially maturing Japanese eels than in those from wild longfins. The biochemical basis for these differences is being investigated in more detail by electron microscopy and Western blot analysis to assess whether they are physiologically important or whether they relate to species-specificity.

PP-111

DIFFERENT EFFECTS OF SALMON GONADOTROPIN I AND II ON VITELLOGENESIS IN JAPANESE EEL,*ANGUILLA JAPONICA.*

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In Japanese eel, we have already demonstrated that weekly injections of salmon gonadotropin (sGTH) were effective for inducing ovarian maturation. It was found that sGTH caused increases of body weight (BW) and moisture contents in ovary during vitellogenesis. sGTH contained sGTH I and II. In this study, changes of BW, moisture contents in ovary and oocyte diameter after injection of sGTHI or II were examined in Japanese eel during vitellogenesis. sGTH I and II were purified by the method of Suzuki et al. Japanese eel were induced vitellogenesis by pretreatment with sGTH. Vitellogenic females received a single injection of sGTH I or II. sGTH I and II treatment groups were compared with daily changes of BW for a week after administration. These were also compared changes of moisture contents of ovary and oocyte diameter before injection, and at 3 and 7 days after injection. In the sGTH I group, BW and moisture contents in ovary decreased during a week. Oocyte diameter increased at 3 day and was constant at 7 day. In the sGTH II group, BW increased to 3 day and then decreased. Moisture contents in ovary increased at 3 day and was constant at 7 day. Oocyte diameter increased to 7 day. Thus, it is suggested that sGTHI stimulates increase of diameter in oocytes by uptake of vitellogenin, while sGTH II stimulates increase of diameter in oocytes by uptake of vitellogenin and water absorption.

PP-112

CLONING OF VITELLOGENIN cDNAS FROM THE JAPANESE EEL, *ANGUILLA JAPONICA*

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Vitellogenin (VTG), the egg yolk protein precursor, is synthesized in the liver, secreted into the bloodstream and transported to the ovary. Subsequently, VTG is sequestered into the oocyte and cleaved into yolk proteins. Although estrogens are known to be important modulators of transcription of the VTG gene, the finer details of its transcriptional control remain unknown. Thus, as a first step towards improving our understanding of the endocrine control of VTG gene transcription in the Japanese eel, we set out to clone the VTG cDNA. In a previous study, a partial-length Japanese eel VTG cDNA was isolated by immunoscreening and characterized. We used this cDNA in the present study to screen a lZap II cDNA library constructed from hepatic poly (A+)RNA of artificially maturing Japanese eel. Accordingly, two VTG cDNAs were isolated and found to encode proteins of 1759 (eel VTG 1) and 1734 (eel VTG 2) amino acid residues, respectively. Moreover, these cDNAs hybridized to a 5.8 kb RNA species in liver from estrogen- or salmon pituitary-treated female eels by Northen blot. Both Japanese eel VTGs are 89.9% identical,while homologies of 62.4 % to trout VTG, 50.4 % to *Fundulus* VTG I, and 53.2 % to *Fundulus* VTG II were found with eel VTG 1, and 59.1 % to trout VTG, 48.4 % to *Fundulus* VTG I, and 48.1 % to *Fundulus* VTG II with eel VTG 2. Thus, there appear to be at least two VTG genes in Japanese eel, both of which contain the anticipated lipovitellin I, phosvitin, lipovitellin II, and β'-component domains. However, VTG of Japanese eel shows a remarkable difference to that in other fish, i.e., it has been shown to be incorporated into the oocyte without any structural conversion. Absence of proteolytic enzyme activity, such as that conferred by for instance cathepsin D, remains unexplained, but is possibly attributable to artefacts of artificial maturation.

PP-113

REPRODUCTIVE STATUS OF WILD-CAUGHT FEMALE SUMMER FLOUNDER

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The summer flounder, *Paralichthys dentatus*, ('fluke') is a migratory fish indigenous to coastal waters off the East Coast of the United States. Fluke move offshore to spawn in the fall and winter and return to warmer estuaries in the summer. Pressure on the fishery has necessitated a finer resolution of the reproductive biology and the maturity schedule for the females. Reproductive maturity in females is reached as early as after the second summer. We collected 673 female fluke, 13-68 cm in total length, between Massachusetts and North Carolina during 1996-97 on groundfish trawl surveys conducted by the National Marine Fisheries Service. Seven stages of oocyte development and correlated estradiol levels are described. Estradiol concentrations increased in developmental stages prior to the appearance of yolk proteins. Seasonal changes in ovarian status of wild-caught females suggest ovarian recrudescence shortly after spawning. Over half the females examined in the winter had atretic follicles. Fish with vitellogenic oocytes were present in collections as early as September.

PP-114

SPARUS AURATA EGGS: MATURATION AND QUALITY

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Egg is the final product of oocyte growth and development. All the contents of the egg, which will determine its quality, are incorporated by the egg when it is an oocyte within the ovary. Egg quality is determined by the intrinsic properties of the egg itself, by its gene, by the maternal mRNA transcripts and by the nutrients contained within the yolk. In all teleosts, oocytes appear to undergo the same basic pattern of growth; in those laying pelagic eggs, two distinct proteolytic processes occur during oocyte maturation. Previous studies in seabream demonstrated that in the first cleavage, the vitellogenin (VTG) synthesized in the liver under hormonal control, transported by the blood flow, and incorporated in oocytes by a receptor mediated endocytosis, is proteolytically processed by cathepsin D into yolk proteins: lipovitellin and phosvitin. Additional cleavages by cathepsin L of yolk proteins during final oocyte maturation, followed by a pronounced water uptake, occurs concomitantly with oocyte maturation. The acquirement of buoyancy, through the hydration process, represents a key event in seabream reproduction. In this study, seabream eggs were used as a system to establish what factors determine egg quality. The eggs were distinguished as being of good or poor quality in virtue of their ability to float or sink in sea water. With the aim of identifying a possible marker of egg quality, we studied the vitelline envelope components, the lysosomal enzymes activities, and NAD+metabolism in these two types of eggs. Differences of egg envelope components and lysosomal enzymes activity were observed between floating and sinking eggs, while studies on NAD+ metabolism are currently in progress.

PP-115

CHANGES OF SERUM GROWTH HORMONE AND VITELLOGENIN LEVELS IN CUTTHROAT TROUT (*ONCORHYNCHUS CLARKI*) DURING SEXUAL MATURATION

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Growth hormone (GH) is not only involved in somatic growth but also regulates sexual maturation in teleosts. However, there are few reports on changes of serum GH levels during sexual maturation. The aim of this study was to develop a highly sensitive chemiluminescent immuno-assay (CLIA) for GH. A further objective of the study was to observe the profile of serum GH levels as compared with serum estradiol-17β (E2) and vitellogenin (Vg) levels in cutthroat trout (*Oncorhynchus clarki*) during sexual maturation. A highly sensitive and specific CLIA was developed for quantification of GH in salmonid species. The measurable range of salmon GH in the CLIA was 39-1250 pg/ml using a short assay (one day) protocol and 3.9-125 pg/ml in a longer (two day) assay. The dilution curve of serum from cutthroat trout in the CLIA was parallel to the standard curve of recombinant chum salmon GH. Seasonal changes of serum GH levels in groups of one-year-old and two-year-old cutthroat trout were observed. In one-year-old fish, the concentration of GH showed a temporary peak in November. In two-year-old fish, serum GH levels were elevated from November to December. Seasonal changes of serum E2 and Vg levels in maturing female fish coincided with these changes of serum GH levels. These data suggest that GH has a close relation, not only to somatic growth, but also with maturation and that high concentrations of GH may play a role in endocrine regulation of sexual maturation.

PP-116

CHANGES IN SERUM CHORIOGENIN AND VITELLOGENIN LEVELS IN MASU SALMON (*ONCORHYNCHUS MASOU*) DURING SEXUAL MATURATION AND AFTER ESTROGEN TREATMENT

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In several teleosts, it has been suggested that the precursor to the vitelline envelope protein, choriogenin (Chg), is synthesized in the liver stimulated by estradiol-17β (E2), secreted into the bloodstream, and then incorporated into the growing oocyte. Two vitelline envelope-related proteins have been purified from serum of vitellogenic masu salmon (*Oncorhynchus masou*) and identified as choriogenins (Chg H and Chg L). In this study, single radial immunodiffusion (SRID) assays for Chgs and vitellogenin (Vg) of masu salmon were developed and serum levels of these proteins during sexual maturation and after injection with E2 were measured. Estrogen induction of Chgs in male fish was investigated in terms of E2 dose and time interval, as compared with Vg levels in the same fish. Chgs and Vg were induced in a dose-depended manner by a single injection of E2 from 0.01 to 5mg/kg body weight (BW). For the 0.01 mg/kg E2 dose, Chgs levels exceeded Vg level. Contrary, from 0.1 mg/kg BW E2, Vg concentrations were higher than Chg levels. In 2-year-old females, serum Vg and Chg levels were increased in concent with gonadosomatic index and serum E2 concentrations, and then decreased at ovulation. Comparison of serum Vg, E2 and Chg levels revealed higher concentrations of Chgs than Vg at low E2 levels. In addition, serum Chg H levels usually exceeded Chg L levels in fish with E2 levels lower than 10ng/ml. Collectively, the results of the present study indicated that serum Chg H, L and Vg concentrations are regulated directly by circulating E2 levels.

PP-117

PROLIFERATION OF OVARIAN FOLLICLE CELLS AT DIFFERENT DEVELOPMENTAL STAGES OF OOCYTES IN RED SEABREAM, *PAGRUS MAJOR*

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The number of proliferating follicular cells (granulosa and thecal cells) in oocytes of red seabream at different developmental stages was analyzed using the 5-bromodeoxyuridine (BrdU) technique. During the spawning season, fish were given an i.p. injection of 50 mg/kg/BW of BrdU in 0.1 M Tris-HCl-buffered saline, pH 7.6, at 9:00 or 15:00, and ovaries were fixed in Bouin's solution 3 hrs later. BrdU- incorporating cells were detected immunocytochemically with a mouse monoclonal antibody against BrdU and the numbers of proliferating cells were counted using light microscopy. BrdU immunoreactivity (proliferating cells) was mainly present in both thecal and granulosa layers of oocytes. Proliferating granulosa cells were first found in follicles at the lipid stage (7.0 ± 0.1 cells/largest cross section of follicles) and increased in the number during the vitellogenic stage (11.2 ± 0.3 cells at the primary yolk globule stage), but were never found at the tertiary yolk globule stage, the migratory nucleus stage, and the mature stage. In the thecal layer, proliferating cells were also found at the lipid stage (5.1 ± 0.4 cells) and this number was maintained during the vitellogenic stage but decreased significantly at the tertiary yolk globule stage (0.6 ± 0.1 cells) and remained low thereafter. The increasing number of proliferating cells during the vitellogenic stage correlates well with the previous immunocytochemical observations of IGF-I in the ovarian follicles of red seabream, suggesting that IGF-I may be involved in follicle cell proliferation in this species.

PP-118

EFFECT OF EXTREME RANGED TEMPERATURES ON FISH OOGENESIS

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The question about influence of extreme ranged temperatures on fish oogenesis is very important because they are one of the limiting factors for fish growth and maturation. To another side it is possible to use them as instrument for investigation of oogenesis mechanisms. The purpose of this research is to study differential sensitivity of oocytes at different stages of fish to action of low and high temperature. The main objects of research were tilapia mossambique (*Oreochromis mossambicus*) and rainbow trout (*Oncorhynchus mykiss*). Tilapia was cultivated under the temperature 25-28°C, as low as 20-22°C and as high as 34-36 °C, and rainbow trout at 14-18 °C, 3-4 °C and 22-23 °C, respectively. Prolongation of extreme ranged temperature action was different and depended upon the aim of the experiment. Fishes of different ages with different gonad condition were used. It is established that low temperature causes retardation of gonado- and gametogenesis, destruction of germ cells, blocked of reproduction of follicular cells in early vitellogenic oocytes, and a decreasing gonadosomatic index (GSI). It is accompanied by lower body growth rates. After transfer of fishes to normal temperature their compensatory growth and gonad development were observed. But rate of gonadal recovery depends upon their initial state at time of low temperature action and low temperature-treated fishes can mature ealier, at the same time and later than untreated ones. Action of high temperature on tilapia increased development of oocytes of early stages and stimulated growth of juvenile fishes, but damaged some previtellogenic oocytes, follicle cells in early vitellogenic oocytes and provoked the total atresia of ripe oocytes. Extreme high temperature blocked early stages of gonad development in rainbow trout. As a result of this process only some previtellogenic oocytes developed. Six months after the action of extreme high temperature seminal ducts appeared in some ovaries.

PP-119

RELATIVE ESTROGENICITY OF STEROIDS IN INDUCING ZONAGENESIS IN ATLANTIC SALMON (*SALMO SALAR*) HEPATOCYTES *IN VITRO*

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Oogenesis represents a complex series of cellular and molecular events, which culminates in oocytic maturation and meiosis. While hepatic vitellogenesis has long been recognized as essential to egg maturation, recent work in several groups has allowed the definition of a novel aspect of hepatic participation in egg maturation. In Atlantic salmon the major part of the eggshell (the *zona radiata*) is composed of only three proteins, which are synthesized in the liver for transport to the ovaries. Estradiol (E2) will induce this synthesis in juvenile salmon of both sexes *in vivo*. After E2-treatment, primary hepatocyte cultures from juvenile salmon of both sexes will synthesize and secrete zona-proteins in a manner which suggests that zonagenesis may somewhat precede vitellogenesis in this species (Celius & Walther, J. Endocrinol. 158: 259-266, 1998). We have used this system to further investigate events during the onset of sexual maturation in salmon. Primary cultures of hepatocytes were exposed to various estrogens for periods of 24-96 hours, and the relative potency of zonagenetic induction evaluated. All hormonal inductions were blocked by the estrogen inhibitor ICI 182,780, suggesting that all inductions ultimately involved action on the estrogen-receptor. While E2 was the most potent estrogen in this system, estrone, estrone-sulphate and estriol were active beyond what was predicted from their affinities for the estrogen receptor in other systems. Progesterone and hydroxy-pregnenolone were not active in this system. *In vitro* zonagenesis provides a sensitive system in which to pinpoint the endocrine initiation of sexual maturation in Atlantic salmon.

Natural Environmental Influences on Reproduction

OP-41

DIFFERENTIAL EFFECTS OF LIGHT INTENSITY ON GROWTH, MATURATION AND PLASMA MELATONIN IN ATLANTIC SALMON AND ITS IMPORTANCE IN AQUACULTURE

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Growth and maturation, both critical to the profitability of Atlantic salmon culture are seasonal events which are synchronised by environmental conditions, in particular the seasonally changing photoperiod. Photoperiod manipulation provides a simple and effective tool with which to alter the timing of maturation and increase growth rate in covered tanks. However, its use is complicated in open sea cages due to interference form the ambient photoperiod. Furthermore, the endocrine mechanisms responsible for conveying light information to the brain and reproductive access are still little understood. This work aims to review the effects of varying light sources and intensities on plasma melatonin levels in Atlantic salmon maintained in uncovered sea cages and relate these findings to harvest weight and grilse levels under commercial conditions. The data clearly show that plasma melatonin synthesis responds in a differential manner to variations in light intensity and this in turn may act as an internal zeitgeber to synchronise developmental parameters. Using additional illumination it was shown that it was possible to increase growth and reduce grilsing. However it is thought that the intensity of illumination required to produce a significant influence on these parameters depends on reducing dark phase melatonin levels below a threshold level. Not only is this information important commercially but it also provides insight into the mechanisms by which daylength differentially affects melatonin secretion and in turn how this mediates internal rhythms of growth and maturation in fish.

OP-42

CIRCANNUAL RHYTHMS OF REPRODUCTION IN RAINBOW TROUT

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The reproductive cycle of rainbow trout is thought to be controlled by an endogenous circannual rhythm, or clock, which is entrained by the seasonal changes in daylength. However, there have been no long-term studies of the phase-shifting effects of photoperiod on the entrainment of this clock. In this study, individually tagged fish (50 per group) were transferred from ambient daylength to either short (6L:18D) or long (18L:6D) daylengths at monthly intervals throughout the year. Over a 4-year period, spawning times were compared with those of control fish maintained under simulated natural photoperiod. During the first reproductive cycle, clear phase-response curves were obtained under each photoperiod, with advances in spawning observed in fish transferred to short days at monthly intervals during January to August, and delays in spawning observed in fish transferred to long days during the same period. During subsequent cycles, whilst remaining significantly delayed in comparison with the controls, the differences in spawning time between groups maintained under long days were dissipated. In fish transferred to short days at monthly intervals between February and May, the spawning rhythm appearred to split into two components, with some fish spawning in advance of the controls, and others exhibiting a slight delay relative to the controls. These results suggest that circannual rhythms in fish, like circadian rhythms, are rather labile. This is the first long-term study of changes in the phase-response of an endogenous circannual rhythm in fish.

OP-43

EFFECT OF EXERCISE AND CONTINUOUS LIGHT ON EARLY SEXUAL MATURATION IN FARMED ATLANTIC COD (*GADUS MORHUA* L.)

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Farmed Atlantic cod usually reach sexual maturity at an age of two years. This is accompanied with reduced somatic growth and decreasing harvest quality. Compared with wild cod, farmed cod display higher growth, larger energy stores, and earlier sexual maturation. Several experiments have demonstrated that restricted feeding of cod has only minor effects on the proportion of mature fish, while exposure to continuous light (LL) can arrest sexual development. This study addresses the combined effects of photoperiod treatment and exercise on somatic growth, energy deposition (liver size), gonadal development, and proportion of cod spawning at an age of two years. Individually tagged juvenile Atlantic cod, hatched spring 1996, were exposed to high water current speed (1 BL/s), medium water speed (0.5 BL/s) or low water current speed from July 1997 to August 1998. The fish were distributed among 12 tanks; 6 of the tanks were exposed to LL and 6 to natural light (NL), creating 6 experimental groups with two replicates pr. treatment. Exercise had only minor effect on the growth of the fish, and no effect on the proportion of sexually maturing fish. By contrast, all cod reared under NL reached sexual maturity during winter and spring 1997, while LL delayed gonadal development, and no maturation was detected during the study. The LL groups had higher growth rate during late winter and spring compared with the NL groups.

PP-120

INTRICACIES OF THE PINEAL MELATONIN IN ENVIRONMENTAL SEX CONTROL AND FITNESS IN FISHES

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It has been unequivocally accepted that the pineal gland is involved in photoperiodic regulation of reproduction in almost all classes of vertebrates. It relays environmental information by regulating the melatonin level. Many environmental challenges are recurrent and the interaction of melatonin is having an ecological relevance on the species survival and fitness. Evidences are accruing that pineal melatonin regulates thyroid hormones and sex steroids and thus have strong impact on reproductive functional perspectives and fitness of the species for survival. The role of hypothalamus in the neuroendocine sex control was recorded for the first time in hermaphroditic teleost, blue wrasse, *Thalassoma fasciatum*. While pineal melatonin was found to have impaired with the environmental and hormonal sex determination in cichlids (*Oreochromis mossambicus*) in our studies. Thermolabile sex - determination and 17α methyltestosterone (17α MT) induced masculinization in tilapia were observed to fetch 85-87% masculinization whereas androgenization with 17α MT under long photoperiod (16L : 8D) provided consistently 100% male population. However, the amenability of the masculinization with the use of 17α MT or different temperature regions (19-23°C and 29-33°C) was found to be influenced by exogenous administration of melatonin and also possibly by the environmentally (photoperiod and temperature) induced changes in pineal melatonin. Such induced sex reversal in tilapia was further studied using exogenous thyroid hormones (T_4 and T_3) in different doses (0.25 µg/l and 0.50 µg/l). The observations did not elicit any significant change in sex ratio and growth except an early fin differentiation and survival of the experimental fishes. But it influenced maturity of the fish. It was observed that the doses of steroid for inducing absolute sex reversal could be reduced by manipulating the melatonin level or even the melatonin itself could bring about endogenous steroid level for sex reversal. This paper discusses the intricacies of pineal melatonin on amenability of sex reversal and fitness of the progeny in a particular environment.

PP-121

PULSATILE STEROID HORMONE PROFILES, DIEL METABOLIC RHYTHMS AND REPRODUCTIVE SEASONALITY IN THE FEMALE RAINBOW TROUT, *ONCORHYNCHUS MYKISS*

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There is increasing evidence that biological clock or "calendar" type mechanism(s) control the seasonal reproductive cycle of many fishes. These mechanisms involve the perception of photoperiodic information, which is integrated with various endogenous rhythms or timekeeping mechanisms so as to give the organism a "sense" or measure of time. Most experiments investigating photoperiodism in fish have considered the organism as a 'black box' (i.e. manipulating photoperiod and measuring changes in the timing of gamete release). As an alternative to this approach we examined some of the underlying endocrine and photoperiodic mechanisms that are involved in the transducton of these clock mechanisms. Steroid profiles were examined over 24-hours at different stages in the annual reproductive cycle using dorsal aorta catheterization (and after manipulation by melatonin implants). By using a sensitive ELISA we were able to measure several hormones within the same individual at a high sampling frequency. This revealed a highly pulsatile profile of testosterone and estradiol. Each pulse lasted approximately 2 hours, when levels increased up to 20 times the basal levels. These pulses were not synchronized with the time of day or between steroids. Pulse frequency was higher in winter than summer. Using flow through respirometry we examined the effect of photoperiod/melatonin manipulation on the diel metabolic activity patterns of individuals so as to elucidate any circadian basis for rhythmic activity. Metabolic activity was highly synchronized to the light regime but showed little evidence of any endogenous circadian component. The results are discussed in relation to possible models of reproductive seasonality.

PP-122

BROODSTOCK MANAGEMENT AND ENDOCRINE CONTROL OF REPRODUCTION OF THE MATRINCHA (*BRYCON CEPHALUS*), AN AMAZONIAN FISH WITH POTENTIAL FOR AQUACULTURE

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The matrincha, a popular food fish in the Amazon region, has shown promising growth rates in experimental culture systems. However, the neuroendocrine control of gonadal function has not been investigated in this species and attempts to spawn matrincha in captivity have been largely unsuccessful. Consequently, there is a shortage of larvae and fingerlings for aquaculture, which is therefore principally dependent on the capture of wild stocks. To determine whether environmental factors may influence sexual maturation in this species, potential broodstock matrincha, originating from wild-caught larvae, were maintained in either 150 m^2 ponds (incoming water originating from a well) or in an enclosure constructed within an igarape (a small stream with water flow rate and chemical composition varying according to season). At regular intervals, blood samples were taken for measurement of reproductive steroids and fish were examined for external signs of sexual maturation. Preliminary results indicate differences in the incidence and timing of sexual maturity in the two environments, which are reflected in the seasonal changes in circulating steroids, and differences in the fertilization rates of eggs obtained under each regimen.

PP-125

TEMPERATURE EXPERIENCED BY FEMALE WOLFFISH (*ANARHICHAS LUPUS* L.) DURING VITELLOGENESIS: SEX STEROID PROFILES AND INFLUENCES ON THE TIMING OF OVULATION

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Common wolffish, which are coldwater stenothermal marine fish, were exposed to different temperatures during vitellogenesis. Plasma sex steroid profiles and timing of maturation were investigated. Three groups, held under simulated natural photoperiod (70 N), experienced 3-4 °C from December until mid-April. Temperature was then raised to 8 and 12 °C for two groups, and the third remained at 4 °C. These regimes were maintained until October (start of the spawning season) and temperature was then adjusted to 4 °C for all groups. Monthly samples were taken, and plasma concentrations of testosterone and oestradiol determined using radioimmunoassay. Temperature treatment influenced the temporal changes in plasma sex steroid concentrations, but was without effect on either the general shape of the profiles or peak values. In fish held at 8 and 12 °C the peaks of both testosterone and oestradiol were about four and five weeks delayed compared to the 4 °C group. These differences resulted in corresponding shifts in the timing of ovulation. Elevated levels of testosterone and oestradiol, indicating onset of vitellogenesis, were recorded from May-June onwards, but peak concentrations were not recorded in the 8 °C and 12 °C fish until they had been transferred to 4 °C. Peak steroid concentrations were recorded in all groups about one month prior to ovulation. The results indicate that sex steroid synthesis, secretion and/or metabolism in wolffish are influenced by temperature treatment during vitellogenesis, and that the temperature experienced at that time also influences the timing of ovulation.

PP-126

GROWTH AND SEXUAL MATURATION OF ATLANTIC COD (*GADUS MORHUA* L.) UNDER DIFFERENT LIGHT INTENSITIES

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Sexual maturation at young age imposes a problem of economical importance in fish farming of Atlantic cod (*Gadus morhua*). Previous studies demonstrated that use of continuous light in covered seawater tanks arrested sexual development, whereas cod exposed to continuous additional light on sea cages postponed spawning for some months. The aim of the present study was to determine the effects of continuous light of different intensities on growth and sexual maturation. Cod hatched spring 1997 were exposed to continuous light from July 1998 in sea cages and were transferred to eight circular seawater tanks on October 1. The fish were reared under four different light regimes; natural light, continuous light in covered tanks or continuous additional light of low or high intensity. The effect of light intensity on growth, gonadal development and endocrine status are discussed.

PP-127

DISULFIDE BONDS IN CHUM SALMON STANNIOCALCIN, A HORMONE REGULATING CALCIUM HOMEOSTASIS

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Calcium plays an essential role in regulation of many physiological process including reproduction. Staniocalcin (STC) is a hormone produced by the corpuscles of Stannius in bony fish. The primary function of STC is to regulate Ca^{2+} homeostasis by preventing the development of hypercalcemia. STC express its hypocalcemic function by lowering the rate of calcium influx through gills and intestine and by stimulating phosphate reabsorption in kidney. Recently, mammalian homologues (STC1 and STC2) of STC have been isolated from human and rodent cDNA libraries. The molecular structure of mammalian STCs is closely related to fish STC. Although 11 disulfide linkages are crucially important for three-dimensional structure of STCs and for expression of their activity, nothing has been reported about positions of disulfide bonds. In the present study, we determined for the first time all disulfide bonds in chum salmon STC. The chum salmon STC was deglycosylated and digested with several proteases in series. Six fragments, each of which consisted of two peptides connected with a single disulfide bond, were isolated by HPLC. Disulfide bonds were determined by sequence analysis after reduction and S-alkylation of each peptide fragment. Chum salmon STC is a homodimer connected by a single intermonomeric disulfide bond at Cys169. The monomer consists of 179 amino-acids contains five intramonomeric disulfide bonds formed between Cys12-Cys26, Cys21-Cys41, Cys32-Cys81, Cys65-Cys95 and Cys102-Cys137.

PP-128

GROWTH AND SEXUAL DEVELOPMENT IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) MALES EXPOSED TO VARIOUS LIGHT REGIMES

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Day length is known to be the determining factor controlling the seasonal pattern of reproduction in rainbow trout. This investigation was designed to study the effect of photoperiod on growth and gonadal activity in males approaching sexual maturity. Sexually non-mature 2 year-old milters, kept in 2000 l circular tanks supplied with a steady flow of 1 l/sec of brook water, were exposed to one of 3 light programs for a period of 18 weeks: A) normal day light cycle from August (13 h light) to January (8 h light), B) constant 8 h light : 16 h dark, C) permanent darkness. At two weeks intervals animals were anesthetized, weighed, checked for the presence of spermatozoa by gentle stripping and bled from the tail vein. In the three treatment groups, 52, 46, and 42%, respectively, ($P > 0.05$) reached sexual maturity. In these individuals, growth rates were identical for Groups A and B, but substantially lower ($P < 0.05$) for Group C (permanent darkness). The same applied to plasma testosterone concentrations. These rose steadily until a sudden increase from 2 weeks before to 2 weeks after the onset of milt production. This surge was more pronounced ($P < 0.05$) in Groups A and B than in Group C ($P > 0.05$). Peak levels reached were also different (in Groups A and B: 138 and 137 vs Group C: 92 µg/ml, $P < 0.05$). In all groups the increase in body weight ceased with the occurance of the surge in testosterone. Under controlled light regimes individual variability for both growth rate and plasma testosterone concentration was much reduced.

PP-129

EFFECTS OF DIFFERENT PHOTOPERIODS ON GROWTH AND SEXUAL MATURATION IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) IN SEA WATER CAGES

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Two different populations of individual tagged rainbow trout juveniles were transferred to sea water cages at Matre Aquaculture Research Station, Western Norway (61°N), in July (experiment 1) and October (experiment 2) 1995 and grown to June 1996 and 1997, respectively. Each of the populations was distributed into two cages, one experiencing natural conditions (NL) and the other exposed to additional continuous light (LL). Subgroups of fish were transferred from one light regime to the other in October, January and April (exp. 1) and January, April an June (exp. 2), giving rise to 8 groups per experiments. At monthly intervals fork length and live body weight of all fish were measured, and sexual maturity was assessed by external examination; spermation in males and ovulation in females. The weight of the fish increased from 180 g to 3500 g and from 150 to 6500 g in experiment 1 and 2, respectively. No significant differences were found in live body weight or forklength between groups. The growth rate did not show any differences between groups. Condition factor showed an increase in all groups and no consistent significant differences were found between groups. The proportion of fish sexual maturation was higher in all groups which experienced transfer from NL to LL compared with NL group or groups transferred from LL to NL. These studies support earlier work indicating that photoperiod treatment affect sexual maturation in rainbow trout. However, growth was not affected as would be expected from earlier findings in Atlantic salmon.

PP-130

THE EFFECTS OF DIFFERENTIAL LIGHT INTENSITIES ON THE DIEL RHYTHM OF MELATONIN RELEASE IN RAINBOW TROUT

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The use of artificial light to advance and delay spawning in rainbow trout has become an important tool in the year-round production of eggs. However, the response of fish to this light depends on whether the artificial light is superimposed upon natural or subdued natural daylight. To determine how differences in light intensity affect the perception of photoperiod, rainbow trout were exposed to varying combinations of light intensity: L2800: D0lux; L300: D0 lux; L2800: D30 lux and L300: D30 lux. After a two-week acclimation period, fish were blood sampled at seven time points over a 24-hour period (four 'day' samples and three 'night' samples), and the plasma melatonin levels determined by radioimmunoassay. In the groups exposed to 30 lux during the night the plasma melatonin levels were significantly depressed ($p<0.05$), but the rhythm was maintained. No difference in daytime melatonin titre was observed between the four groups. This implies that circulating melatonin levels are related to differentials, rather than absolute light intensities. Expansion of this work may reveal the intensity of artificial night-time illumination required to completely suppress the diel melatonin rhythm of fish held under natural day-light.

PP-131

MELATONIN RECEPTOR AND *PER1* GENE EXPRESSION IN THE TELEOST FISH BRAIN

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Fish exhibit both daily and seasonal rhythms in their behaviour and physiology. These circadian and circannual rhythms are entrained to the ambient photoperiod, but persist in the absence of photic input indicating that they are governed by an endogenous clock mechanism. The pineal hormone, melatonin, a neuroendocrine transducer of photoperiod, has been implicated in the entrainment of both circadian and circannual rhythms in mammals, although it's role in fish is unclear. To determine the location of a potential clock mechanism in fish and any interaction with melatonin we have investigated the distribution of melatonin receptor gene expression by *in situ* hybridization together with that of the period gene (*Per1*) in the rainbow trout and the cod brain and pituitary. *Per1* has been identified as a component of the clock mechanism in several species ranging from insects to mammals. *Per1* gene expression in fish was studied using a riboprobe derived from a 404 bp cDNA fragment of the ovine period gene *oPer1*. A riboprobe was derived from a 500 bp cDNA fragment of the rainbow trout Mel1b receptor sub type and used to localise melatonin receptor gene expression. Melatonin receptor mRNA was found in brain areas known to be involved in the processing of visual information in both species of fish. *In situ* hybridization with the *oPer1* probe had a similar pattern of distribution to that of the melatonin receptor with high levels of labelling for both genes present in the optic tectum and the cerebellum.

PP-132

AGE AT FIRST MATURITY AND SEX DEPENDENT GROWTH OF ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*) GROWN ON FOUR DIFFERENT LIGHT REGIMES

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Age at first maturity and sex-dependent growth was investigated in 166 (79 females and 87 males) Atlantic halibut (*Hippoglossus hippoglossus*) reared at 11°C on four different light regimes (simulated natural photoperiod, continuous light, constant 8 h light and 16 h dark switched to continuous light on 4 May 1996, constant 8 h light and 16 h dark switched to simulated natural photoperiod on 6 July 1996). The experiment lasting from 10 February 1996 to 9 February 1998. Only males matured (21.8%) at age 2+. No significant differences in % maturation between the light groups were found (chi-square test) but an inverse relationship between photoperiod and maturity was seen. Independent of photoperiod and maturation, females were significantly bigger than males from 14 April 1997 onwards. In females the significantly highest mean weights were seen on continuous light during the first year (13 April to 3 August) and at the end of the experiment. For males, a similar growth enhancing effect of extended photoperiod was seen only from 13 April to 28 September 1996. Immature males were bigger than maturing males from 23 March 1996 onwards. The results suggest an overall growth enhancing effect of continuous light in females, but not in males. Continuous light reduced maturation at age 2+ in males. Females showed overall better growth than males.

PP-133

SEXUAL MATURATION IN COD (*GADUS MORHUA* L.) IN RELATION TO STRAIN, TEMPERATURE, SEX, SIZE AND HAEMOGLOBIN TYPE

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The main objective of the study presented here was to investigate the effect of temperature, strain and haemoglobin type on life history traits of fish. Culture raised one-year old cod originating from two strains, Northeast (NE) Arctic and coastal cod, were individually tagged and kept in tanks at three water temperatures, 8, 12 and 15°C from May to March. Commercial dry feed were given in surplus and natural light regimes corresponding to latitude 60°N (Bergen, Norway) were simulated. Rate of maturation and gonadosomatic index (GSI) in their second year of life was recorded and is presented here as function of strain, individual growth rate and haemoglobin type within the temperature groups. Except for one female kept at 8°C and two raised at 15°C all coastal cod were maturing or ripe at the temination of the experiment (5th March). Among the NE Arctic cod 11, 25 and 23 percent of the females raised at 8, 12 and 15°C respectively were still immature. The corresponding numbers for NE Arctic cod males were 37 and 10 percent immature among those raised at 8 and 12°C, while all individuals raised at 15°C were maturing or ripe.

PP-134

THE EFFECT OF ALTERED PHOTOPERIODS ON MATURATION OF MALE AND FEMALE ATLANTIC SALMON (*SALMO SALAR*), OBSERVATIONS OF DIFFERENT RESPONSES AND MECHANISMS ?

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Two experiments were conducted on groups of mixed sex one sea winter Atlantic salmon. In one experiment, a group (P) of 40 fish was maintained under constant light from February to May before being transferred to a short day (8 hours). The control group (C) was treated identically, but maintained under a natural photoperiod. Ovulation was advanced 21 days (first ovulated fish) or 17 days (50% ovulated) in group P, compared to group C. Milt production was advanced 6 weeks (first fish with flowing milt) or 23 days (50% of fish with milt). In a second experiment, 3 groups of 50 fish were given 4 months of long daylength (20 hours), initiated in December (group D), February (group F) and April (group A), in an otherwise short daylength (8 hours) photoperiod. Control groups were maintained on constant short daylengths (group S) and constant long daylengths (group L). Percentage maturation for males and females respectively was 16 and 0, group D; 24 and 0, group F; 50 and 10, group A; 49 and 21, group S and 32.5 and 16, group L. Two females were observed to ovulate, one in October, group F and one in November, group A. Milt production was first observed in June, group D; July, groups F and L; August, group A and September, group S. These results support suggestions that male salmon mature at a smaller size or age than female salmon. The results also suggest that testis development is more flexible in time required to complete maturation and/or environmental conditions required for successful completion of maturation.

PP-135

PHOTOPERIOD AND TEMPERATURE AFFECTS GONADAL DEVELOPMENT AND SPAWNING TIME IN ATLANTIC SALMON (*SALMO SALAR* L.)

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The present study addresses the combined effects of photoperiod and temperature treatment on gonadal development and timing of ovulation in farmed Atlantic salmon. Previously immature salmon (n = 1000) maintained under simulated natural photoperiod (SNP; 60° N) in sea-water tanks for 1.5 years from smolting, were exposed to either SNP or an accelerated photoperiod regime (AP; 24:0LD from February, thereafter 8:16LD from May), using three tanks per treatment. From August onwards all tanks were exposed to brackish water. On September 6, one tank from each photoperiod treatment was exposed to cooled water (cooled from 14 to 8 °C and maintained 6 °C below ambient). The two remaining tanks from each photoperiod treatment were maintained at ambient temperature. On monthly intervals, 10 fish from each tank were sacrificed and sampled for gonads and blood plasma. Gonadal development and ovulation time were advanced following exposure to AP compared with SNP. An additional advancement and synchronisation of ovulation was seen following exposure to cooled water in both the AP and SNP treatments. The present study indicate that exposure to cold water prior to spawning advances and synchronises ovulation in Atlantic salmon, and that combined use of photoperiod and temperature can be used to control spawning time in salmon broodstock.

Anthropogenic Environmental Influences on Reproduction

OP-44

ENDOCRINE DISRUPTING CHEMICALS IN THE AQUATIC ENVIRONMENT

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It is now widely accepted that there are chemicals present in the aquatic environment that have the ability to disrupt the endocrine system, and hence cause adverse effects to aquatic organisms. These chemicals range from natural and synthetic oestrogens (e.g. oestradiol and ethynyl oestradiol, respectively) to man-made xenoestrogens (e.g. nonylphenol, methoxychlor). Other chemicals with other activities are also present in the aquatic environment; for example, p,p-DDT is primarily anti-androgenic, and many PAHs are anti-oestrogenic. It is likely that many more chemicals with endocrine activity will be discovered in the future, and it is equally likely that many chemicals will be shown to have a number of distinct endocrine activities; for example, a chemical may have oestrogenic and anti-androgenic activities, and also interfere in steroid hormone synthesis. Despite this rapidly increasing knowledge about chemicals capable of endocrine disruption, in most cases it is very unclear whether these chemicals are present in the aquatic environment at concentrations high enough to cause effects, and if they are, what these effects are. Even if effects are observed (such as intersexuality in wild fish in many rivers in the UK), it is uncertain presently whether such effects at the level of the individual fish translate into effects at the population level. My talk will be an overview of our present state of knowledge in this rapidly expanding field of research. I will discuss the chemicals of interest/concern; what is known about their modes of action, and results from controlled laboratory experiments in which the reproductive effects of known concentrations of individual chemicals can be assessed. I will then attempt to extrapolate these results to wild populations of fish, which can be exposed to complex mixtures of chemicals potentially capable of causing endocrine disruption. I will highlight the many gaps in our knowledge, and suggest approaches that will help us to make a better judgement of how serious an environmental problem endocrine disruption is to the reproductive physiology of fish.

OP-45

SUITABILITY OF TESTING STRATEGIES TO EVALUATE ENDOCRINE DISRUPTING CHEMICALS (EDCS) IN FISH

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There is world-wide interest in developing short-term testing methods to evaluate the potential of individual chemicals and complex effluents to affect reproduction. We have evaluated the utility of measures of response at the receptor, tissue and whole animal level to predict the effects of EDCs in fish. Our studies demonstrate that estimates of the activity of suspected EDCs based on receptor binding studies should be viewed with caution. For example, studies with androgen receptors revealed marked species (goldfish, rainbow trout) and tissue (brain, testis and ovary) differences in the binding of EDCs. Moreover, the use of mammalian receptor binding assays to predict effects of EDCs in fish is not recommended owing to major differences in the potency of EDCs, including DDT, DDE, sitosterol and hydroxylated PCBs, measured in teleost estrogen and androgen receptor assays. We also showed that potency estimates for EDCs based on receptor binding often do not accurately reflect *in vivo* activity. This was due in part to the observation that estrogenic EDCs (sitosterol, nonylphenol, pulp mill effluent) function as partial agonists *in vivo* which makes estimation of their potencies difficult at best. As well, individual compounds (sitosterol) and complex mixtures (pulp mill effluent) exert their effects through multiple mechanisms of action which limit the ability to make extrapolations of potency back to receptor binding data. Collectively, these studies illustrate the need to focus attention on *in vivo* testing of the activity of suspected EDCs.

OP-46

ESTROGENIC POTENCY OF XENOBIOTICS: DETERMINATION OF THE MOLECULAR MECHANISMS OF ACTION USING TWO COMPLEMENTARY BIOASSAYS

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To investigate the endocrine disrupter potencies of chemical mixtures found in environment and their interactions with estradiol receptor (ER), a genetically engineered yeast strain stably transformed with reporter plasmids and rainbow trout ER gene, as well as an aggregate culture system of rainbow trout hepatocytes were developed. In these two bioassays 49 chemical compounds were analysed for their estrogenic potency. Different metabolites were also tested in both the recombinant yeast which possesses low biotransformation activity and hepatocytes exhibiting most of the xenobiotic biotransformation capacity of the organism. Among the estrogenic compounds, 70 % were able to activate ER in yeast and hepatocytes, however 30 % of these active compounds exhibited estrogenic activity in only one of the bioassays. This suggests that some of the metabolic transformation can either increase or reduce the estrogenic potency of the chemicals. In another term the parent compounds do not necessarily exhibit estrogenic action as a result of direct ER binding but they may act either through their metabolites or via different gene activation pathways. Both bioassays were also used to elucidate molecular mechanisms of action of some xenobiotics such as 4-n-nonylphenol (4-NP, a potent estrogenic-like compound) or cadmium (Cd, an inhibitor of vitellogenesis). Our recent results showed that compared to estrogens, 4-NP induced differential ER conformation which results in a differential gene activation. Moreover, Cd-mediated inhibition of vitellogenesis could be correlated to modification in the transcriptional activity of ER.

OP-47

LONG-TERM EFFECTS OF NONYLPHENOL ON VITELLOGENIN AND ESTROGEN RECEPTOR EXPRESSION DETERMINED BY QUANTITATIVE COMPETITIVE RT-PCR AND ON SEX DETERMINATION IN JUVENILE RAINBOW TROUT

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Nonylphenol (NP) is a degradation product of surfactants that enter aqatic systems mainly via sewage treatment plants. It has been demonstrated that after short-term exposure, vitellogenin (VG) in juvenile male fish is induced. Hitherto, the estrogenic activity of NP has not been analysed after long-term exposure. The aim of this study is the analysis of low concentrations of NP on sex determination and vitellogenin expression in rainbow trout after long-term exposure. Vitellogenin is a precursor of the egg yolk protein, synthesized in the liver under the control of the female sex hormone estradiol and transferred to the ovaries via blood. Induction of VG can therefore serve as a biomarker for estrogenicity. In this study, rainbow trout eggs were exposed 20 d after fertilization to NP concentrations of 1 and 10 µg/l under controlled laboratory conditions. After 6 and 12 months expression of VG mRNA and estrogen receptor (ER) mRNA was analysed in the liver by quantitative RT-PCR, and VG protein using polyclonal antibodies in western blots. Quantitative competitive RT-PCR included primer design and use of heterologous standards for quantification. Both VG mRNA and protein were induced in NP-exposed rainbow trout in a dose-dependent manner. Increase of VG mRNA and protein were already observed at 1 µg/l NP. No effect on sex determination has been found at 1 and 10 µg/l NP, respectively. The sex ratio did not change and no ovo-testes have been found by histological analysis. This study shows that chronic exposure of fish early life stages to environmentally realistic concentrations of NP leads to induction of vitellogenin in juvenile fish, but not to negative influence on sex determination.

Zrf induit par dose 10 µg/l exposition 1 an

OP-48

THE THREE-SPINED STICKLEBACK AS A BIOMARKER FOR ANDROGENIC XENOBIOTICS

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Xenobiotics can act as endocrine disruptive chemicals (EDCs) by mimicking, antagonising or affecting the synthesis and metabolism of endogenous hormones and their receptors. The three-spined stickleback (*Gasterosteus aculeatus*) offers the potential for the assessment of reproductive disturbances caused by xenobiotic androgens. Male sticklebacks have pronounced androgen-dependent secondary sexual characteristics during their breeding season. One of these is in the kidney, which hypertrophies due to the production of a 'glue' protein, which is used to build a nest. Androgens were administered to sticklebacks via the ambient water. Kidney epithelium height (KEH) measurements, which provide an objective measure of the kidney hypertrophy, indicated significant stimulation in both males and females. As far as the authors are concerned, this is the first time that the kidney hypertrophy has been demonstrated in both females and males exposed to androgens administered via the water. Furthermore, the 'glue' protein, spiggin, was localised histochemicaly in the tubules of the secondary proximal segment. The effect was clear despite the fact that the fish were exposed to the hormones for only two weeks. Environmental chemicals, suspected to have endocrine modulating activity, are currently being screened. In order to improve the sensitivity and the speed of the bioassay for environmental androgens, the glue protein, spiggin (first characterised by S. Jakobsson, Stockholm University) was collected from the urinary bladders of fish in breeding condition as well as from nest threads and was purified with SDS-PAGE and injected into rabbits. An ELISA is being developed.

OP-49

CONTRACEPTIVE PILL RESIDUES IN SEWAGE EFFLUENT ARE ESTROGENIC TO FISH

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The estrogenicity of sewage effluent water to fish has been described in several countries. Natural oestrogens have been confirmed as major causing agents in several British rivers. In a recent study (Larsson et al., Aquatic Toxicology 1999, 45 (2-3) pp 91-97) we identified estrogenic substances by GC/MS in effluent water from a Swedish sewage treatment works receiving mainly domestic wastewater. Substances found include the synthetic estrogen used in contraceptives 17α-ethynylestradiol (4.5 ng/L), the natural estrogens estrone (5.8 ng/L) and 17β-estradiol (1.1 ng/L) and the weaker non-steroidal estrogens 4-nonylphenol (840 ng/L) and bisphenol A (490 ng/L). Ethynylestradiol exceeded levels shown to be estrogenic to fish by 45 times. The estrogenicity of the effluent water was investigated by introducing juvenile rainbow trout (*Oncorhynchus mykiss*) in cages downstream of the sewage treatment works. All estrogens indicated above were present in the bile of the fish. The estrogen inducible protein, vitellogenin, was found in large amounts in the plasma as far as two kilometers downstream as determined by ELISA and Western blotting. Our studies suggest that a widely used synthetic estrogen affects the endocrine systems of fish exposed to sewage effluent water.

Effekt norethisterone 2,5 mg/kg - Part the convert
in ethinylestradiol per kg — to be microorganisms

PP-136

CHARACTERISATION OF VITELLOGENIN IN ARCTIC CHAR (*SALVELINUS ALPINUS*)

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Vitellogenin (VTG) is the major yolk protein produced under estrogen control in all oviparous vertebrates. Several VTG-genes have been isolated and characterised from a large number of oviparous vertebrate species. In these species a small family of genes encodes VTG. The number of groups in the VTG-gene family varies depending of the species investigated. In teleost fish there is a lack of information concerning the number of genes encoding VTG. The present study was aimed at characterising VTG from Arctic char (*Salvelinus alpinus*). VTG was purified from plasma of 17-ß-estradiol treated juvenile Arctic char by precipitation and fast protein liquid chromatography (FPLC). The purified VTG was used to obtain polyclonal antibodies and the specificity was investigated by Western blot and enzyme linked immunosorbent assay (ELISA). A cDNA library constructed from estrogen treated Arctic char liver was screened for VTG. Several clones with high homology to rainbow trout (*Oncorhynchus mykiss*) VTG were isolated. The sequences suggest that the isolated clones code for at least two different VTG transcripts. The obtained VTG cDNA and the antibodies are being used to study the effect of substances with suspected estrogenic effect on vitellogenesis in Arctic char.

PP-137

THE USE OF JUVENILE ATLANTIC COD TO MONITOR ENVIRONMENTAL ESTROGENS

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Environmental estrogens have been found to affect both freshwater and marine fish, although most studies have focused on freshwater environments. Recent studies have indicated that there may also be effects in marine and estuarine areas. The aim here is to assess the use of plasma vitellogenin (Vg) in juvenile Atlantic cod (*Gadus morhua* L.) as a tool to monitor environmental estrogens in marine systems. Juvenile cod have been used in experimental studies and for monitoring purposes. In all studies, blood was sampled from the caudal vein using a syringe treated with heparin and aprotinin. Plasma Vg was determined by ELISA using an anti-cod Vg primary antiserum*. Basal levels of Vg in juvenile cod is 1-2 ng/ml plasma or below, similar for both sexes, and there does not appear to be any large seasonal variation. Following injection of estrogens, the Vg response in juvenile cod is similar to that found for juvenile Atlantic salmon. Vitellogenin synthesis peaks at one week in juvenile cod held at 10-11°C and exposed to a natural estrogen (17-ß estradiol) or a model xenoestrogen (4-nonylphenol) through injection, or through water. Preliminary results do not indicate any seasonal effects in the Vg response of juvenile cod to estrogens in water. Biochemical and physiological characteristics of juvenile cod thus support its use for monitoring estrogens in marine ecosystems.

*Antiserum against cod vitellogenin was a gift from Drs. Silversand and Haux, Göteborgs Universitet, Sweden.

PP-138

MOTILITY AND MORPHOLOGY OF FISH SPERM AS MONITORS OF REPRODUCTIVE DISRUPTION BY HEAVY METALS

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The reproductive system of fish is increasingly threatened by a cocktail of anthropogenic chemicals, such as environmental oestrogens, heavy metals, pesticides and industrial chemicals. Although the very high concentrations of these toxicants, associated with spillage incidents, often results in dead fish in waterways, low-level pollution which does not cause such a visual impact is often ignored. Such low-level contamination in the environment, however, may be more detrimental to aquatic wildlife, than the high toxicant levels associated with gross pollution. Very low concentrations of pollutants can affect the reproductive capacity of fish, either by direct action on the gametes or indirectly via the endocrine system and consequent changes in hormone balance. In this paper we examine the direct effects of low concentrations of the heavy metals, mercury, lead, tin and copper, on sperm motility and morphology in a salmonid, the rainbow trout (*Oncorhynchus mykiss*) and a cyprinid, the goldfish (*Carassius auratus*). Motility and morphology parameters of metal-exposed sperm can be assessed easily, rapidly and quantitatively using computer-assisted sperm analysis (CASA) and the Hobson morphology software package. Initial results show that goldfish sperm had decreased motility at 0.1 mg l^{-1} mercury and was immotile at concentrations of 1 mg l^{-1} mercury, and above. The application of this methodology to monitoring the effects of metal-induced endocrine disruption on sperm quality will be discussed.

PP-139

THE INFLUENCE OF HEAVY METAL CATIONS ON THE MOTILITY OF SPERM CELLS OF LANDLOCKED STURGEON *ACIPENSER RUTHENUS* L.

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The effects of cations of copper, zinc, lead and mercury on the motility characteristics of spermatozoa of sterlet, *Acipenser ruthenus* L., the landlocked sturgeon species inhabiting European rivers, were examined. Sperm cells were exposed to a range of concentrations of pollutants in water at the moment of activation or while held in isoosmotic extender for 8-24 hours at 2°C. Video records of spermatozoan movement were examined using Computer Assisted Sperm Analysis (CASA). Data showing disturbance to the motility characteristics of sterlet sperm by heavy metals will be presented.

PP-140

AN UNIQUE OCCASION OF THE APPEARENCE OF DWARF INDIVIDUALS OF SILVER CARP *HYPOPHTHALMICHTHYS MOLITRIX* IN THE COOLING RESERVOIR OF THE CHERNOBYL NUCLEAR POWER PLANT AFTER THE ACCIDENT

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In 1988-1992 and 1995-1997 we studied the reproductive system of silver carp from the netcages in cooling reservoir of Chernobyl NPP (the accident happened in 1986). The structure of germ cells was investigated using light and electron microscopy. In 1989-1992 the progeny of fishes that survived in the accident were obtained. Reproductive indices (fecundity, percentage of fertilization and hatching of normal larvae, ejaculate volume and sperm concentration) were studied. In 1996 two dwarf sex-matured individuals (female and male) were found among 10 fishes hatched in 1991. The female was 0,24 m and 250 g; the male was 0,31 m and 461 g (usually 60 cm and 3000g). The destruction of previtellogenic and vitellogenic oocytes of dwarf female was observed. The appearence of dwarfs was connected with the specificity of radiation conditions and the low level of nutrition.

PP-141

EFFECTS OF ALKYLPHENOLS ON LIPID TRANSPORT IN COD (*GADUS MORHUA*) DURING GONADAL DEVELOPMENT

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Alkylphenols are suspected to be environmental estrogenes causing endocrine disruption in fish. Oil production in the North Sea results in large disposal of produced water into the sea. The alkylphenols constitute an important group of organic pollutants in the produced water and are usually found at 0,6-10 ppm (mg/l) levels. Phenol and cresol (C_1) stand for around 80%, but higher alkylated phenols from C_4 to C_7 are also reported at low concentrations (2-237 ppb (mg/l)). At the Institute of Marine Research a comprehensive study is conducted aimed to investigate whether or not low levels of alkylphenols give any endocrine disruption in cod and thus directly or indirectly affect the reproduction of this species. The investigation is done on first-time spawning cod (2 year-old fish). The fish were divided into a control group and two exposed groups. The fish were followed from September to January (spawning), and sampled once a month. The exposed groups were given a mixture of 4-tert-butylphenol (C_4), 4n-pentylphenol (C_5), 4n-hexylphenol (C_6) and 4n-heptylphenol (C_7) mixed in the food (wet pellet) at doses of 5 ppb and 500 ppb per fish per day, respectively. The lower dose represents an expected realistic concentration, and the high dose a positive control. We are in this presentation comparing the fatty acids profiles from liver, plasma and gonad to invest if the phenols affect the levels or composition of fatty acids.

PP-142

HORMONES, ENZYMES, GAMETES AND THE ENVIRONMENT

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Most teleost fish have a well-defined seasonal cycle of reproduction, which is regulated by the endocrine system in response to environmental cues. Out-of-season spawning can now be induced in many species by manipulation of photoperiod and/or temperature so that gametes can be produced at any time of the year for aquaculture. Anthropogenic influences can also alter the reproductive capability of wild fish, either by artificially warming the waters in which they live or by chemically disrupting their reproductive endocrine system. Normal gonadal development and production of viable sperm and eggs is dependent upon a delicate balance between a number of key enzymes and the hormones that they produce. This balance changes in response to natural seasonal cues, but can be disrupted by both experimental manipulation to induce out-of-season spawning and by anthropogenic chemicals, resulting in changes in gamete quality. Measurement of gamete quality can therefore play a valuable role in both monitoring the effects of endocrine disruptors of human origin, and determining whether artificial manipulation of seasonal cycles can affect fertility. This paper describes the use of computer assisted methods for the analysis of sperm motility and morphology, and for the measurement of egg size and number which may have widespread application in assessing the effects of altered environmental conditions on the fertility of fish.

PP-143

CHLORINATED FATTY ACIDS AS ENVIRONMENTAL POLLUTANTS

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Chlorinated fatty acids are the major contributors to extractable, organically bound chlorine in fish lipids. A known anthropogenic source for chlorinated fatty acids is chlorine bleached pulp production. Additional anthropogenic sources may exist e.g. transformation of known chlorinated pollutants, and a natural production may also occur. Chlorinated fatty acids have a wide geographic distribution, they have so far been identified in fish both from Alaskan waters, the whole of the Scandinavian west-and north-coast as well as from the Baltic. Chlorinated fatty acids are bound in lipids such as triacylglycerols and phospholipids, and because of that, chlorinated fatty acids have a theoretical potency to disturb any biological process involving lipids. They have for instance been shown to disturb cell membrane functions. It is not known how chlorinated fatty acids become incorporated in lipids, but in uptake experiments with perch (*Perca fluviatilis*) orally ingested dichlorostearic acid was assimilated similarly to stearic and oleic acids. In toxicological studies of chlorinated fatty acids, the most pronounced effects have been found on reproduction related processes. It has been indicated that chlorinated fatty acids are degraded by beta-oxidation mainly down to chlorinated myristic acid (14-C). In the present study we have indications that dichlorostearic acid can be transferred in a laboratory food-chain. If chlorinated fatty acids are assimilated like "normal" fatty acids and are recalcitrant to beta-oxidation, they may be regarded as environmentally stable pollutants, enduring in the biosphere.

PP-144

ESTROGENIC EFFECTS OF BUTYL BENZYL PHTHALATE AND DI-N-BUTYL PHTHALATE IN JUVENILE ATLANTIC SALMON (*SALMO SALAR*)

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Environmental contaminants of different origin, may interfere with the endocrine system in animals. One group of these endocrine disruptors known as environmental estrogens or pseudoestrogens are known to mimick the female sex hormone 17β-estradiol. Some phthalates, which are common contaminants in water, have been identified as weak pseudoestrogens in several *in vivo* tests. The effects of these compounds on fish *in vivo* are, however, largely unknown. In this work, we have exposed juvenile Atlantic salmon (*Salmo salar*) to the phthalates butyl benzyl phthalate (BBP) and di-n-butyl phthalate (DBP) under flow-through conditions in order to investigate possible estrogenic effects *in vivo*. Induction of the yolk precursor protein vitellogenin (Vtg) and eggshell (zona radiata) protein (Zrp) synthesis in juvenile non-vitellogenic fish has been used as a biomarker for estrogen receptormediated effects. The results are discussed with reference to the *in vitro* binding of BBP and DBP to hepatic estrogen receptor and plasma steroid hormone binding proteins.

PP-145

SEXUAL DISRUPTION IN GUDGEON (*GOBIO GOBIO*) IN UK RIVERS

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A recent study of roach (*Rutilus rutilus*) in UK rivers demonstrated a widespread occurrence of intersexuality (the simultaneous presence of both testicular and ovarian tissues in the gonad). The occurrence of the intersex condition was correlated with exposure to oestrogenic effluent from sewage treatment works (STWs). To investigate whether the intersex condition was species specific or a more general phenomenon in fish in UK rivers, a second cyprinid fish, the gudgeon (*Gobio gobio*), was studied: a species with a different ecological niche and different reproductive strategy compared with the roach. Four hundred and five gudgeon were collected from sites on two rivers, both receiving STW effluent discharges (Rivers Aire and Lea) and from two stillwater Lakes that did not receive inputs from STWs. Gonads were dissected out and examined histologically, and plasma concentrations of vitellogenin (a biomarker for oestrogen exposure) measured by ELISA. Intersexuality was found in gudgeon derived from all the study sites (the incidence varied between 6% and 15%). The highest degree of intersexuality (proportion of the gonad affected) was seen in gudgeon at the more polluted sites; sometimes there was almost complete sex reversal. In contrast, in the 'intersex' fish at control sites, only very few oocytes occurred. The plasma concentrations of VTG, and the gonadosomatic index data, support the data published for the roach, and indicate that intersexuality in gudgeon in the UK rivers has resulted from a feminisation of males.

PP-146

MODULATORY EFFECTS OF 4-NONYLPHENOL ON LH PRODUCTION IN AFRICAN CATFISH PITUITARY

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Alkylphenols are considered to be environmental contaminants with oestrogenic capacity. They have been implicated in the disruption of endocrine controlled functions in wildlife. To test the hypothesis that endocrine disruption not only affects peripheral organs (gonads, liver), but also the brain-pituitary neuro-endocrine system, we studied the influence of an alkylphenol (4-nonylphenol (4-NP)) on the luteinizing hormone (LH) production by the African catfish pituitary. To this end, juvenile catfish (10-15 weeks of age) were treated daily with 4-NP (10 mg/l aquarium water). After 7 days, pituitaries were collected, homogenized and analyzed for LH content by radioimmunoassay. Male individuals showed a significant ($P<0.05$) increase in LH content from 1.1 ± 0.3 to 5.3 ± 1.4 ng/pituitary, whereas only a slight but not significant effect was observed in female fish (2.9 ± 1.2 to 3.8 ± 0.9 ng/pituitary). For both sexes, no changes in LH plasma level were found. To investigate whether 4-NP affects the LH releasable pool, the effect of chicken GnRH-II (cGnRH-II) on LH release by pituitary cells in primary cultures was studied. Treatment of the cells with chicken GnRH-II (cGnRH-II) dose-dependently stimulated LH release (EC50 = 0.1 nM). However, pretreatment with 4-NP (1 mM; 24 h) neither altered basal LH release nor the EC50 for cGnRH-II, but the maximal cGnRH-II stimulation was decreased by 73% ($P<0.05$). In contrast, 17β-estradiol (2 nM; 24h) did not affect the dose-relationship of cGnRH-II. In conclusion: these data indicate that 4-NP acts as an oestrogenic endocrine disruptor by increasing the amount of LH in African catfish gonadotrophs. It seems not to affect the basal LH release, but certainly has an effect on the GnRH-responsiveness and/or the LH releasable pool.

PP-147

NONYLPHENOL AS A XENOESTROGEN IN TILAPIA - HYPOPHYSEAL EFFECTS

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p-nonylphenol (NP) is a non-steroidal compound occurring in the aquatic environment and has estrogenic activity. A project has been initiated to examine its effect on the brain-pituitary gonadal axis in fish. We report here on possible *in vivo* and *in vitro* effects of NP or estradiol on the gonadotropins and on gonadal development in maturing male blue tilapia (*Oreochromis aureus*). Maturing fish (19-50 g bw; initial GSI 0.13±0.02) were exposed to estradiol 17-β (E) or NP (both at 1 µg/l) added every 48 h to 100 liter glass aquaria and the fish were examined after 3 and 5 weeks. GSI increased after 5 weeks in the controls, while in E or NP-treated fish it did not change. Plasma taGtH levels in E or NP groups were significantly lower than in the control fish after 5 weeks. The mRNA levels of FSH beta showed a dramatic decrease after 3 weeks, and remained lower thereafter. Such a decrease was not seen in the mRNA levels of LHbeta. In order to examine the possible direct effect of NP on the secretion of taGTH (LH), dispersed pituitary cells were challenged for 48 h in culture with NP (0.1-100 micromolar). The secretion of taGtH increased in response to NP in a dose-dependent manner. The results of the present work indicate that NP exerts an estrogenic effect at the pituitary level and may, therefore, interfere with the normal reproductive process in fish exposed to contaminants of this kind.

PP-148

NONYLPHENOL AS A XENOESTROGEN IN TILAPIA - GONADAL EFFECTS

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Nonylphenol (NP) is a xenobiotic compound used in resins, plasticizers and surface active agents and its para isomer is considered as estrogenic. Apparently, the formation of vitellogenin (Vg) in male is the most sensitive estrogenic effect in fish. In order to establish an assay for determining xenoestrogens' effect in tilapia (*Orecochromis aureus*), Vg was isolated from plasma of estradiol treated males by discontinuous NaBr density gradient ultracentrifugation (Wallaert & Babin, J. Lipid Res 35; 1619-33, 1994), after incubating plasma with Sudan black. Each fraction was subjected to native and SDS PAGE and Western blot analysis. The fraction containing Vg was identified by a positive reaction to polyclonal antiserum generated against the ca. 170 kDa subunit of *Sparus aurata* Vg. Vg was not detected in the lipoprotein fractions of control untreated fish. A polyclonal antibody is being prepared against the ca. 190 kDa subunit of tilapia Vg and will be used in an ELISA for determining Vg levels in tilapia exposed to xenoestrogens. The possibility that estrogens may directly affect the secretion of testosterone from testes of fish was examined *in vitro*. Testicular tissue from immature fish (GSI=0.15±0.01) was rinsed for 2 h in BME and then exposed for 4 h to estradiol (10 nM to 10 µM). Testosterone determined in the medium showed a dose-dependent increase in response to estradiol. The assay did not cross react with the estrogen. The effect of NP in this system will be reported.

PP-149

THE IMPORTANCE OF APPROPRIATE SPECIES SELECTION FOR UNDERSTANDING REPRODUCTIVE ENDOCRINE EFFECTS OF XENOBIOTICS ON FISH: *FUNDULUS HETEROCLITUS* USE IN EASTERN CANADA.

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Evidence demonstrates that environmental contaminants have toxic effects on the reproductive endocrine systems of fish which can result in impaired reproductive performance. Many fish species have been used in field, mesocosm, and laboratory studies monitoring contaminated bodies of water and identifying effects of endocrine-disrupting compounds. However, species that are not environmentally relevant or are suitable to one of only lab or field have been used in some studies. This hampers the ability to interpret findings and to make estimates of effects on wild populations. Species selection needs to follow ecotoxicological principles: species should be relevant, suitable for lab and field, sedentary, have an understood physiology, and be capable of integrating the hazard. We have used mummichogs (*Fundulus heteroclitus*) to monitor coastal waters in eastern Canada for reproductive endocrine dysfunction from contaminated water. A hardy, endemic, estuarine species, mummichogs are sedentary, dimorphic, a good size for field and lab studies, and have characterized lunar spawning cycles. Wild fish living close to an industrialized port city show reproductive endocrine cycles that differ from fish living further from pollution sources. Mummichogs exposed to pulp mill effluent in a mesocosm system remain healthy when kept for up to 60 days; reduced plasma steroid levels in adults and decreased juvenile growth indicate they are responding to pulp mill effluent in ways similar to other species. To best integrate and interpret our understanding of endocrine disruption effects on fish, therefore, it is important where possible to select species such as the mummichog.

PP-150

MODULATION OF PITUITARY GONADOTROPIN SUBUNITS mRNA LEVELS IN JUVENILE ATLANTIC SALMON BY THE ENVIRONMENTAL ESTROGEN, 4-NONYLPHENOL

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Degradation products of environmenal alkylphenol polyethoxylates such as 4-nonylphenol (NP) are reported to have estrogenic effects such as induction of synthesis of vitellogenin (Vtg), eggshell zona radiata proteins (Zrp) and modification of gonadal growth and morphology that may lead to reproductive disturbances in fish. To examine the effects of NP on the expression of potential target genes in the pituitary, we isolated partial cDNA sequences of Atlantic salmon (*Salmo salar*) gonadotropin subunits FSHb (GTH Ib) and LHb (GTH IIb). The effects of NP and E2 on the mRNA levels of pituitary gonadotropin subunits FSHb and LHb were investigated in juvenile Atlantic salmon after i. p injection with NP (125 mg/kg body weight) and E2 (2 or 5 mg/kg body weight), 3 or 4 days post injection. Northern blot and RT-PCR analyses showed that NP mimics E2 in modulating FSHb and LHb mRNA levels. Both NP and E2 resulted in significant ($p < 0.001$) elevation LHb mRNA synthesis in females. E2 caused a slight decrease in FSHb mRNA synthesis in both sexes. NP also caused a small, not significant decrease in FSHb mRNA levels but only in females. The data show that NP mimics E2 in inducing LHb mRNA synthesis suggesting that NP and related compounds have the potential to perturb neuroendocrine regulation of gonadotropins by E2 in the brain-pituitary-gonadal axis during reproductive cycle in fish.

PP-151

THE POTENTIAL USE OF SVALBARD CHARR (*SALVELINUS ALPINUS* L.) IN DETECTION OF XENOESTROGENS IN ARCTIC REGIONS

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Animals in Arctic regions are exposed to persistent environmental contaminants through atmospheric and oceanic transport. Some of these pollutants are known or suspected endocrine disruptors, and much attention has been drawn against the xenoestrogens, compounds mimicking the female sex hormone. Due to lack of knowledge of effects on Arctic species, there is a need for establishing relevant *in vitro* and *in vivo* tests for such effects. The Svalbard charr (*Salvelinus alpinus* L.) represents a potential aquatic bioindicator for endocrine disruptors in the Arctics. In this work, we have characterized plasma steroid hormone binding protein (SHBP) and hepatic estrogen receptor (ER) from male, female and juvenile individuals. Maximum specific binding (Bmax) and dissociation constant (Kd) for SHBP and ER from Svalbard charr has been determined through saturation studies with radio labeled estrogen ([2,4,6,7-3H]Oestradiol). In addition interactions of both natural hormones and pseudoestrogens has been tested *in vitro* for possible agonistic or antagonistic effect. Based on the results from these findings, the need of further *in vitro* and *in vivo* studies on this species will be discussed.

PP-152

THE USE OF SMALL FISH SPECIES FOR ENVIRONMENTAL EFFECTS MONITORING OF INDUSTRIAL EFFLUENTS: A COMPARISON OF REPRODUCTIVE RESPONSES IN LARGE AND SMALL SPECIES

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New Canadian Federal Government Regulations for Environmental Effects Monitoring (EEM) require the industrial sector to examine their receiving environments for impacts. These regulations were initiated in the pulp and paper sector in 1992 and first cycle results submitted in 1995 identified a number of limitations. The most cited problem for cycle 1 was the inability to capture sufficient numbers of two sentinel species. The mobility of large sentinel species was also identified as a potential problem as one was unsure of exposure. For these reasons we have been examining the use of small forage fish species for use in the EEM program. The program requires the examination of fish populations for a number of parameters including age structure, growth and reproduction. We have initiated studies at a number of sites comparing the responses of various forage fish species to that of different large sentinel species. Previous studies at these sites identified alterations in gonadal development and corresponding reductions in plasma sex steroid hormone levels where measured. Our studies indicate that it is possible to examine all measures of fish health required for the EEM program in forage fish species. We have used the *in vitro* production of reproductive steroids as a substitute to circulating steroid levels. Examination of results demonstrates similarities between the two species at some sites but differences at others. There are several possible reasons for these differences including different species sensitivities to the effluents, different exposure conditions and the greater potential mobility of the larger species.

PP-153

REPRODUCTIVE BIOMARKERS IN WHITE STURGEON: IS ENDOCRINE DISRUPTION OCCURRING IN THE COLUMBIA RIVER?

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We investigated the reproductive physiology of wild white sturgeon (*Acipenser transmontanus*) from the Columbia River. Low reproductive productivity in sturgeon from some reservoirs behind hydroelectric dams led us to hypothesize that this low productivity could be attributable to endocrine disruption. This species is of great importance to the Pacific Northwest of the US where, for example, the harvest in the lower Columbia River is about 45,000 fish per year. Wild white sturgeon were sampled after harvest in the Columbia River in 1996 and 1997. Plasma levels of sex steroids and vitellogenin, and liver EROD activity (an indicator of contaminant exposure) were measured. Males had significantly higher levels of testosterone and 11-ketotestosterone than females; however, estradiol was similar between the sexes and the levels were much lower than those reported from females undergoing vitellogenesis. Vitellogenin was detected in plasma from a few males; however, these levels were far below those found in white sturgeon females undergoing vitellogenesis and below levels found in male rainbow trout or in carp that had been exposed to sewage treatment plant effluent. In white sturgeon collected from two reservoirs, hepatic ethoxyresorufin-O-deethylase (EROD) activity indicated induction by exposure to contaminants, but the low levels of steroids found in general in sturgeon from the Columbia make it difficult to conclude any disruptive effect on the endocrine system or reproduction. However, these elevated EROD activities occurred in reservoirs where condition factor of the fish was also reduced.

PP-154

ENDOCRINE DISRUPTORS AND REPRODUCTIVE TOXICOLOGY, A MOLECULAR APPROACH

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The study of how anthropogenic environmental pollutants can disturb hormonal balances and alter reproductive processes in an organism, has become a relevant subject of environmental toxicology. It has been shown that many compounds can mimic the natural hormone estradiol, inducing estrogenic processes. Chemicals interfering with the endocrine system in this or other ways have been named "endocrine disrupting chemicals". Alkylphenol ethoxylates (APEs) are non-ionic surfactants broadly used in a variety of activities (pulp and paper industry, agriculture, textile manufacturing, petroleum production, plastic production, detergents, cosmetics etc.) They have been shown to be extremely toxic to aquatic organisms. Their toxicity increases with the increasing length of hydrophobic chain. Therefore, the degradation products such as nonylphenol (NP), are always more toxic than the parent compound. The estrogenic effects of NP in Atlantic salmon (*Salmo salar*) have been previously studied in our laboratory both *in vitro* (hepatocyte culture) and *in vivo*, and antibody as well as cDNA probes have been developed and used in these studies. In both systems, NP was shown to induce the egg-yolk precursor protein vitellogenin (Vtg) and the eggshell zona radiata proteins (Zrp). To obtain additional information about estrogenic effects at the molecular level, we have started to search for other differentially expressed genes in NP-exposed salmon versus control. Atlantic salmon juveniles were experimentally exposed to 20 mg/kg b/w of NP with a single intraperitoneal injection *in vivo*. Liver mRNA was extracted and used in a replica colony screening (RCS) strategy to study the differential transcription of genes in NP-exposed fish. This strategy may identify differentially expressed genes which may be interesting for elucidating the mechanisms behind these responses and for the development of new biomarkers for endocrine disrupting compounds.

PP-155

VITELLOGENIN IN MALE FLATFISH; POSSIBLE INVOLVEMENT OF ENDOGENOUS OESTRADIOL

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A recent survey of UK estuaries has revealed significantly elevated (and, in at least three estuaries, enormously elevated) concentrations of vitellogenin in the blood plasma of male flounders (*Platichthys flesus*). Although the evidence points strongly to the involvement of exogenously-derived 'xenoestrogens', the possibility has not yet been fully explored that the vitellogenin, whether whole or in part, is produced as the result of endogenous oestradiol secretion. This is not far-fetched. In the closely-related North Sea plaice (*Pleuronectes platessa*) reproductively mature males, collected from the middle of the North Sea, have concentrations of oestradiol of up to 3 ng/ml (as measured by radioimmunoassay). Although these are not a high as those found in reproductively mature female plaice (100 ng/ml) they nevertheless appear to be sufficient to stimulate vitellogenin production of about 2.5 mg/ml. The results from the flounder indicate that mature females produce similar amounts of oestradiol to female plaice. Mature males, however, collected at the same time and in the same areas as the plaice, have considerably lower concentrations of both oestradiol (30 pg/ml) and vitellogenin (60 ng/ml). Interestingly, males from the Mersey and Tyne estuaries, which have massively elevated concentrations of vitellogenin (of up to 60 mg/ml), have slightly elevated oestradiol concentrations (between 60 and 150 pg/ml). However, it is unlikely that these are high enough to have been the cause of the high vitellogenin concentrations.

PP-156

CHANGES IN SECONDARY SEX CHARACTERISTICS AND GONADAL SIZE IN FISH EXPOSED TO PULP MILL EFFLUENTS OVER THE PERIOD OF MILL MODERNIZATION

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Studies conducted near a large bleached kraft pulp mill between 1988 and 1991 demonstrated dramatic alterations in reproduction, including delays in sexual maturity, reduced gonadal sizes and reduced secondary sex characteristics. The installation of secondary treatment in the fall of 1989 was associated with dramatic improvements in effluent quality, but the fish reproductive responses were not improved. Detailed studies showed that the low production of steroid hormones in fish was associated with dysfunctions within the steroid synthetic pathway, and that the biochemical site of these breaks could change with season and mill sites. Exposure of goldfish to effluent demonstrated steroid hormone depressions within 4 d of exposure. However, after a shutdown associated with increasing the chlorine dioxide substitution of the mill, the ability to detect these steroid responses in laboratory exposures, and in wild fish was reduced. It is not believed that the change in chlorine dioxide substitution level was associated with this improvement. Three years later, the fish are showing improvements in gonad size and secondary sex characteristics in male fish, but 50% of female fish are now displaying male secondary sex characteristics. While recovery is not complete, it is apparent that some unidentified mill process changes can be associated with improvements in the reproductive performance of wild fish. The incomplete recovery of prespawning steroid hormone levels and the appearance of male secondary sex characteristics in female fish suggests that all issues are not resolved at this site. The presentation will update findings with data from May 1999 collections.

PP-157

SKEWED EMBRYONIC SEX RATIOS IN A VIVIPAROUS FISH – A RESULT OF ENDOCRINE DISRUPTION?

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Sex ratios in catches of wild adult fish are often affected by sex related differences in growth, behaviour and survival. Therefore, such sex ratios may not reflect the relative number of males and females that are recruited to the population. However, this can be circumvented by studying unborn fish embryos. We have found skewed sex ratios among embryos from the viviparous eelpout (*Zoarces viviparus*) which could indicate exposure to endocrine disrupting chemicals. The sex ratio was close to 50/50 at two presumably clean sites from Kattegat/Skagerrak and two from the Baltic Sea. On the Swedish Baltic coast near a large pulp mill the relative number of female embryos was significantly lower (42 %; p=0,006) but approached 50% further south in the discharge gradient. Treatment of females during early pregnancy with methyltestosterone inhibited oocyte development in all embryos, and instead testis-like tissue was formed. Thus, masculinization found in the field could be caused by exposure to androgen mimics or substances interfering with steroid-synthesis, activity or excretion. This may arise from exposure to the pulp mill effluents, alternatively water from the river Emån with a long history of organochlorine and metal pollution, entering ten km north of the pulp mill outlet. Irrespectively of the cause, reduced numbers of female offspring may have negative impacts on the recruitment capacity of a population.

PP-158

AFFINITY OF ESTRADIOL RECEPTORS FROM RAINBOW TROUT HEPATOCYTES FOR PHYTOESTROGENS

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The fish liver, as the liver of other classes of egglaying vertebrates, responds to exogenous or endogenous estrogens. The highly sensitive response is the vitellogenin (VTG) synthesis which expression is estradiol-dependent. For the VTG to be expressed, estradiol must bind to its receptors (ER) and form a complex, which activates the transcription of mRNA-VTG when the ER concentration is sufficient. Thus, a competitive binding assay is used to determine the affinity of the estradiol receptor to estrogenic xenobiotics. In this case, xenobiotics are phytoestrogens, considered as dietary estrogenic contaminants as they induce vitellogenin synthesis *in vivo*. This has been previously demonstrated in our laboratory for the isoflavonoids : genistein, daidzein, formononetin, biochanine A and equol. It seems then very interesting to test their affinity compared to estradiol for ER. This experiment consists in incubating estradiol receptors (from liver nuclear fractions) with tritiated estradiol (10nM) and to displace this binding by adding increasing concentrations of cold competitors. Phytoestrogens concentrations used were generally 1000 times higher than estradiol concentrations, except for genistein and formononetin which seem to have the higher binding activities. For each compound, the determination of the concentration of phytoestrogens showing a 50% displacement (DC50) allow us to classify phytoestrogens according to their affinity for the rainbow trout estradiol receptor. In that scale, estradiol (DC50=7nM) > formononetin (DC50=300nM) > genistein (DC50=500nM) > equol (DC50=5µM) > daidzein (DC50=7µM) > biochanin A (DC50=100µM). These results proved that these phytoestrogens mimicking estradiol, can pertube the endocrine system by competing for estrogen receptors. Therefore they can change the pattern of natural estrogens and have estrogenic activities. These phytoestrogens can act as estrogenic or antiestrogenic compounds depending on their concentrations. The anti-estrogenic activity is revealed when the phytoestrogen concentrations are low and when the compounds inihibit the binding of estradiol to its receptor.

PP-159

THE DEVELOPMENT OF A QUANTITATIVE RT-PCR ASSAY FOR MEASURING P450 AROMATASE EXPRESSION IN THE FATHEAD MINNOW (*PIMEPHALES PROMELAS*) WITH A VIEW TO ITS APPLICATION FOR STUDYING ENDOCRINE DISRUPTION

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Some of the effects caused by endocrine disrupting chemicals are believed to be mediated through alterations in the steroidogenic pathways. The cytochrome P450 enzyme aromatase converts androgens into oestrogens thus in females, it plays a key role both in sexual differentiation sexual maturation. The aim of this study was to measure aromatase expression in the fathead minnow and to asses its suitability as a molecular biomarker for endocrine disrupting chemicals. A quantitative RT-PCR (QC-PCR) assay was set up with specific primers designed to regions in the open reading frame of the aromatase sequence in the ovary of the fathead minnow. Using the same primes, a competitive template was constructed, which was smaller in size. Known amounts of competitor cRNA were introduced into the RT reactions together with the RNA to be analysed in PCR. Due to their size differences, the target and competitor template could subsequently be distinguished on gels. Using quantitative PCR, P450 aromatase expression was detected in ovary, testis and brain of sexually maturing fathead minnows. Aromatase expression in the gonads was around 10-100 fold lower compared with brain samples. The higher expression of aromatase in the brain compared with the gonads was confirmed by Northern hybridisation, in which a transcript size of 2.7 kb could be detected in brain, but not in the gonads. This quantitative PCR assay is now being applied to asses alterations in aromatase expression in fathead minnow which have been exposed to endocrine disrupting chemicals.

PP-160

G-GLUTAMYL TRANSPEPTIDASE AS A POSSIBLE MARKER OF SERTOLI CELL FUNCTION IN FISH IN RELATION TO ESTROGENIC EXPOSURE

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In fish, estrogenic chemicals are known to have marked effects on the male reproductive system. Therefore finding markers of reproductive effects is of great importance and interest. In mammals, g-glutamyl transpeptidase (g-GTP) has been used as a marker of Sertoli cell function. If this also applies to fish, g-GTP could be a possible marker of effects on testes after exposure to estrogenic chemicals. In earlier studies we have measured the testicular activity of g-GTP in two viviparous fish using a commercial kit. In the marine eelpout *Zoarces viviparus* we found a marked reduction in the testicular activity of g-GTP concomitant with an altered (squamous or degenerated) Sertoli cell structure after nonylphenol as well as estradiol treatment (intraperitoneal injection). However, treatment (water exposure) of the freshwater platyfish *Xiphophorus maculatus* with nonylphenol or estradiol had no significant effect on the activity of g-GTP in the testes despite an observed hyperthrophy of the Sertoli cells. However, a tendency of increased activity could be observed. At present we are validating the g-GTP as an enzyme marker in fish testis. This includes studies on the seasonal changes of testicular g-GTP activity of the eelpout, and examination of the localisation of g-GTP in the testes. The need for histochemical studies to localise g-GTP specifically to the Sertoli cells in fish is essential before any conclusions about g-GTP as a possible Sertoli cell marker in fish can be made. Results from these ongoing studies and from experiments with estrogenic chemicals will be presented and discussed.

PP-161

ESTROGEN IN FOOD OR WATER SEVERELY EFFECT THE MALE GOLDFISH (*CARASSIUS AURATUS*) SEXUAL BEHAVIOUR

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Endocrine disrupting chemicals (EDCs), mimicking the female hormone 17β-estradiol (E2), and the hormone itself have been found in significant concentrations in rivers and lakes. Several studies, in lab or by using caged fish in nature, have shown that fish are affected by exposure to different EDCs. It was recently reported that wild populations of roach (*Rutilus rutilus*) showed a high incidence of intersexuality (i.e. feminised testicles) in rivers throughout the United Kingdom. Further, in studies of estrogenic activity in effluents from sewage-treatment works (STW) in British and Swedish rivers it was shown that the estrogen activity originated mainly from three sterols, the natural hormones E2 and estrone and the synthetic hormone 17α-ethynylestradiol (used as contraceptive). The aim of this study was to examine how E2 affects pheromone induced sexual behaviour and physiology in mature male goldfish. E2 was administered to mature male goldfish in physiological concentrations and by two routes, the food or the water. After four weeks, the sexual behaviour of the male interacting with a sexually active female for 15 minutes was recorded. A blood sample was taken for sex hormone analysis and the fish were sacrificed to measure gonadosomatic index, presence of spawning tubercles and milt. As predicted, the physiology was significantly affected but the results also showed that the male sexual behaviour were almost totally inhibited. The study indicates that the concentrations of EDCs measured in certain rivers would be high enough to significantly and adversely affect the male goldfish reproduction.

PP-162

EFFECTS OF ESTROGENIC SUBSTANCES ON DIFFERENT DEVELOPMENTAL STAGES IN THE SEXUAL DIFFERENTIATION OF ZEBRAFISH, *DANIO RERIO*

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A number of structurally diverse chemicals are suspected to act as endocrine mimics in vertebrates. In order to investigate possible adverse effects of xenoestrogens on sexual differentiation and reproduction of fish, we use *Danio rerio* as experimental model. This species completes its life cycle from the fertilized egg to the mature adult within 3-4 months. Histological differentiation of ovaries occurs from 4 weeks after hatching onwards, whereas testes development occurs only 4-6 weeks later. Our objective was to reveal at which developmental stage of zebrafish estrogenic substances are able to induce vitellogenin synthesis, and if premature vitellogenesis would be correlated with altered gonadal normogenesis. In the present study, zebrafish fry were exposed to 1.7 x 10-7, 9.1 x 10-7, 5.6 x 10-6 and 3.4 x 10-5 µmol/L of the synthetic estrogen 17α-ethynylestradiol for a peroid of 65 days post-hatch. At the end of exposure time, whole body vitellogenin levels were quantitated by means of ELISA and Western blot using a polyclonal antibody raised against purified egg-yolk proteins of zebrafish. In parallel, the induction of vitellogenesis in gonads and liver was evaluated qualitatively by immunohistochemistry in paraffin-embedded tissue sections. In support of the results classical histological examinations of gonad development were performed additionally.

PP-163

MULTIVARIATE MODELLING TO EVALUATE CHANGES IN "ENDOCRINOLOGICAL -BEHAVIOURAL PATTERN"; EFFECTS OF OESTROGEN ON MALE GOLDFISH (*CARASSIUS AURATUS*)

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17β-estradiol (E2) and other hormones have been identified in effluents from sewage-treatment works at British and Swedish rivers. These and other endocrine disrupting substances may pose as a hazard to wildlife and humans. Multivariate methods such as Principal Component Analysis (PCA) and Partial Least Squares Regression Projection to Latent Structures (PLS) are powerful tools in the evaluation of complex biological experiments (large numbers of data, highly dependent variables, naturally varying variables and missing data) in that all systematic information can be extracted without loosing information. The aim of this study was to apply multivariate modelling in the evaluation of E2 induced effects on mature male goldfish. E2 was administered via the food (FE2) or the water (WE2). After four weeks of exposure seven behavioural (pushing, spawning etc.) and eight physiological variables (milt production, GSI etc.) were measured. The multivariate models were constructed by using the fish plasma E2 concentrations and the recorded behavioural and physiological responses. The obtained PCA model (FE2 and WE2 together) explained 72.4% of the variation. For the two resulting PLS models (FE2 and WE2 separately) the explained variation (R^2) and the predicted variation (Q^2) were also satisfactory. R^2=88.4%, Q^2=51.0% (FE2) and R^2=72%, Q^2=44.1% (WE2). In conclusion, the multivariate models describe the relation between E2 exposure and multiple responses in fish behaviour and physiology. The models had a high ability to predict fish plasma E2 concentrations and consequently whether the male had been exposed to E2. In addition they illustrated to what extent the measured variables were effected by E2 exposure. Modelling the change in "endocrinological-behavioural pattern" results in promising and useful tools to investigate substances or mixtures with unknown endocrine disturbing action.

PP-164

IMPACT OF HEAVY METALS AND ORGANIC POLLUTANTS ON HATCHING OF ATLANTIC HALIBUT

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The hatching in halibut embryos can be controlled by light manipulation. We have used this ability to try to distinguish effects of pollutants direct on the hatching process from developmental effects influencing hatching. Three different experiments were performed with heavy metals (Cd, Cu, Hg, Zn and As): Long-term exposure with natural hatching, long-term exposure with induced hatching and short-term exposure with induced hatching. One experiment was performed with natural hatching after exposure to organic compounds (PCB 77, 118, 153, BNF and BaP) for one day half way through the egg phase. In general the embryos were able to hatch in higher concentrations of pollutants after short-term exposure than after long-term exposure. The highest concentration of heavy metals resulted in 100% mortality (except Cd and As) prior to hatching. The intermediate concentrations delayed hatching, while the lowest concentrations seemed not to influence hatching. Hg, Cu and Zn appears to inhibit hatching in a dose-dependent manner both after short- and long-term exposure. Deformations in the yolk sac and body axis were observed after exposure to BNF and to the highest concentrations of Cd, PCB 77 and BaP. Deformations in the yolk sac were also observed after exposure to Hg.

Aquaculture

OP-50

ENDOCRINE AND MOLECULAR STRATEGIES FOR THE MANIPULATION OF SPAWNING IN FARMED FISH: CURRENT AND FUTURE PERSPECTIVES

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As aquaculture has intensified during the last three decades, it has become increasingly obvious that commercial hatcheries must gain complete control over the reproductive cycle of the cultured fish and produce fertilized eggs on demand. The early spawning induction technologies used pituitary extracts or mammalian gonadotropins. With the determination that in many farmed fish the failure to spawn in captivity reflects a lack of gonadotropin II (GtH II) release from the pituitary, research and development efforts focused on the use of gonadotropin-releasing hormones (GnRHs). Analogues of GnRH were designed which are resistant to enzymatic degradation, have a higher affinity to the GnRH receptor and are potent stimulators of GtH-II release and ovulation. These analogues were incorporated into a range of sustained-release delivery systems that have been optimized for the induction and synchronization of final oocyte maturation (FOM), ovulation, spermiation and spawning in multiple farmed species. The discovery that the brains of perciform fish contain 3 forms of GnRH, and information about their relative roles in the regulation of reproduction, is now being used to tailor more physiologically-compatible GnRH spawning induction therapies. The cloning of the genes and cDNAs coding the multiple GnRH forms and their receptors, and our current understanding of the regulation of GnRH synthesis and release, is shedding new light on the nature of the hormonal failure responsible for the absence of FOM, ovulation and spawning in many farmed fish, and will lead to the development of novel strategies for their induction.

OP-51

EFFECTS OF SUSTAINED ADMINISTRATION OF GnRHa ON GONADOTROPIN-2 (GTH-2) AND GONADAL STEROID LEVELS IN ADULT MALE SEA BASS (*DICENTRARCHUS LABRAX*)

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This study examined the effects of different modes of administration of GnRH analogue (GnRHa; [D-Ala^6, Pro^9 NET]-mGnRH) on GtH-2 and gonadal steroid plasma levels. Groups of sea bass received either GnRHa injection in saline (I; 25 µg/Kg body weight [BW] or one of the three types of GnRHa sustained release polymeric device: a fast releasing implant (ethylene-vinyl acetate copolymer [EVAc]; 100µg/Kg BW); a slower releasing implant (ethylene-vinyl acetate copolymer [EVSL] 100 µg/Kg BW); or biodegradable microspheres (M; 50 µg/Kg BW). Fish were sampled at various intervals for 44 days. Untreated males showed low levels of GtH-2 (3-8 ng/ml) over the 44 day experimental period. All GnRHa treatments stimulated a significant increase in GtH-2 plasma levels peaking by day two (I: 15.60±4.40, M: 17.98±3.46; EVAc: 17.03±2.38; ng/ml) or day 7 (EVSL: 17,76± 2.36 ng/ml). However M treatment exhibited a second peak on day 28 (M: 23,61±3.95 ng/ml) and EVSL maintained high levels of plasma GtH-2 until the end of the experiment. Mean plasma levels of gonadal steroids (T, 11-KT and 17,20βP) did not show significant differences among groups. Nevertheless differences were observed in the plasma steroid profiles when fish were individually examined. This data show that sustained administration of GnRHa increases and prolongs the GtH-2 pituitary surge which in turn affect plasma steroid profiles and finally results in a substantial increase of milt volume.

OP-52

OVULATION OF TASMANIAN ATLANTIC SALMON MAINTAINED AT ELEVATED TEMPERATURES: IMPLICATIONS OF CLIMATE CHANGE FOR SUSTAINABLE INDUSTRY DEVELOPMENT

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Atlantic salmon culture in Tasmania is conducted at latitudes where ambient water temperatures approach the upper limits of thermal tolerance. The potential impacts of global warming along with El Niño events of increasing frequency and magnitude (which result in high autumn temperatures) threaten sustainable industry development. Against this background, experiments are being conducted to examine how elevated water temperatures influence reproductive development in Tasmanian Atlantic salmon. Fish were maintained at water temperatures of 16, 11 and 6°C from late vitellogenesis (late March). Blood samples were collected at weekly intervals and plasma steroids were measured by RIA. From the start of the recognised spawning season (early May) fish were checked for ovulation at 3-4 day intervals and ova were fertilised and incubated to provide fertility and survival data. Ovulation in fish held at 11°C was delayed relative to 6°C while at 16°C, ovulation was inhibited until water temperature was reduced. Pre-ovulatory declines in 17β-estradiol (E2) and peaks in testosterone and 17α,20βdihydroxy-4-pregnen-3-one occurred first in fish held at 6°C then in fish at 11°C but did not occur at 16°C unless temperature was lowered. The quality of ova from fish held at 16°C was significantly lower than those from fish held at lower temperatures. These results demonstrate apparent temperature inhibition of endocrine processes associated with maturation but suggest that acute temperature manipulation may be enough to maintain ovulation in fish exposed to high autumn temperatures. However, E2 levels were significantly lower in fish held at 16°C than those at 6°C and 11°C, suggesting that elevated temperatures may also affect vitellogenesis.

OP-53

FERTILITY AND MOTILITY OF SPERM FROM MALE ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*) TREATED WITH GONADOTROPHIN-RELEASING HORMONE AGONIST

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We report on studies in which spermiating male Atlantic halibut were implanted intramuscularly with pellets containing different doses of a gonadotrophin-releasing hormone agonist (GnRHa). Blood and milt samples were taken at various intervals. GnRHa induced a rise in both androgens and progestogens. The major progestogen in the blood of both wild-caught and hatchery-reared males was identified as sulphated 5β-pregnane-3α,17,20β-triol. 17,20α-dihydroxy-4-pregnen-3-one was also identified. Concentrations of both steroids increased as a result of the GnRHa treatment. The major effect of GnRHa on milt was to increase its fluidity. We have performed fertilisation trials and used computer aided sperm analysis to determine whether the quality of the sperm is different in milt from treated and non-treated fish.

OP-54

CRYOPRESERVATION OF COMMON CARP *CYPRINUS CARPIO* AND TENCH *TINCA TINCA* FOR GENE RESOURCES CONSERVATION

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In this study, cryopreservation methods were elaborated for *ex situ* conservation of Bohemian common carp and tench (7 strains of common carp and 9 strains of tench). Sperm of tench is usually contaminated with urine, therefore the sperm was collected directly into modified extenders of Kurokura. Common carp sperm was diluted 1:5 in a Kurokura medium and equilibrated for 10 minutes at 4°C. Diluted sperm of both species was transferred to 2 ml cryotubes and 10 % of DMSO was added, than the cryotubes were directly transferred to pre-programmed PLANER Kryo 10 series III and cooled from +4°C to -9°C at a rate of $4°C.min^{-1}$, then from –11°C to –80°C at a rate of $11°C .min^{-1}$, hold for 6 min at -80°C and finally transferred into liquid N_2. The spermatozoa were thawed in a water bath 35°C for 90 s and checked for fertilization rate, hatching rate and larval malformations. Sperm motility was evaluated for the percentage and velocity of motile video frames. ANOVA showed significant influence of sperm (frozen and fresh $P<0.0001$) on fertilization rate and hatching, but insignificant on larval malformation 0-6.8 % and insignificant influence of different males on fertilization rate, hatching and larval malformation. Multiple range analysis (LSD) assessed difference between frozen sperm and fresh sperm on fertilization rate 54.73 % and 82.6 % ($P<0.01$), hatching rate 50.58 % and 59.44 % ($P<0.01$), respectively ($1.8\text{-}2.4.10^5$ spermatozoa per egg) in common carp. In tench, ANOVA showed significant influence of sperm (frozen and fresh $P<0.0001$) on hatching and insignificant influence of different groups of males on hatching. Larval malformation was zero. Multiple range analysis (LSD) assessed difference between frozen sperm at solution A, B, C and fresh sperm on hatching rate 25.49, 33.13, 34.84 % and 86.92 % ($P<0.05$), respectively ($3.6 - 16.5.10^5$ spermatozoa per egg).

OP-55

PROLONGATION OF SPERM MOTILITY IN THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AND ITS CONSEQUENCES FOR ARTIFICIAL INSEMINATION

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In *Oncorhynchus mykiss* the influence of metabolic inhibitors, substrates, co-enzymes and oxygen concentrations on spermatozoal parameters during motility was studied. Sperm motility parameters were significantly reduced in the presence of inhibitors of respiration, by anaerobic conditions and inhibition of the tricarboxylic acid cycle. When the sperm motility activating saline solutions were optimized in aspects of ionic composition and energy supply, about 50% of the motile spermatozoa swam progressively (> 20µm/sec) for about 3 min, about 10% swam progressively for around 30 min. The efficiency of the optimized sperm motility activating solution as fertilization solution during artificial insemination was tested. When low sperm to egg ratios were used or when the semen was of low quality the optimized sperm motility activating saline solution was highly significantly superior to the routinely used fertilization solutions.

OP-56

ASSESSMENT OF EGG QUALITY OF HADDOCK (*MELANOGRAMMUS AEGLEFINUS*) IN PAIRED MATINGS

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In order to forecast egg quality for subsequent aquaculture production, a series of tests were made of egg batches from wild captured haddock (5 months captive holding) to assess their relative usefulness for inclusion in juvenile production operations. Egg criteria evaluated were sequential batch number for each individual female, egg diameter, egg dry weight, ash dry weight, total lipid and fatty acid composition, fertilization and hatching rates. Results indicate that high ash as % of dry matter greater than 20% and sometimes over 40% was an indicator of poor quality, as reflected in low fertilization and hatching rates. Good quality eggs consistently had low ash content (12-18% ash). There was no correlation between egg quality and total dry matter, total lipid or fatty acid composition. Certain spawning pairs outperformed others and consistently yielded eggs characterized by high fertilization and high hatching rates. Number of egg batches per female ranged from 5 to 10. Egg diameter declined seasonally ranging from 1.6 to 1.3 mm. These results support the use of captive paired matings as a means of generating eggs for juvenile production and which in turn could evolve as a method to develop broodstock selection programs for gadoids. The repeated use of pairs over several years will also permit the evaluation of nutritional effects on successive spawning events. The fish in this study had derived the majority of the egg nutrients from wild feeding prior to capture. The use of eggs from communal matings in which 30-50 mature broodstock co-occur in a single tank does not permit the following of gametes from selected broodstock members.

OP-57

NORWEGIAN AQUACULTURE

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The Norwegian aquaculture production will in 1999 exceed 420.000 tons. Of these, Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) make up close to 98% with production volumes of 370.000 and 45.000 tons, respectively. Arctic charr (*Salvelinus fontinalis*) is another salmonid that are established in a niche market with small volumes (400 tons). During the last years the salmonid industry has shown an annual increase of 8-9% per year and can reach 1 million tons during the next decade. The Norwegian production constitute 50% of the world production of Atlantic salmon. Commercial aquaculture production of marine finfish is still low. Methods for production of halibut (*Hippoglossus hippoglossus*) and wolffish (*Anarhichas* spp.) are being developed. In 1998, 250 tons of farmed halibut were produced, but further research and development are required in order to enable commercial production. Production technology of Atlantic cod (*Gadus morhua*) is available but commercial aquaculture production is limited (500 tons) by a low market price due to large landings of wild fish. Also small quantities (125 tons) of turbot (*Scoptalamus maximus*) is produced in heated waste water from the metallurgic industry. The knowledge about the reproductive biology and the current research focus on these species are very dependent on how far their aquaculture industry has been developed and are briefly discussed.

PP-165

FEASIBILITY OF CULTIVATION COLD-WATER MARINE FISH SPECIES: AN ONTOGENETIC APPROACH

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Large-scale production of juvenile marine fishes for culture is not always possible owing to complex and expensive technology requiring for their raising from the eggs. The aim of this study is to assess the feasibility of the cultivation of several present or potential objects for cold-water marine aquaculture (cod, turbot, halibut, and wolffish) based on the features of embryonic, larval, and juvenile development. In addition, the ontogeny of eelpout (*Zoarces viviparus*), a viviparous species is analysed. From the ontogenetic point of view, cultivation of the latter species is the simplest, because the young possess maximum protection from external environment and reach a large size (35 mm or 17% of adult fish) at parturition. However, both its commercial value and absolute fecundity (9-132 eggs) are low. Among oviparous species, early ontogeny of wolffish is comparatively independent from environment: the embryos, 22 mm in length, hatch at an advanced stage of development, the larva state (i.e. readiness to external feeding) begins before hatching, and free embryo phase is absent. Cod, turbot, and halibut possess prolonged periods of metamorphosis and small larvae (5-12 mm in length) at a low degree of morphological development, and the latter species has the longest free embryo phase. Therefore, start-feeding and subsequent rearing of wolffish are much easier than these in other species. Thus, the features of ontogeny and related complexity of rearing technology should be applied for the assessment a feasibility of cultivation of different fish species.

PP-166

withdrawn

PP-167

A RAPID AND QUANTITATIVE METHOD FOR THE ASSESSMENT OF STORAGE AND CRYOPRESERVATION TECHNIQUES FOR FISH SPERM

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A novel method for the rapid, objective and quantitative assessment of different techniques for the storage and cryopreservation of fish sperm is described using computer assisted sperm analysis (CASA), sperm viability and ova hatching rate. It is applied to evaluate the quality of African catfish (*Clarias gariepinus*) sperm after two-day storage at 4°C or freezing at -196°C. The effect on sperm quality of storage in an extender before and after refrigeration and that of dilution in four different cryodiluents before and after freezing were evaluated. The motility of sperm stored at 4°C decreased significantly after 48 hours. Frozen sperm had a shorter duration of movement compared to fresh sperm and had a path with greater curvature. Sperm movement expressed by the straight line velocity (VSL), the curvilinear velocity (VCL) and the average path velocity (VAP) was highly correlated with hatching rates obtained from fertilisation using minimal sperm:egg ratios. Post-thaw hatching rates reflected the viability of frozen spermatozoa, which was cryoprotectant dependent. The cryodiluent containing dimethyl sulphoxide (DMSO) as a cryoprotectant in combination with 10% egg yolk provided the best cryoprotection to the spermatozoa for all post-thaw sperm quality measurements. The methodology described is applicable to the rapid and objective measurement of the quality of semen of any fish species.

PP-168

THE EFFECT OF SALINITY ON PLASMA STEROID CONCENTRATIONS IN ADULT MALE AND FEMALE BLACK BREAM *ACANTHOPAGRUS BUTCHERI*

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Black bream is a euryhaline sparid that spawns in the middle to upper reaches of estuaries. It has been suggested that salinity may play a role in regulating the onset of reproductive activity in black bream. The aim of this study was to investigate the effect of salinity on reproductive development of wild fish acclimated to laboratory conditions in order to assess the suitable salinity range for broodstock maintenance. The steroids estradiol (E2), testosterone (T), 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) and cortisol were measured by radioimmunoassay. Fish were held at salinities of 5, 20 and 35 ppt from May to November and blood samples taken in August, September and November. Ovarian recrudescence was not affected by salinity with only 1 fish failing to undergo vitellogenesis. Similarly, all males were fully spermiated at the end of the experiment. Concentrations of plasma cortisol in both males and females were lowest in August and high in September and November in all three salinities. In males, plasma T and 11KT levels were not affected by salinity in August and September, however, steroid levels in November were higher in fish held at 35 ppt. Plasma 17,20βP concentrations were highest in September in male fish held at 35 ppt but dropped to levels similar to those in fish held in 5 and 20 ppt by November. In females, plasma 17,20βP concentrations were highly variable and not effected by salinity. At all three salinities, plasma E2 levels were high in August and November but low in September, whereas, plasma T levels were highest in November. These results indicate that reproductive development proceeds normally over a wide range of salinities and that, fish can be held from brackish through to full saline conditions.

PP-169

IN VITRO CONTROL OF JAPANESE EEL SPERMATOZOA MOTILITY BY MANIPULATION OF THE ENVIRONMENTAL IONIC CONCENTRATION

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Spermatozoa from artificially matured Japanese eel, *Anguilla japonica*, begin to swim under hyperosmotic conditions. Before incubation (initial control), the percent motility just after dilution with 450 mM NaCl was 53.4% (range 7.4 to 91.5%; n=10). The percent motility increased significantly after 60 min incubation with isotonic artificial seminal plasma (ASP), which corresponded in terms of ionic constituents to the seminal plasma, consisting of NaCl + KCl + $CaCl_2$ + $MgCl_2$ + $NaHCO_3$ buffered with TAPS-NaOH at pH 8.1 (83.4%), and with Ca^{2+}, Mg^{2+} free-ASP (86.1%). Motility, however, decreased rapidly in K^+ free-ASP (1.8%) and in HCO_3^- free-ASP (5.7%). The percent motility increased with increasingconcentrations of K^+ ions (0-30 mM) and HCO_3^- ions (0-20 mM) in ASP. The acquisition and loss of the potential for motility could be induced several times by varying the ASP K+ and/or HCO3- ion concentrations. Initiation of motility of spermatozoa demembranated with Saponin required increasing ionic concentration (above 400 mOsm/kg) and raising the pH (between 7.7 and 8.0). These results indicate that the percent motility of eel spermatozoa can be adjusted to optimal levels by the potassium and bicarbonate ion concentrations of the incubating medium irrespective of the initial potential for motility. In addition, the extracellular high concentrations of both ions would be essential for the preparations for increase of intracellular potassium concentration and rise of intracellular pH under hyperosmotic conditions.

PP-170

CRYOPRESERVATION OF AFRICAN CATFISH, *CLARIAS GARIEPINUS*, SPERM

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In African catfish culture, it is often necessary to kill valuable male broodstock in order to obtain milt, since stripping of males is difficult, even after hormonal therapy. Storing multiple batches of sperm by cryopreservation would significantly improve the reproductive potential of a single catfish male. However, to date no reliable technique for catfish sperm cryopreservation has been developed. Using a standard freezing medium (10% MeOH in Ginzburg fish ringer) we compared different freezing rates and freezing depths in two-step and three-step freezing protocols. In two-step programmes, sperms were slowly frozen at -2°C/min, -5°C/min, or -10°C/min until different sub-zero temperatures (range -25°C -70°C,) and then plunged into liquid nitrogen (LN_2). Highest hatching rates (not significantly different from control fertilisations) were obtained when sperm was frozen at -5°C/min until -45°C (89.4% vs. 93.7% control). The transition point from slow freezing rates to fast freezing (plunging) was found to be critical within the range of -40°C to -45°C. Fertilisation rates equal to control were obtained when sperm was frozen until -43-45°C, while fertilisation was close to zero when sperm was only frozen until -41°C or higher temperatures. In three-step programmes the effect of holding time (0, 2 or 5 min) at different transition temperatures before fast freezing in LN_2 was analysed. Fertilisation rates of sperm frozen at -5°C/min until either -35°C or -40°C, with no holding, were less than 1%. Holding samples for 5 min. at these temperatures improved survival of sperm to values not significantly different from controls (85.1% and 86.8% vs. 85.8%). Similar results were obtained with freezing rates of -2°C or -10°C/min. These results show the importance of completing proper crystallisation during slow freezing rates before fast freezing is commenced. Our best protocol (-5°C/min until -40°C, 5-min hold followed by fast freezing) was tested in on-farm conditions. Again no difference in fertility was found between frozen and unfrozen sperm. One cryovial containing 0.5 ml 1:10 diluted sperm was sufficient to fertilise 20g (approx. 15 000) catfish eggs.

PP-171

EFFECT OF COOLING RATES ON THE MOTILITY AND FERTILITY OF TWO STURGEON SPECIES SPERM AFTER CRYOPRESERVATION

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The gametes cryopreservation is essential aspect for breeding of endangered species in culture with the goal of biodiversity conservation. Semen of twosturgeon species (*Acipenser stellatus* and *Huso huso*) from Volga river were cryopreservated. The cryoprotrectant included 37.1% sucrose and 5.7% dimetilsulfoxide in the tris-HCl buffer (pH=7.96). Two variants of cooling rates up to -34°C with following quick transfer into the nitrogen liquid was used in experiment: 4°C/min and 10°C/min. After storage in the nitrogen liquid samples were thawed in water thermostat (+15°C) and activated with the river water. The post-thaw sperm motility was varied from 40% up to 50 % in all trials. The sperm, cooled by 10°C/min, showed 0% fertility in both species. The sperm, cooled by 4°C/min, fertilized 22% of *A. stellatus* eggs and 36% of *H. huso* eggs. Evidently, high cooling rate influences negative on the sturgeon sperm fertility after cryopreservation.

PP-172

CRYOPRESERVATION OF SEABREAM SPERMATOZOA (TELEOST, *PERCIFORMES, SPARIDAE*)

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The *Sparidae* family contains a number of economically important species throughout the world. Among them four seabreams i.e., yellowfin seabream (*Acanthopagrus latus*), black porgy (*A. scheligeli*), red seabream (*Pagrus major)* and gold lined seabream (*Sparud sarba*) are the most popular for aquaculture in Taiwan. The effects of both osmolality and cation in the initiation of sperm motility were examined in these *Sparidae*. Various factors involved in the cryopreservation of seabream spermatozoa on motility are discussed. Extender containing only glucose proved to be a suitable medium for freezing seabream spermatozoa to -196°C. Glycerol seems to have a direct osmotic effect on seabream sperm cells, and it induced sperm motility before freezing and during thawing. However, this exhausted the energy needed for sperm motility for fertilization. Dimethyl sulfoxide (DMSO) proved superior to ethylene glycerol, propylene glycerol, glycerol and methanol as a cryoprotectant. Prolonged equilibration time had a detrimental effect on both prefreezing and post-thawing sperm motility. The estimated optimum freezing rate was in the range of -20 to -154°C/min. More frozen-thawed than fresh spermatozoa are required to achieve comparable fertilization rates.

PP-173

CORTISOL AND SEX STEROIDS PROFILES IN STELLATE STURGEON FEMALES DURING MATURATION UNDER PITUITARY PREPARATION TREATMENT IN AQUACULTURE

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The concentrations of serum steroids levels (ng/ml): cortisol, testosterone and progesterone were measured by ELISA in Stellate sturgeon females after different time of holding (12-15 or 47 days) in the ponds at the fish farm. Final maturation was induced by sturgeon pituitary preparation (PP). PP treatment included 2 injections: the low dose (5% of total dose) and the main dose (95% of total dose), the interval between injections was 10 h. Females after 12-15 days (I, n=6) and after 47 days (II, n=5) of holding were investigated (four- or triple samples of blood were taken). Cortisol levels in females (I) after capture (20-50 min.) from holding pond were high; 90.7± 25.89, because of stress (netting) reaction. After staying during 28 h in the tanks, cortisol levels decreased significantly to 17.1± 4.69 ($P<0.05$) and no reaction to low PP dose treatment was observed, but after the main PP injection cortisol levels elevated to 54.5± 13.78 (I)($P<0.05$). Testosterone levels did not change during staying in the tanks and significantly increased after low PP dose injection in both females groups: from 109.7± 21.92 to 209.3± 39.47 (I)($P<0.05$) and from 137.9± 20.95 to 227.9± 13.06 (II) ($P<0.01$). After final maturation testosterone levels decrease were observed; 97.8± 23.21(I)($P<0.05$) and 118.0± 7.07(II) ($P<0.001$). Serum progesterone levels demonstrated the strong tendency to elevate after the low PP dose as well as after the main dose treatment. Differences between individuals are discussed.

PP-174

STEROID HORMONES IN REPRODUCTIVE FUNCTION REGULATION OF STURGEONS IN AQUACULTURE

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The maintenance of wild sturgeon stocks in Caspian region is realised mainly throughout artificial reproduction, using induced spawning of wild caught stock. Brood fishes are taken from commercial landings and transported to the fish farms, held here for different time from 1–60 days to 6 month and induced final maturation by hormonal treatment. Serum cortisol and sex steroids (testosterone, progesterone) concentrations in spring and winter forms of Giant sturgeon (*Huso huso*, L.), Russian sturgeon (*Acipenser gueldenstaedti,* Brandt) and Stellate sturgeon (*Acipenser stellatus,* Pallas) at the beginning of anadromous migration, at the fish farm after different time of holding and after final maturation under pituitary preparation treatment were measured by ELISA. Netting and transportation significantly increased serum cortisol levels and decreased testosterone levels in fishes. Cortisol concentrations decreased to the basic level after fish's delivery to the fish farm. Holding for a long time in the tanks or ponds influenced on hormonal status of fishes: the basic serum steroids concentrations, the stress reaction, the reaction on pituitary preparation treatment modified. Acute stress (handling, asphyxia) affected dramatically short-term increase of serum cortisol levels, but did not alter on success of final maturation and gamete quality in females. Chronic stress (crowding) was accompanying by slow serum cortisol elevation and induced negative effect on gamete quality in males.

PP-175

STERIODS PROFILES IN GIANT STURGEON FEMALES (*HUSO HUSO*, L.) AT THE BEGINNING OF ANADROMOUS MIGRATION AND AT INDUCED OVULATION AFTER RESERVATION

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The cortisol (C) and testosterone (T) levels (ng/ml) in blood serum of beluga sturgeon migrating to Volga river in spring and in the fish farm were measured by ELISA. At the beginning of river anadromous migration of the spring forms (SF) beluga (n=4) just before spawning in spring, serum steroids concentrations were 231.3±58.36 for C and 69.4±23.92 for T. After short-term reservation of the SF (7-14 days) in tanks in the fish farm and ovulation, induced by sturgeon pituitary preparation, the steroids levels decrease significantly (n=32); C: 65.6±7.09, T: 21.2±1.78 in comparison with fishes at anadromous migration ($p<0.01$ for C, $p<0.05$ for T). The winter form (WF) beluga enters the river in autumn, spawning take place next spring. After long-term reservation of the WF females from autumn till spring (6 months) in the fish farm, successful ovulation was received after the same hormonal treatment (n=18), the steroids levels being significantly lower then in ovulated SF females; C: 34.4±3.57, $p<0.001$, T: 12.5±1.85, $p<0.01$. The interrenal of ovulated SF and WF females was functionally active with sings of exhaustion in some WF females. The data show the possibilities exploitation of different beluga seasonal races in aquaculture and the significant decrease of serum steroids concentrations after reservation, especially for WF, the progeny quality being high as for WF as for SF. The role of steroids in the reproductive function regulation in sturgeons (*Chondrostei*) is discussed.

PP-176

INDUCED MATURATION IN CASPIAN INCONNU, *STENODUS LEUCICHTHYS* (GULD.), *SALMONIDAE*

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Caspian inconnu, a diadromous teleost, inhabits the Caspian Sea. It migrates for spawning to Volga River. The mean body weight of females and males are 7-8 kg, and 5-6 kg, respectively. C. inconnu spawning season begins usually in November at 6-5°C. Ripe eggs and sperm can be obtained after stimulation of prespawning fishes by environmental temperature but there are great difficulties in holding fishes in captivity. After Volga dams were build up population of C. inconnu has decreased sharply and now this fish survives only due artificial reproduction at the fish farms. In females after reservation in cages during 3-4 weeks two repeated injections of GnRH-analog ([D-Ala^6, Pro^9 NHEt]-LHRH) or pacific salmon pituitary preparation induced ovulation and shortened the maturation period as compared with untreated females. The effective doses of these preparations are 30-62 mg/kg b.w., and 17-26 mg /kg b.w., respectively. There were no significant differences in egg quality between the two groups, with egg fertilisation averaging 85%. In males a single injection of GnRHa (14-20 mg/kg) or pituitary preparation (3.6-4.3 mg/kg) was followed by stimulation of milt volume in 3-4 days after treatment at temperature 8-7°C without negative effect on sperm quality. Inducing maturation of Caspian inconnu with hormonal preparations is the effective way of preservation of this endangered species.

PP-177

CHANGES OF SERUM CORTISOL AND TESTOSTERONE LEVELS IN CHUM SALMON DURING SPAWNING MIGRATION AND FINAL STAGES OF MATURATION

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Serum cortisol (C) and testosterone (T) concentrations (ng/ml) in chum salmon, *Oncorhynchus keta,* in nature and at a fish farm in Magadan region were determined by ELISA. Females at early stages of vitellogenesis with oocyte average weight (AWO) 90± 6.6 mg had low C levels (10.2±3.0, n=5). In mature females with AWO 210-255 mg (ovulation) from nature (n=2) and from cages (n=5), C levels increased to 348±30.5 ($P<0.001$) and 521±94.6 ($P<0.001$), respectively. Females (n=9) at intermediate stages of vitellogenesis (AWO 140± 8.4 mg) could be divided on two groups: with low (7.1±0.9) and high (209±81.2) C levels. The serum T concentrations in all females from nature were high (migrating fishes: 161±31.9, n=16; ovulated ones: 107±0.5, n=2) but decreased in ovulated females after reservation in cages during 5-7 days to 60±14.4 (n=4) or in tanks during 30-40 days to 33±9.8 ($P<0.001$). Males at early stages of spermatogenesis had low C levels - 5.8±0.14 (n=4). The males in prespawning or spawning state from nature had high C levels (208±50.7, n=3), or moderate levels (31±7.8). As well males from cages (5-7 days of reservation) had high degree of variation of this index - from 20 to 327. Serum levels of T at early stages of spermatogenesis were less than in females with AWO 90±6.6 mg (54±7.5, n=8), but increased in prespawning and spawning fishes up to 112±18.3, (n=8, nature) and 104±19.8 (n=8, cages). Long-term reservation in tanks during 30-40 days was followed by sharp decrease of T levels to 20±7.7, n=4 ($P<0.001$).

PP-178

NUTRITION-BASED ENHANCEMENT OF SPAWNING PERFORMANCE OF ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*) BROODSTOCK

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Atlantic halibut, *Hippoglossus hippoglossus,* is one of the most promising aquaculture species for cold waters. Broodstock management, and nutrition in particular, are important determinants of egg quality. Halibut broodstock in the UK have traditionally been fed trash fish or moist diets. The replacement of wet diets is a high priority for the prospective halibut industry as they are not consistent in composition and may carry the risk of inadvertently introducing pathogens, such as VHS, nodavirus, etc. Nutrient intake and health status can be more fully controlled using fabricated diets, with comparable spawning performance to that obtained from moist-trash fish diets. The present work describes experiments conducted over three spawning cycles. During the first year of the experiment, two groups of Atlantic halibut were placed under two dietary regimes: Control, moist diet using wet fish components; and Enhanced DHA, elevated AA and DHA pellet including a Northern Hemisphere fish meal and tuna orbital oil. Once the effectiveness of the dry diet was confirmed during the first spawning season, a second experiment was set up to concentrate more on improving the levels of AA and its relevance to the production of eicosanoids. This group of locally produced highly active compounds derived from AA is now suspected to be important in ovarian final maturation and the early development of eggs. Therefore, two populations of fish were again fed different diets: Standard: good quality Northern Hemisphere fishmeal and oil, and Enhanced AA: Standard diet enhanced with AA rich oil. These diets were fed over two successive spawning seasons to the same groups of fish. Data to be presented includes: Spawning performance and egg quality, and the total lipid and fatty acid composition of eggs derived from the two dietary treatments.

PP-179

INDUCTION OF SEX REVERSAL IN THE SEVENBAND GROUPER, *EPINEPHELUS SEPTEMFASCIATUS*

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The sevenband grouper, *Epinephelus septemfasciatus*, is a protogynous hermaphrodite which matures first as a female then changes to a male in the later stage of its life cycle. Techniques for accelerating the sex reversal to obtain male brooders were required for the success of induced breeding in this species. Administration of methyltestosterone (MT) was reported to effectively induce sex reversal in groupers. However, the optimum dose and treatment method have not been determined for the sevenband grouper. Thus, we examined the effects of oral administration (10 mg MT/kg diet, twice a week for two months; group A) and implantation (1 mg (group B) or 4 mg (group C) MT in a silastic capsule/fish) for immature females (mean BW: 2.0 kg). The total quantity of MT administered to group A was estimated to be 1.5 mg/fish. Gonads exhibited various transitional phases at the end of treatment. The effect of oral administration was incomplete and varied according to the feeding response of individual fish. Ten months after the treatment termination, all group A fish reverted to resting females. In contrast, group B and C fish transformed into active males two months after the implantation of MT, and some of them spermiated three months after the implantation. One year after implantation, the group C fish remained resting males, while the group B fish reverted to a transitional phase. In conclusion, MT-implantation at a dose of 2 mg/kg fish is an effective and sustainable treatment for induction of sex reversal in the sevenband grouper.

PP-180

CHANGES IN FATTY ACID COMPOSITIONS OF THE MUSCLE, LIVER AND OVARY OF THE JAPANESE EEL, *ANGUILLA JAPONICA*, DURING ARTIFICIAL MATURATION

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Salmon pituitary homogenate (SPH) injections were used extensively to induce maturation in female Japanese eel. However, the hatching and survival rate of the larvae are very low. In several fish, it has been reported that lipids, especially n-3 highly unsaturated fatty acids (HUFA) in eggs, are related to larval growth and survival. It is considered that egg fatty acids are provided from the adipose tissue via the liver during vitellogenesis. In order to clarify the effects of SPH injections on lipid transportation to eggs, changes in lipid contents and fatty acid compositions of the muscle, liver and ovary of SPH injected Japanese eel were examined. Fatty acid composition of ovulated eggs in artificially maturing eels were compared to that of some naturally maturing teleosts. Female Japanese eels were injected with SPH (30 mg/kg-BW/week). Total lipid of each organ in each vitellogenic stage and ovulated eggs was extracted with chloroform/methanol 2:1 (v/v). Fatty acid compositions were analyzed by gas-liquid chromatography. Serum lipid levels were examined by a commercial kit. Lipid contents in eggs and serum increased in the early and mid-vitellogenic stage. Percent of n-3 HUFA in liver and ovary decreased during artificial maturation. Percent of n-3 HUFA of ovulated eggs of eels was remarkably lower than that of other fish. These results suggests that fatty acid transportation to eggs in eels injected with SPH is not normal.

PP-181

PARENTAL EFFECTS ON EARLY DEVELOPMENT OF THE EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*)

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Parental effects on precocious performances of the sea bass (*Dicentrarchus labrax*) have been investigated. Seven females have been chosen for their homogenous prematurational stage and simultaneously stimulated with synthetic LHRH (Sigma) to induce ovulation. Egg release obtained by hand stripping was asynchronous between females and extended from 70H45 to 75H08 post induction. Each spawn was fertilised *in vitro* with the sperm of 6 males according to a triplicated diallele experimental design. Sperm concentrations were adjusted to get a discriminating number of 20 000 spermatozoa per ovule. Fertilisation rates were found significantly different between females. On the contrary, neither effect of the males nor interaction between males and females could be detected. Each batch of fertilized eggs were distributed among three incubators where they were maintained for 48h. Hatching rates of living eggs at 48H post fertilization were evaluated in triplicate for each family. They were not found to be significantly different between males but significant difference between females and a significant interaction were detected. Finally, total length of larvae sampled at the first feeding age of 8 days post hatching was not different between males. However, a significant difference between females and a significant interaction between males and females was observed. In conclusion, most of the parent effects on eggs and larvae development are due to females and a factorial analysis has been performed in order to characterise maternal effect.

PP-182

POST VITELLOGENIC MODIFICATIONS OF SEA BASS OOCYTES IN CAPTIVE POPULATION. MORPHOLOGICAL AND FUNCTIONAL DESCRIPTION

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Growth and morphological modifications of sea bass oocytes were studied from the beginning of vitellogenesis (late October) to the end of the reproductive period (March) in French Mediterranean conditions, by fortnightly biopsies of females maintained for several years in captivity and recorded as regular spawners. This study showed that the growth of the first batch of fully vitellogenic oocytes occurred within only one month and brought follicle diameters from 250 to 800 µm. Then oocytes underwent a long postvitellogenic and/or maturation process lasting 1.5 to 3 months and characterised by 3 main features: stagnation of oocyte diameter; differentiation of the vitellus which consisted in a hyalinisation of the peripheric cytoplasm, progressively spreading toward the centre of the oocyte and driving progressively to oil droplet completion; migration of the germinal vesicle. Four different stages were described using a morphological scale by observation of cleared follicules in toto. The occurrence with time and the duration of the different stages were studied on the first annual cohort of oocytes of 37 females. Since micropinocytosis was observed during the postvitellogenic period, a status of the activity of vitellogenin receptors of postvitellogenic oocytes was assessed and compared to that of either previtellogenic or vitellogenic ones. LHRH analogue stimulation of females showed that oocytes were competent at all posvitellogenic stages for hydration, meiosis resumption and ovulation, however they presented differences in their response (delay, morphology, fertility).

PP-183

FERTILIZATION SUCCESS OF HALIBUT GAMETES STORED UNDER COLD CONDITIONS

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Halibut eggs can be successfully transported a few days prior to hatch. However, lack of sufficient incubation facilities may require the transport of eggs immediately before or after fertilization. Holmefjord and Bolla (1988) showed that halibut eggs are very susceptible to mechanical stress during the first 2 days post-fertilization. This investigation was designed to evaluate the feasibility of transporting unfertilized halibut eggs and milt under cold conditions for subsequent fertilization. Three experiments were conducted to determine the fertilization success of halibut gametes after cold storage over 64 hours. In the first experiment five batches of eggs were obtained from separate females, divided into subsamples and stored at 0.2-0.4°C. Subsamples of fresh milt were stored in bags with oxygen at 1-3°C. Duplicate egg and milt samples were fertilized at regular intervals from 0 to 64 h. Fertilized eggs were incubated at 5°C for 12-18 h before fixation in Stockard's fixative. The percentage of fertilized and viable eggs was determined. Preliminary analysis indicate that at these storage temperatures fertilization success decreases by about 20% over 16 h. To determine whether the decreased fertilization rate was due to milt or egg quality additional experiments were conducted using eggs stored at 1°C and 3°C and fresh milt or milt stored at 1.0°C. Results indicate that fertilization rate is similar using fresh or cold stored milt from the same male. The fertilization rate of eggs stored for 64 h at 3°C decreased by about 10%, and those stored at 1°C decreased by about 20% and fertilization rates were more variable. Statistical analysis will be presented.

PP-184

withdrawn

PP-185

THE RELATIONSHIP BETWEEN SPERM DENSITY AND SPERMATOCRIT IN ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*)

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The goal of this research was to establish the optimum sperm density for the successful fertilization of halibut eggs, and to determine the suitability of spermatocrit as a rapid estimator of sperm density for fertilization studies in this species. Sperm density and spermatocrit were determined by use of a hemocytometer and centrifugation, respectively, for milt samples from 17 males. Sperm density ranged from $2x10^{11}$ (spermatocrit 27%) to $5.8x10^{11}$ (spermatocrit 99%) cells per ml. Regression analysis showed a strong relationship between sperm density and spermatocrit (r^2 of 88%). Fertilization experiments were carried out to determine the range of milt to egg volumes that would not have a negative effect on fertilization success. Sperm to egg volume ratios of 1:15 to 1:8000 were tested, with eggs incubated at 5°C until they reached 8-cell stage, at which point fertilization success were determined. No difference was found in fertilization success throughout this range of sperm to egg volumes.

PP-186

DOSE-RESPONSE OF HUMAN CHORIONIC GONADOTROPIN (HCG) AND INDUCTION OF OVULATION IN COMMON SNOOK, *CENTROPOMUS UNDECIMALIS*

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The common snook is an inshore sportfish whose pelagic eggs contain a single oil globule for buoyancy. Reproductive fish were captured during June and July; the reproductive condition of each female was ascertained by ovarian biopsy. Ovulation can be induced with HCG. To determine the most effective dose, i.e. the lowest dose of HCG that induced a high rate of ovulation and produced highly viable eggs, we conducted a dose-response experiment. HCG was dissolved in 0.9% sterile saline and injected alongside the first dorsal fin at rates of 0 (controls), 50, 100, 250, 500, 1,000, or 2,000 IU/kg. Sample size was five fish per group. Once injected, individual females were confined in net pens so that they could be easily monitored and ovarian biopsies could be taken. None of the controls or the fish receiving an injection of 50 IU/kg ovulated. Fish injected with 100 or 250 IU/kg ovulated inconsistently. Those injected with 500, 1,000, or 2,000 IU/kg of HCG ovulated consistently and produced eggs of good quality. Egg quality was measured by: (1) percent fertilization at the blastula stage, (2) percent hatch, and (3) percent survival to first feeding (72 hours post hatch). From the results, it was determined that the lowest effective dose of HCG was 500 IU/kg, half what our hatchery had formerly used.

PP-187

INDUCTION OF OVULATION IN COMMON SNOOK, *CENTROPOMUS UNDECIMALIS*, USING GONADOTROPIN RELEASING HORMONE

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Injections of the mammalian analogue of gonadotropin releasing hormone (GnRHa) have been effectively used to induce ovulation in a number of commercially important fish. The common snook spawns from late afternoon into the evening hours and can be induced to ovulate by a single injection of HCG (500 IU/kg). Single injections of GnRHa, at doses of 25 or 100 µg/kg produced unsatisfactory results: no viable eggs were produced and most females did not ovulate. GnRHa implants, as 5-day time-release pellets, were highly effective at inducing ovulation in common snook at release rates of 10, 25, 50, or 100 µg/kg*day. Furthermore, four analogues of GnRH (salmon, chicken II, sea bream, and mammalian) were incorporated into pellets and implanted at a dose of 10 µg/kg*day. All but the sea bream analogue were highly effective at inducing ovulation in common snook and producing eggs of good quality. None of the fish ovulated after implantation of the sea bream pellets, although oocytes in some females underwent partial final maturation. Commercially available GnRH analogues, both mammalian and salmon, can effectively induce ovulation in common shook if administered in a time-release form. Although successful in many fish species, a single injection of GnRHa was not effective at inducing ovulation in common snook.

PP-188

HORMONE INDUCED ARTIFICIAL PROPAGATION OF TIGER PUFFER (*TAKIFUGU RUBERIPES*)

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The tiger puffer is one of the most expensive food fish in Japan. Some trials for seed production of tiger puffer have been done for the last few years, however, it was difficult to obtain a stable supply of high quality seed. Recently, we developed an effective method for the induction of artificial propagation of tiger puffer using various hormonal treatments. Higher water temperature (17°C) and long photoperiod (14L:10D) caused the earlier growth of gonads. A single injection with HCG (500 IU/kg BW) and chum salmon pituitary (7mg/kg BW) in combination was efficient for inducing and synchronizing spermiation of males which had fully grown but not spermiated testes. After such treatment, milt stripped out of the fish showed stable motility (time until all spermatozoa cease their forward movement) maintaining values between 60 and 80 seconds for 30 days. Egg diameter of individual fish was monitored by ovarian biopsy before hormonal treatment. A single implantation of LHRHa cholesterol pellet (LHRHa 400mg/kg BW) successfully induced oocyte maturation and ovulation for females which had yolk accumulated ovaries. After implantation, four-hour interval palpations were performed to check expansion and hardening of the abdomen due to hydration of oocyte which indicates the completion of final oocyte maturation. Ovulation occurred between 18 and 36 hours after confirming hydration. The fertilization rate of the eggs collected from ten fish decreased as the time increased, suggesting that artificial fertilization of tiger puffer must be carried out immediately after ovulation in order to obtain high quality eggs.

PP-189

SUPERCOOLING OF RAINBOW TROUT *(ONCORHYNCHUS MYKISS)* EGGS AT DIFFERENT STAGES OF DEVELOPMENT

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In view of the lack of practicable means for cryopreserving rainbow trout eggs, storage for prolonged periods may be accomplished by supercooling. Based on an extensive range of preceding trials indicating that cryoprotective agents such as sucrose and ethyleneglycol exerted a detrimental effect, an experiment was conducted with 5 replications involving unhardened fertilized eggs, morulae (10T°) and eyed eggs of 190, 210 or 230 T°. These were exposed to 0, 1, 2 or 3% carboxymethylcellulose (CMC) or 5% dimethylsulfoxide (DMSO). After 60 min equilibration, eggs were drawn into macrostraws and cooled to - 4°C at a rate of 0.1°C/min. After 24 h, eggs were transferred to an incubator to assess their developmental potential. Fertilized, non-hardened eggs (5 - 48%), morulae (7 - 24%) and advanced (230T°) eyed eggs (6 - 16%) turned out to be the least suited. Eyed eggs of 210 T° gave reasonable survival rates of 42 - 56%, where eyed eggs of 190 T° produced hatching rates of 65 - 84%. Contrary to the other stages, at this particular stage of development water devoid of a cryoprotective produced the best result of 84% hatching. In most of the younger stages , 2% CMC provided the best protection. At the advanced (210 and 230T°) eyed egg stages DMSO at a concentration of 5% proved to be most suitable. Long-term storage experiments, applying the most promising of the above techniques, are underway.

PP-190

THE INDUCTION OF TRIPLOIDY IN THE YELLOWTAIL FLOUNDER, *PLEURONECTES FERRUGINEUS* (STORER)

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The induction of triploidy was attempted in yellowtail flounder eggs using hydrostatic pressure shock treatments. Trials were performed under a range of conditions including pressure levels of 5000 to 10 000 psi, duration of treatments ranging from 10 to 30 minutes and initiation times of 5 minutes to 15 minutes post-fertilization. Induction rates were assessed using flow cytometry to determine DNA content of larval cell suspensions. Rates of induction were determined on 15 individual larvae after yolk-sac absorption, which for yellowtail occurs two weeks post-fertilization at 8°C. High percentages of triploids (>90%) were found using shocks of 7000 to 10000 psi initiated at 5 min post-fertilization, increasing percentages of diploid larvae were found below 7000 psi. In the range of 8000 to 10000 psi, high percentages of triploids could be produced with durations as short as 5 minutes. Pressure shocks initiated 5 min post-fertilization had excellent larval yields but survival appeared to be compromised when egg shocks were initiated later, at 10 or 15 minutes post-fertilization. The recommended treatment is a 10 minute application of 7000 psi initiated at 5 minutes post-fertilization. This treatment yielded a mean triploid induction rate of 99% (n=6) for samples of two week old larvae. Initial experiments in raising triploid larvae has revealed that percentages of triploids remain above 90% when sampled at metamorphosis, the rate of residual diploids being low. These same experiments have also suggested that triploid yellowtail may have reduced growth rates.

PP-191

ADVANCEMENT OF OVULATION IN YELLOWTAIL FLOUNDER (*PLEURONECTES FERRUGINEUS*) USING ENVIRONMENTAL AND HORMONAL STIMULI

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The yellowtail flounder (*Pleuronectes ferrugineus*) is a cold ocean flatfish with considerable potential for aquaculture. The objective of this research is to advance spawning of yellowtail using a combination of photoperiod and thermal manipulation in conjunction with hormone treatment, to enhance broodstock management. Female yellowtail flounder (n=14), separated into two equal groups, were photoperiod, temperature and hormonally manipulated in an attempt to advance the spawning season. Both groups were exposed to compressed photoperiod (8L:16D) and lowered temperature (approx. 5°C) from September to January. Subsequently, photoperiod was increased (17L:7D) as well as temperature (approx. 6°C). One group was administered a sustained-release preparation cholesterol pellet of gonadotropic hormone-releasing hormone analogue ([D-Ala^6,Pro^9-NHEt]GnRHa) intramuscularly. The second group was given a sham pellet in the same manner. Unmanipulated broodstock fish served as normal photoperiod and temperature controls. First ovulation occurred in the environmentally stimulated group given the hormone preparation 4 months prior to the unmanipulated broodstock. Fish photoperiod and temperature adjusted without hormonal stimulation spawned only 2 weeks before the regular broodstock. Eggs were stripped following ovulation, and subsequently assessed for viability and fertilization. These parameters were consistent with unmanipulated broodstock. Viabilities and fertilization rates for the hormone treated, environmentally adjusted, the unmanipulated groups were 86% and 77%, 72% and 80%, 65% and 75% respectively.

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APPLICATION OF STAINING TECHNIQUES TO IMPROVE ASSESSMENT OF VIABILITY OF RIPE TURBOT (*PSETTA MAXIMA*) OVA

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To develop a rapid, simple method for determining viability of stripped ova of turbot, staining-dyes were tested using a vital dye neutral red (viable cells become red, while non-viable cells do not) or the trypan blue exclusion test (dead cells are coloured). Viability rate of ova was established on morphological criteria with or without staining (control), and classes were established relative to staining intensity and cell injuries. When viability rate of the control was high (near 90 %) or low (inferior to 65 %), no significant differences were observed in the assessment of ova viability with or without staining-dyes. When viability was intermediate (between 85% and 70 %) significant differences were observed ($P < 0.05$), and viability rate assessed in staining conditions was close to fertilisation rate. Trypan blue was located both in dead cells and in the layer of the chorion of viable cells in a clear staining ring and therefore this dye was less discriminating than neutral red. Ova kept in ovarian fluid observed from scanning electron microscope (SEM) studies, show a chorion with regularly arranged pores which elucidate penetration of dyes at ova periphery. After activation of ova when the pores disappear, lower staining intensity in neutral red was observed in several cells. This observation was reliable to loss of fertilisation. Staining techniques were rapid and useful in intermediate quality spawns, to assess the viability of translucent ova a few minutes after collection. In addition, neutral red may be used during fertilisation, to assess the development of blastomers which were stained and were easy differentiated from the other cells.

PP-193

GONADAL DEVELOPMENT AND HORMONAL LEVELS OF CAPTIVE MEDITERRANEAN YELLOWTAIL (*SERIOLA DUMERILII*, RISSO) UNDER PHOTOPERIOD CONTROL

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Seriola dumerilii (Risso, 1810) is a promising finfish specie for the aquaculture diversification of production in the Mediterranean area. A major bottle-neck of its culture development is the control of reproduction. Previous studies have shown that maturation in captive conditions usually appeared to be blocked at the onset of vitellogenesis. Therefore, common hormonal techniques are not being effective in the induction of final maturation and spawning of this specie. The employment of artificial cycles of photoperiod has becomes a common practice in several temperate marine cultured teleosts for inducing maturation and spawning. Thus, a controlled photoperiod regime (9 hours of light and 15 hours of darkness from October 1997 to March 1998, and the opposite for the rest of the year) was employed in the rearing of two groups of captive *S. dumerilii*, whilst another group were reared under natural photoperiod. In order to check its effect on the fish maturity state, blood (for sex steroid and GTH analyses) and gonad samples were collected at the pre-spawning and spawning periods. The photoperiod regime promoted a suitable gonadal maturation state (vitellogenesis and sperm production) in some of the fish for being successful hormonal treated for the final maturation and spawning. Although, mean sex steroids (testosterone, 11-ketotestosterone, 17β-estradiol, 17,20β-dihydroxyprogesterone) and GTH II levels did not significant differs in both sexes between controlled and natural photoperiod groups. The individual variability of response for such method of inducing maturation observed in our trial has also been reported in other fish and it is discussed.

PP-194

not used

PP-195

not used

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ASSESMENT OF DIFFERENT EPISODES OF GONADAL MATURATION IN ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*) WITH THE USE OF ULTRASOUND SCANNING

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Although Atlantic halibut (*Hippoglossus hippoglossus*) is regarded as the next major commercial species for aquaculture in cold waters, there are still problems related to egg supply. Little is known about the processes of gonadal development and maturation in halibut. Broodstock fish are still scarce and the practice of culling fish for sampling purposes is not an option. Atlantic halibut is a batch spawner, with several batches of eggs produced during several weeks of the spawning season. Timing of ovulation can be disrupted by handling stress, with negative effects on the spawning and general condition of the fish. Ultrasound scanning appears to be an ideal non-intrusive technique for the assessment of gonadal development throughout the life history of the fish up to spawning. The data presented here illustrate the use of ultrasound scanning as a tool for studying ovarian development in different experiments. A group of hatchery produced broodstock was monitored over a two year period, collecting images of gonadal development and comparing this to histology samples of ovaries of fish at similar points in development. Spawning females were sampled at regular intervals in between egg releases, with ovarian biopsies collected at the same time as ultrasound images. Ultrasound scanning proved to be a valuable tool for sexing Atlantic halibut, studying maturation, assessing gonadal development throughout the year, following hydration and final maturation of egg batches, and resorption of gonadal material after spawning.

PP-197

PHOTOPERIODIC EFFECTS ON HATCHING GROWTH AND SURVIVAL OF *CLARIAS GARIEPINUS* EGGS AND LARVAE

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The effect of photoperiod regimes (hours of light and dark; 12L:12D, 6L:18D, 18L:6D, 0L:24D, 24L:0D and natural photoperiod) on hatching, growth and survival of catfish (*Clarias gariepinus*) eggs and larvae were investigated. Hatching occurred between 22h 30min and 24h. Percentage hatch and deformed larvae ranged from 32.9 to 45.1 and 10.4 to 35.0, respectively. No significant differences occurred in hatching time but significant differences ($P<0.05$) occurred in the percentage hatch and deformed larvae. In the growth performance experiment, 18L:6D and 0L:24D displayed highest and least growths respectively. Percentage survival ranged from 85% to 88% and significant differences occurred in weight gain among treatments. The results suggests that light influences feeding and growth in *C. gariepinus*.

PP-198

EFFECT OF SALINITY ON HATCHING OF EGGS AND GROWTH OF YOLK SAC LARVAE OF *HETEROBRANCHUS BIDORSALIS* (M) X *CLARIAS GARIEPINUS* (F) HYBRID LARVAE

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The effects of salinity on the development of eggs and yolk sac larvae of *Heterobranchus bidorsalis* (m) X *Clarias gariepinus* (f) hybrid larvae were investigated. Incubation temperature was 23°C to 25°C under different concentration of sodium chloride per litre of water (2g/L, 4g/L, 6g/L, 8g/L,10g/L and normal spring water). Percentage hatch and deformity ranged from 30% to 81.5% and 3.33% to 8.5%, respectively. There were significant differences ($P<0.05$) in the percentage hatch and deformity. Rate of yolk absorption was 0.233mm, 0.166mm, 0.133mm, 0.133mm per day in the 2g/L, 4g/L, 6g/L and control experiment respectively. Mortality occurred immediately after hatching in the 8g/L treatment. Highest growth was recorded in the control experiment while the least growth was found in the 6g/L treatment with length 6.0mm and 5.0mm respectively. There were however no significant differences in rate of yolk absorption while significant differences ($P<0.05$) were found in growth. The results suggest that increasing salinity reduces growth rate for HB(M) X CG(F) hybrid larvae.

PP-199

UNIVERSAL EXPEDITIONARY INCUBATOR FOR FISH EGGS

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Universal incubator "Incub-1" for eggs incubations includes six glass containers simultaneously regulated for temperature. Each container is a module with individual preparation of water. The incubator is supported by a "cardano hang up" and occupies a square of about 1 m^2. Each module consists of a container, heat exchanger, electromagnetic valve and checking-measuring block. The checking-measuring block allows to assign and support a temperature with a precision of 0.1C°within the range of -4 to + 35C° and register any changes in temperature to a computer. The present incubator makes it possible to undertake experiments on pelagic, bentos and litho-fitofilin eggs at sea.

PP-200

INDUCTION AND *IN VITRO* FERTILIZATION IN *CICHLASOMA DIMERUS* (*PERCIFORMES, CICHLIDAE*), SPECIES WITH PARENTAL BEHAVIOUR

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As a part of a project oriented to the induction of triploidy in species with parental behaviour, it was necessary to determine the initial time of fertilization and the kariotype; erythrocytes's dimension of *C. dimerus* were also analyzed. Adults were captured in Corrientes province, Argentina (27°25´S, 58°15´W). They were kept in big aquarium at 25+/-5°C and 12L:12D photoperiod. Partner selection is a characteristic of this species. Prior to each spawning so called pre-spawning behaviour is shown by both partners of the pairs. As the female lays eggs on a substrate, the male inseminates them. This process takes about two hours. Both partners fan the eggs and the young fry, and guard their young when these, in a school, start swimming around. At the present study, animals were injected intraperitonealy with HCG on two successive days. Spawn was attained 26+/-3 hr. after second dose. The eggs were fertilized by dry method with milts obtained by gentle abdominal massage, with the aim of determining the initial time of fertilization. This procedure can be carried out successfully every 25 days, under photoperiod and temperature regulated and the partners are keeping in contact just until to the beginning spawning behaviour. The chromosome preparations were obtained from cephalic kidney cells. The diploid number was 2n=48, and the karyotype consists of six meta-submetacentric and eighteen acrocentric pairs (NF=60). To determine erythrocytes sizes, blood smears were prepared by conventional method. Major (M) and minor (m) axes of cells (C) and nuclei (N) were measured with micrometer (MC=10.02+/-0.6µm; mC=7.33+/-0.6µm; MN=3.94+/-0.4 µm; mN=2.43+/-0.8µm).

PP-201

not used

PP-202

INFLUENCE OF CONSTANT AND FLUCTUATING TEMPERATURES ON EARLY DEVELOPMENT AND SURVIVAL IN LOACH LARVAE

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The mortality of fish under natural and also aquaculture conditions is mostly observed in early ontogeny. The optimisation of environmental conditions durring eggs developing and larvae fish developing may essentially increase their survival ability. The influence of constant (16, 18 and 20 °C) and fluctuating (18 ± 2 °C, the frequency of temperature fluctuations being 2 hours) temperatures on survival ability of loach larvae (*Misgurnus fossilis*) was observed. It was established, that loach embryos survival to 20 hours after fecundation under constant temperatures (16, 18 and 20 °C) was 79, 87, 89% and 87% under fluctuating temperature (18 ±2 °C) . Up until hatching the survival was 63, 71, 74% under constant temperatures and 86% under fluctuating temperature. Average survival of loach larvae to 5 and 8 days posthatch in constant temperatures was 60 and 59% at 16 °C, 68 and 66% at 18 °C, 73 and 72% at 20 °C; in fluctuating temperatures it was 84 and 83% at 18 ±2 °C. Incidence of abnormalities was 16-30% under constant temperatures and 15% under fluctuating temperatures. The finding that the survival of Loach larvae increased under fluctuating temperature indicates that these temperature conditions are more favourable than constant ones.

PP-203

VISUAL LIGHT AND MAGNETIC FIELD TREATMENT APPLICATIONS IN TROUT AQUACULTURE: EFFECTS ON REPRODUCTION IN CULTIVATED SALMON (*ONCORHYNCHUS MYKISS*) IN CRIMEA

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For last 5 years we have conducted experiments at the trout farm of Crimean Nature Reservation to demonstrate the efficiency of pineal regulation for piloting of spawning, smolts and recruits growth rates, food consumption efficiency (feeding rate); for curing of bacterial and fungal diseases without any chemicals; for individual curing of fish specimens with pineal tissue transplantation (diffusion chambers method) and - of magnetic field exposure for piloting of melatonin blood and neural levels. The proper illumination regimes evoked increase of the growth rate of gonads maturation by 7-12%, mortality decrease by 75 - 100%, and normalisation of feeding behaviour in extremely infected experimental pond, as well as *Oncorhynchus mykiss* melatonin secretion regulation in native pineal under different illuminations (ranged from 10 to 15 000 lux) during one month experiments with the usual feeding procedure. Various illumination regimes influences on skin pigmentation and blood formula, gonadal maturation degree, somatic growth rate and behaviour were analysed as most demonstrative in 3 groups of *Oncorhynchus mykiss*. They were exposed to the following illumination regimens: 1) L:D = 12:12; 2) L:D = 24:0; 3)L:D = 0:24. Magnetic field (MF) was created by Helmholtz coils (7000 mTesla with 730 A/m intensity). Pineal melatonin production was obviously regulated by artificial photoperiod and MF treatment that was observed in temporal skin coloration shifts, feeding and schooling behaviour. It was shown that illumination regimes could be used as effective tool to pilot maturation and reproduction in trout aquaculture under poor feeding and extensive infectious press.

PP-204

BIO-MARKERS INDICATIVE FOR THE EGG QUALITY DECREASE DURING OVER-MATURATION IN THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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The present study describes potential bio-markers indicative for the egg quality decrease during over-maturation in the rainbow trout, *Oncorhynchus mykiss*. From individually marked rainbow trout portions of eggs were stripped in intervals of 6 days and the samples were investigated on physiological and biochemical changes. The fertilization rate significantly decreased from 71.5 ± 35.9% (mean ± S.D., n =25) in egg batches collected within 3 days after ovulation to 25.1 ± 32.0% in egg batches collected one month after ovulation, the ovarian fluid pH decreased from 8.13 ± 0.11 to 7.97 ± 0.16, the ovarian fluid protein levels increased from 534.3 ± 105.5 mg/100ml to 611.7 ± 163.5mg/100ml. The percentage weight increase during water hardening decreased from 22.4 ± 10.7% to 12.1 ± 6.3% and the egg respiratory activity decreased from 2.13 ± 2.09 µmol/min/mg protein to 1.24 ± 2.35 µmol/min/mg protein.

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POTENTIAL FOR DOPAMINE INHIBITION OF GtH RELEASE IN GREENBACK FLOUNDER *RHOMBOSOLEA TAPIRINA* (GÜNTHER, 1862): INDIRECT ASSESSMENT BY MEASUREMENT OF GONADAL STEROIDS AND OVULATION

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Greenback flounder were treated by intraperitoneal injection with either desGly^{10}[D-Ala^{6}] LHRH-ethylamide (LHRH-a), pimozide (PIM), or LHRH-a + PIM. Treatment with LHRH-a, and LHRH-a + PIM increased the number of ovulations above control levels. LHRH-a was more effective than LHRH-a + PIM and PIM in 1 out of 2 experiments. PIM increased the total number of ovulations in 1 out of 2 experiments. LHRH-a, PIM, and LHRH-a + PIM increased the percentage of fertilised eggs that developed through to 4 cell stage above control levels, and treatment with LHRH-a resulted in significantly more eggs surviving to the 4 cell stage than fish treated with PIM and LHRH-a + PIM. Fish treated with LHRH-a and LHRH-a + PIM had higher plasma 17β-estradiol (E2) and testosterone (T) levels than control fish or fish treated with PIM. Plasma levels of 17α20ß-dihydroxy-4-pregnen-3-one (17,20βP) were not elevated above control levels in fish treated with exogenous hormones, but were elevated above pre- treatment levels in all treatment groups in some cases. Co-treatment with LHRH-a + PIM did not improve the efficacy of LHRH-a in inducing ovulation, nor did it augment plasma levels of T and E2 above levels reported in fish treated with LHRH-a alone. With the proviso that plasma levels of gonadal steroids only provide indirect evidence of GtH release, it appears that DA may not have strong inhibitory action on GtH release in greenback flounder.

PP-207

ANDROGENESIS IN STERLET (*ACIPENSER RUTHENUS*) USING FRESH AND CRYOPRESERVED SPERM

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Acipenserid species are highly vulnerable fishes of great economic importance. As producers of caviar they are valued throughout the world. Their great sensitivity towards environmental problems makes evaluation of new methods for broodstock management and conservation extremely important. One of these methods can be androgenesis. Successful androgenesis was carried out in sterlet (*Acipenser ruthenus*). Genetic material of eggs was inactivated using Gamma ray irradiation. Irradiated eggs were inseminated either with fresh or cryopreserved sperm. For sperm cryopreservation the following protocol was used: extender was composed of 23.4 mM sucrose, 120 mM Tris, pH 8.0, 10 % methanol was used as cryoprotectant. Sperm samples were frozen in 0.5 ml straws in vapour of liquid nitrogen, and were thawed in a 40 °C water bath. A heat shock in a water bath was applied to the eggs to restore the diploid state of embryos. The parameters of irradiation and heat shock had to be evaluated experimentally. During the optimisation of one parameter, the others were standardised. In experiments on the optimal dosage of Gamma ray irradiation the highest percentage of hatched larvae (5.1% using fresh sperm, 3.1% using cryopreserved sperm) was achieved at the dosage of 30 kR. The application of heat shock at 85 minutes post fertilisation yielded the highest number of hatched larvae (5.3%). In further experiments on the parameters of heat shock the temperature of shocking was optimised at 38 °C (5.2% of hatched larvae), and the duration of the shock at 150 seconds (5.2 % of hatched larvae).

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CRYOPRESERVATION OF STERLET (*ACIPENSER RUTHENUS*) SPERM

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Acipenserids are highly endangered chondrostean fishes, that inhabit the Northern hemisphere. They are valued for the production of caviar. There is constantly increasing demand in the market for caviar as a luxury product. Therefore broodstock management and evaluation of new methods for conservation of these fish species is of great importance. One of these methods can be sperm cryopreservation. Experiments were carried out on the smallest of sturgeon species, sterlet (*Acipenser ruthenus*). Sexually mature fishes were collected in the spawning season form the Danube river. They were injected with carp pituitary extract. Sperm was collected into dry beakers and stored at 4°C. It was diluted in a 1:1 ratio with an extender composed of 23.4 mM sucrose, 0.25 mM KCl, 30 mM Tris, pH 8.0. Dimethyl-sulphoxide (DMSO), Dimethyl-acetamide (DMA) and Methanol were used as cryoprotectants in various concentrations. Sperm samples were frozen in 0.5 ml straws using a styrofoam box filled with liquid nitrogen. Straws were placed on styrofoam frames floating on the surface of liquid nitrogen. The height above nitrogen was 3 cm, the duration of freezing was 3 minutes. Then straws were plunged directly into liquid nitrogen. Thawing took place in a 40°C water bath for 10 seconds. Eggs were fertilised in Petri dishes. Fertilisation percentage was recorded at 4 blastomere stage. Best post-thaw motility (46±23%) was achieved using 10 % methanol. DMSO and DMA yielded significantly poorer post-thaw motility results. Promising fertilisation results were achieved upon insemination with cryopreserved sperm.

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DIETARY LIPID AFFECTS SPERM MEMBRANE INTEGRITY AND FERTILITY AFTER CRYOPRESERVATION IN RAINBOW TROUT

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The effect of dietary lipid on sperm fatty acid (FA) and cholesterol contents, and sperm viability after cryopreservation in rainbow trout was evaluated after fish were fed for 16 mo. with one of four diets differing only in the source of lipid (12 % of diet): herring oil, menhaden oil, safflower oil, or tallow. Sperm from 40 fish was analysed for FA. Sperm membrane integrity was assessed fluorometrically, and fertility trials were conducted.Dietary ratios of unsaturated:saturated and n-3:n-6 FA were 5.2, 1.4 (herring oil), 3.3, 2.7 (menhaden oil), 6.8, 0.1 (safflower oil) and 1.8, 0.3 (tallow), respectively. Sperm FA content was significantly affected ($P<0.05$) by source of dietary lipid. Monounsaturated FA for sperm from tallow or herring oil-fed fish were significantly higher than those of the menhaden oil group which were higher than those fed safflower oil. Polyunsaturated FA in sperm from the safflower oil group were significantly higher ($P<0.05$) than those in sperm from the other groups. Sperm cholesterol levels were higher for the tallow group ($P<0.05$) than for the other treatments. Cryopreserved sperm from tallow-fed fish exhibited the least membrane damage ($P<0.05$) and produced the highest percentage ($P<0.05$) of eyed embryos compared to sperm from the other treatments. Cryopreserved sperm from menhaden oil-fed fish had the highest level ($P<0.05$) of damaged sperm, and lower percentage of eyed embryos than other treatments. The altered FA and cholesterol levels of the sperm from fish fed the tallow diet afforded more protection from cryopreservation damage than those obtained with the fish or safflower oil diets.

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CRYOPRESERVATION OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) SEMEN USING DIMETHYLACETAMIDE OR DIMETHYLSULFOXIDE AS CRYOPROTECTANT AND THREE SIZES OF STRAW

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The objective of this study was to determine the effect of straw size and cryoprotectant on the fertility of frozen-thawed rainbow trout sperm. In experiment 1, semen was frozen in liquid nitrogen vapour using 0.5 or 5.0 mL straws and extender consisting of 0.3 M glucose with 10% dimethylsulfoxide (DMSO) or 10% dimethylacetamide (DMA) as cryoprotectant. In experiment 2, extender containing 10% DMA was used to freeze semen in 0.5 mL, 1.7 mL (flat) or 5.0 mL straws. Freeze rates inside the straws were measured using a type-T thermocouple. Success of treatment was determined by the fertility of frozen-thawed semen expressed as a percentage of eyed eggs obtained with fresh semen.In experiment 1, freeze rates between -15° C and -35°C for 0.5 mL and 5.0 mL straws were 35.9 and 15.6°C/min, respectively. The mean (± SEM) fertility of semen frozen using DMA (55.9 ± 5.6%) was higher ($p < 0.01$) than that of semen frozen using DMSO (28.9 ± 5.6%). The fertility of semen frozen in 0.5 mL straws (57.8 ± 5.6%) was higher ($p < 0.001$) than that of semen frozen in 5.0 mL straws (27.0 ± 5.6%). In experiment 2, the freeze rates were 88.9, 84.2 and 46.7°C/min for 0.5, 1.7 and 5.0 mL straws, respectively. Mean fertility of semen frozen in 0.5 mL, 1.7 mL and 5.0 mL straws was 72.6 ± 11.5%, 84.4 ± 11.5% and 76.3 ± 11.5%, respectively ($p > 0.05$). In experiment 1, frozen-thawed rainbow trout sperm had higher fertility when DMA was used as cryoprotectant than when DMSO was used. Semen frozen in 5.0 mL straws had reduced fertility compared to that frozen in 0.5 mL straws. When freeze rates were increased in experiment 2, straw size did not affect the fertility of frozen-thawed semen.

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REPRODUCTIVE PERFORMANCE OF AFRICAN CLARIID CATFISH (*HETEROBRANCHUS BIDORSALIS*) UNDER DIFFERENT FEEDING REGIMES

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There is an increasing demand for artificially produced seeds of *Heterobranchus bidorsalis* in Nigeria for pond stocking because of their economic importance as food fish. However, this is hampered by inadequate knowledge of appropriate feeding regimes for broodstock. This has considerable effect upon gonadal growth, fecundity and progeny. Reproductive performance of *H. bidorsalis* broodstock were assessed over 8 weeks in a feeding experiment. Four feeding regimes (starved (control), 2% of body weight/day, 4% of body weight/day and satiation) were involved and treatment replicated thrice with eight females in each replicate. Gonad weight, oocyte and gonadosomatic index (GSI) increased as the rearing period increased and there was a significant difference ($P < 0.05$) in the relative fecundity, gonad weight, weight of oocyte, GSI, % fertilisation, % hatchability, % deformation and progeny survival of control and fed treatments. The best results were achieved at 2% bw/day hence, recommended for improving the reproductive performance of *H. bidorisalis*

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THE EFFECTS OF INDUCED TRIPLOIDY ON THE REPRODUCTIVE PHYSIOLOGY IN THE EUROPEAN SEA BASS, *DICENTRARCHUS LABRAX*

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Triploidy in the sea bass may be relevant to achieve sterile fish with a better performance. Triploidy induction was carried out in this species by cold shock resulting in 95-97% of indeed triploid fish. Triploidy did not affect sex ratio, thus males outnumbered females (3:1) similarly to the diploids ($P > 0.05$). Triploids had a lower somatic growth than diploids at the adult stage ($P < 0.05$), however, significant differences were observed in the gonadal development around 9 months of age in females and 20 months of age in males. At 4 years of age, the gonadosomatic index in adult triploid males was 1.4 times lower than that of diploids, i.e., 1.79±0.09 vs 2.53±0.09%. Diploid males showed primary and secondary spermatocytes and were sexually mature, releasing sperm by gentle abdominal massage. Triploid males exhibited a similar histomorphology, however, no mature fish were observed after 4 years of age. Adult diploid females had well-developed ovaries with vitellogenic oocytes, while triploid ovaries were filiform and not weight up to 0.12 g. Triploid ovaries contained mainly oogonia and primary oocytes arrested in leptotene and zygotene stage. A few vitellogenic oocytes were present. When diploid fish accomplished sexual maturity, triploid males and particularly females, exhibited a lower hepatosomatic index than diploids ($P < 0.05$). Essentially, triploid showed a higher carcass index than that of diploids, however, useful biomass yield was superior in diploids. In conclusion, these results show that triploid sea bass are functionally sterile. Thus, this condition may be useful for marketing proposes and it can be advantageous as tool for reproductive endocrine research and for regulatory aspects of transgenesis.

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MODULATION BY bGH OF OVULATION RESPONSE INDUCTION WITH GnRHa ON THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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In salmonid aquaculture the spawning synchronization is made through the administration of gonadotropin releasing hormone analogues (GnRHa). This treatment can induce deleterious effects in egg quality if the endocrine signals chain are modified at the periovulatory time. Our interest was to evaluate the role of GH-IGF pathway in this process. In the present study we assayed intraperitoneal injections of 20 mg/Kg des-Gly^{10}[D-Ala^{6}]-LHRH-ethylamide (GnRHa), with or without bovine growth hormone (bGH) 100 mg/Kg. These hormones were injected through saline or oil suspensions in rainbow trout (*Oncorhynchus mykiss*) females. We scored weekly the ovulation time in Control, Placebo (injected only with saline/oil suspension), GnRHa saline, GnRHa+bGH saline, GnRHa oil and GnRHa+bGH oil groups, and evaluated the embryonic development performance in all of them. This experimental procedure always showed that the hormonal oil system gave the fastest spawning response; but gave also a significant diminution (45-67%) of spawning induced rate in the GnRHa-bGH coinjected groups relative to the GnRHa treated group, both in saline and oil systems. When we analysed the effects of these treatments on egg quality we observed a tendency to better embryonic development rate in the case of GnRHa-bGH coinjected oil hormonal system than in the GnRHa oil treated group. Then the GH system effectively could modulate the GnRHa induced ovulation response in rainbow trout, but the mechanism involved should be determined.

PP-214

EFFECTS OF REDUCED SEASONAL CYCLE AND LHRHa INJECTIONS DURING THE VITELLOGENIC PHASE ON SEXUAL MATURATION IN PERCH (*PERCA FLUVIATILIS*)

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The effects of a reduced seasonal cycle and injections of LHRHa and pimozide on sexual maturation in perch (*Perca fluviatilis*) were investigated. 150 tagged perch (1+; 104.4±34.5 g) were split in two groups and subjected to a reduced season; two months with decreasing temperature (14'8 °C) and day length (11'6h) followed by two months with increasing temperature (8'15 °C) and day length (6'15h). One group was treated monthly with LHRHa (30 µg/kg) and pimozide (10 mg/kg). At the start of the experiment in October, the immature perch were sexed by the serum levels of sex steroids. The level of 17β-estradiol (E2) in females was 0.22 nmol/l vs. < 0.07 nmol/l in males ($p<0.01$), and the testosterone (T) level was 0.83 nmol/l vs. 1.57 nmol/l respectively ($p<0.01$). From October to December the T levels in males and females increased to 16.9 nmol/l vs. 16.4 nmol/l while E2 levels in females increased to 6.6 nmol/l. No further increase in hormone levels were, however, observed in January and February. The levels of sex steroids were not significantly higher in the hormone treated group than in the control group. At the end of the study in March the gonadosomatic index of maturing females (70-80%) and males (100%) was 6.6% and 4%, respectively, in both the treated and control group. Whereas 3% of the treated females spawned at this time, no spawning was observed in the control group. High larval mortality indicates that reduced seasonal cycle may have negative effects on the egg quality.

PP-215

COLORATION IN CROSSES BETWEEN TWO VARIANTS OF GOLDFISH, *CARASSIUS AURATUS*

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Crosses involving two varieties of goldfish (*Carassius auratus*), the red comet and the variegated or the transparent-white variants of blue shubunkin, were performed in order to test possible acceleration of early coloration in their crossbreeds. Three cross-combinations were performed in triplicate (a, b and c) matings: (1) Comet x Comet (CGFxCGF), (2) Comet x White-transparent Blue Shubunkin (CGFxWBS) and (3) Comet x Variegated Blue Shubunkin (CGFxVBS). The offspring were maintained for three months in flow-through 30L funnel-shape incubators. Random samples of fish taken from each group were examined in 2-4 weeks intervals. In progenies derived from spawners in whom one or both parents were blue shubunkins, the light coloured larvae appeared soon after the incubation was accomplished and could be easily identified among the hatchlings. All offspring of the CGFxCGF combination remained wild-type colored during first three months of maintenance. All offspring of the CGFxWBS cross were light-coloured immediately after hatching and progeny of CGFxVBS segregated into wild-type and light-coloured fish at the expected Mendelian 1:1 ratio. Fish belonging to a-triplicate (three groups) were equally divided and parallel grown in three incubators and in three $1m^3$ cages suspended in an earthen pond, in order to compare environment-dependent coloration. After one month the fish grown in cages attained 2-3 fold bigger size than their sibs that have been grown in funnels. All light-coloured fish, offspring of CGFxWBS and CGFxVBS, obtained contrast red patches and colours, similar to fish, popularly named by aquarists as Jericho shubunkin. A part of the wild-type coloured fish, in groups CGFxCGF and CGFxVBS, started to obtain the orange coloration, characterizing the red comet. At the same time, no color changes could be observed in all groups grown in funnels. The results suggest that at least two different interacting genes, R and B control the colors in crosses between the comet and shubunkin, according to simple Mendelian ratios (significant by (2-test). Assuming that the phenotype of red comet represents the homozygous RR genotype, while B2B2 the white-transparent blue shubunkin and the heterozygous B1B2 (B1 co-dominant to B2) the variegated blue shubunkin (Rothbard et al. 1997. Israel. J. Aquacult. - Bamidgeh, 49:25-33). The heterologous crosses between shubunkin and red comet yield the heterozygous Jericho shubunkins (B2 epistatic to R) and absence of B2 results with heterozygous red comets (R dominant to B1).

PP-216

DISRUPTION OF VITELLOGENIN AND STEROID PLASMA LEVELS IN RAINBOW TROUT FED GENISTEIN ENRICHED DIETS

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Genistein is one of the major phytoestrogens found in fish diet. It is brought by soya meal increasingly used as a protein source by fish diet manufacturers. Genistein was found in soy meal at concentration ranging from 120 ppm to 6000 ppm. We therefore, set up an experiment to check the effect of this compound on fish reproduction. Rainbow trout are fed three diets for one year until spawning. One diet is a control diet based on fish meal, one diet, also based on fish meal is enriched with 500 ppm genistein and another with 1000 ppm genistein. Plasma samples are collected every 2 months. All fish were weighed and some of them killed for histological examination of the gonads. Vitellogenin, testosterone, 11-ketotestosterone and oestradiol were measured at each sample time. Vitellogenin was significantly increased in males fed the 1000 ppm diet. In females the effect was not noticeable when endogenous vitellogenin synthesis was fully engaged. Testosterone plasma levels were significantly decreased in males and females fed the 1000 ppm enriched diet except when the gametogenesis was fully engaged. Estradiol plasma levels were not altered by the diets. Spawning was not dramatically altered by the diet. From this experiment we can conclude that genistein is a weak endocrine disrupter in the rainbow trout which can alter vitellogenin and testosterone plasma levels in farmed fish. However, the disruption is not strong enough to perturb definitively gametogenesis nor spawning. Concern must remain about the effect of this compound at higher concentrations especially if soy is used as a major source of protein in fish meal. In addition, genistein is usually not alone in soy meal but can act synergistically with daidzein, coumestrol or other environmental oestrogens.

PP-217

GONADOTROPINS AND SEX STEROID HORMONE PROFILES IN RANCHED, DIPLOID AND TRIPLOID ATLANTIC SALMON (*SALMO SALAR* L.)

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Ranched triploid Atlantic salmon released in May 1996 returned to the freshwater release site during 1997 with diploid salmon but at a significantly lower rate (χ^2; $p<0.05$). Ovarian development was significantly reduced in triploid females. Gonadosomic indices (GSI) in triploid and diploid fish were 0.16 and 12.43 respectively for females and 4.08 and 3.65 respectively for males, in September 1997. During the period July to September 1997, as fish returned to freshwater, the blood plasma levels of estradiol were significantly lower in triploid females than diploid females. Conversely triploid females had significantly higher levels of gonadotropin I in July and September. Gonadotropin II levels were not significantly different between ploidy groups over the period monitored. A control group of diploid and triploid Atlantic salmon, held in sea cages, were sampled from November 1996 to May 1997. Levels of estradiol were consistently low until May when a significantly higher level was detected in the diploid group. Gonadotropin I & II and testosterone levels were not significantly different when male triploid and diploid fish were compared in control and in ranched return salmon. However, testosterone levels increased significantly during September in both diploid and triploid groups.

PP-218

GONADOTROPIN-RELEASING ACTIVITIES IN STRIPED BASS (*MORONE SAXATILIS*) OF THE THREE NATIVE FORMS OF GONADOTROPIN-RELEASING HORMONE (GnRH) AND OF NOVEL AGONISTS

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The gonadotropin (GtH) II-releasing activities of the three native forms identified in striped bass were evaluated during vitellogenesis. Fish were injected with 10 ug of salmon (s), seabream (sb) or chicken II (cII) GnRH and bled 0, 2, 4 and 8 h afterwards. ChickenII-GnRH was the most potent GtH II releaser, followed by sGnRH and sbGnRH. Obviously, all three GnRHs identified in striped bass brains are able to induce pituitary GtH II release, but sbGnRH, which is the form most abundant in the pituitary has the least bioactivity. These results are in agreement with findings in another perciform (*Sparus aurata*). With the objective of developing improved, highly potent GnRH agonists (GnRHa), the GtH II-releasing activities of [desGly^{10}-Pro^{9}-NEt]-GnRHas based on the sbGnRH and cII-GnRH forms were evaluated in vitellogenic females. At the 5 ug/kg dose, [DArg^{6}]-cII-GnRHa induced the highest increase in plasma GtH II, with [DAla^{6}]-cII-GnRHa resulting in the next highest increase, followed by [DAla^{6}]-sbGnRHa and [DTrp^{6}]-sbGnRHa. Similar to the situation with the native forms, c II GnRHas have the highest potency, while none of the novel GnRHas is more potent than the widely-used [DAla^{6}]-mammalian GnRHa. Apparently, although the striped bass GnRH receptors in the pituitary are mostly exposed to sbGnRH, they may have a higher affinity for the not-so-common cII-GnRH form and its agonists. It is also possible that the cII-GnRHas have a slower degradation rate than the GnRHas based on the more abundant sbGnRH form.

PP-219

ARTIFICIAL SPAWNING OF CARP (*CYPRINUS CARPIO* L.); DIFFERENCES BETWEEN THE EFFECTS OF REPRODUCTION IN FEMALES OF HUNGARIAN AND POLISH PROVENANCE TREATED WITH CARP PITUITARY AND OVOPEL

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The effects of reproduction were investigated in females of Hungarian and Polish provenance after two hypophysation treatments (0.3±2.7 mg/body weight), pituitary applied as the priming dose of 0.3 mg and Ovopel as the resolving dose of 1 pellet/kg body weight, and Ovopel in two doses of 1/5 ± 1 pellet/kg body weight. The application of two doses of Ovopel induced the spawning in all the females irrespective of their origin. The lowest percentage of fish spawned after two hypophysation treatments. Statistically significant ($P \leq 0.05$) differences were evidenced between the investigated groups of fish in the weight of obtained eggs both in grams and in percentage of female body weight. The greatest weight of eggs was obtained after repeated hypophysation and the smallest after the pituitary and Ovopel treatment. No significant effect of the group was assessed on traits associated with the quality of eggs i.e. the percentages of fertilization and of live embryos. Differences in the weight of eggs and in percentages of fertilization and living larvae were statistically non-significant for the classification associated with the provenance of females. The interaction between the provenance of females and the ovulation stimulators was statistically non-significant for the weight of obtained eggs and percentage of live embryos, being significant ($P \leq 0.05$) for the fertilization percentage. The ovulation time did not depend upon the provenance of females, yet it was related with the applied stimulators.

Pituitary

OP-58

PUBERTY IN TELEOSTS: NEW INSIGHTS INTO THE ROLE OF PERIPHERAL SIGNALS IN THE STIMULATION OF PITUITARY GONADOTROPIN

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In fish, as in other vertebrates, the first sexual maturation ("puberty") is characterised by an activation of the brain-pituitary-gonadal axis. Regulatory mechanisms underlying this activation are still poorly understood. This paper focuses on the role of peripheral signals in pubertal stimulation of pituitary gonadotropin (GtH2) in teleosts, with special reference to the European eel. Sexual steroids exert a positive feedback on GtH2, in juveniles from various teleost species, such as salmonids, catfishes, and eels. Steroid effects involve both indirect (brain) and direct pituitary actions. Variations in steroid specificity and mechanisms of actions occur between teleost species. The positive feedback can play a specific role in teleosts as an amplifier of pubertal activation of GtH2. However, it remains doubtful whether steroids would also be responsible for the initiation of puberty. Field and aquaculture data show that age at puberty is strongly related to metabolic status and body growth rate in teleosts as in other vertebrates. We investigated the potential role of growth and metabolic hormones in the stimulation of GtH2. IGF1, insulin-like growth factor produced by the liver under the control of growth hormone (GH), exerts both a negative feedback on GH and a direct stimulatory effect on eel GtH2 synthesis. Cortisol, adrenal hormone involved in the response to stress and the regulation of intermediary metabolism, also stimulates eel GtH2 synthesis by a direct pituitary action. These data suggest the potential role of growth and metabolic hormones as triggering signals for the onset of puberty in teleosts.

OP-59

GONADOTROPINS- FROM GENES TO RECOMBINANT PROTEINS

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We have cloned the cDNAs encoding for the β LH and FSH from a number of commercially important fish species, including seabream, mullet, tilapia and black carp. A striking feature is the high level of conservation found between the deduced amino acid sequences of the LH β subunits compared with the high sequence divergence observed between the different FSH subunits. The temporal gene expression of the LH and FSH subunits was studied in the seabream. Both genes were found to be expressed throughout the year. At all times the levels of β LH transcripts were higher than FSH, while the latter were higher in males than in females. The genes coding for β LH and FSH were isolated from black carp and tilapia. We are currently identifying putative regulatory sequences. Recombinant LH and FSH β subunits were produced using bacterial and baculovirus expression systems. The bacterial system yielded milligram amounts of recombinant proteins which had to be *in-vitro* refolded in order to immunologically resemble the native gonadotropins. However antibodies against these proteins could not identify circulating levels of the gonadotropins. The recombinant gonadotropins produced by the baculovirus expression system appear to be immunologically similar to the native hormones. The difficulties associated with this system are the low levels of recombinant hormones produced and their purification. The production of pure recombinant LH and FSH subunits, either for the development of immunoassays or for the study of their biological activities could provide an important breakthrough in the study of these hormones.

OP-60

HYPOTHALAMIC CONTROL OF GLYCOPROTEIN-HORMONE α SUBUNIT mRNA LEVEL IN TILAPIA

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Gonadotropic hormone (GtH) release in teleosts is regulated by hypothalamic factors, delivered to the anterior pituitary. Much less is known about hypothalamic effects on GtH biosynthesis. In tilapia, GnRH stimulates release and synthesis of the GtH subunits while dopamine inhibits only GtH release. In order to further clarify the hypothalamic effects on GtH synthesis, the transcript levels of the glycoprotein hormone alpha (GP α) subunit were measured in dispersed pituitary cells of tilapia. Cells were cultured for 3 days before exposure for 24 h to pituitary adenylate cyclase-activating polypeptide (PACAP27 or PACAP38; 0.001-10 nM), vasoactive intestinal peptide (VIP; 0.001-10nM), neuropeptide-Y (NPY; 0.01-500 nM) or norepinephrine (NE; 0.1 nM-10 microM), with or without salmon GnRH (sGnRH; 10 nM). All these factors stimulated the release of taGtH from the pituitary cells. In addition, incubation of the cells with PACAP 27 led to a dose-dependent increase in the steady-state levels of the GP α mRNA, reaching 400% of the control at 0.01 nM. It also potentiated by 200% the GnRH response of the cells. A similar but less pronounced effect was also seen with PACAP 38 and VIP. NE and NPY barely increased the subunit mRNA levels but augmented the response to GnRH. These results indicate that the hypothalamic factors examined stimulate the release of GtH and elevate the GP α gene transcription. Moreover, they facilitate the GnRH effect on these parameters. The possible pathways mediating their effects on the GP α as well as on the other GtH subunits remain to be elucidated.

OP-61

DISTINCT EXPRESSION AND STRUCTURE OF THE GOLDFISH GTH-Iβ AND GTH-IIβ GENES

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In teleosts, it is considered that two types of GTH, GTH-I and GTH-II, are produced in the pituitary, and their molecules are comprised of common α and distinct β subunits. In order to understand regulation of gonadal development by two GTHs at molecular level, we examined gene expression of GTH-I and -IIβ subunits, and then characterized GTH-Iβ and -IIβ genes in goldfish. Testosterone and estradiol-17β exerted an inhibitory effect on GTH-Iβ gene expression but a stimulatory effect on GTH-IIβ gene expression in immature and early recrudescent goldfish of both sexes. In sexually mature fish, no clear effects were observed on the mRNA levels in both sexes. In both sexes, GTH-IIβ mRNA levels reached peak levels in April (early spawning season). Changes in GTH-Iβ levels were different between sexes: the levels increased in April in females and unchanged in males. Plasma steroids reached peak levels in February in males, but in May in females, probably explaining the different seasonal changes in the GTH-Iβ levels between sexes. Both genes consisted of three exons separated by two introns. However, the location of the putative regulatory elements was distinct between the 5'-flanking regions of the GTH-Iβ and -IIβ genes. These studies indicate that the gene expressions of GTH-Iβ and GTH-IIβ are differentially regulated by sex steroids in goldfish, probably via upstream regulatory regions of the genes. The results also suggest that the responsiveness of GTH β subunit gene expressions to sex steroids differs depending on gonadal maturity.

OP-62

EFFECTS OF RECOMBINANT HUMAN INHIBIN ON GTH I AND GTH II SECRETION FROM DISPERSED PITUITARY CELLS OF FEMALE RAINBOW TROUT DURING THE REPRODUCTIVE CYCLE

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The influence of human recombinant inhibin was on gonadotropin release was studied at different stages of female rainbow trout gametogenesis, using the culture of dispersed pituitary cells in the presence of inhibin (with or without sGnRH). Inhibin was found to decrease the basal GtH I release in immature fish and during the vitellogenesis, having no effect on the GtH I secretion at the beginning of vitellogenesis and before ovulation. After ovulation, inhibin decreased the secretion of GtH I only in fish stripped of eggs, while it had no effect in unstripped females, suggesting the interference with factors contained in the ovarian fluid. The basal secretion of GtH II was stimulated by inhibin from the stage of vitellogenesis up to 2 weeks after ovulation, its potency increased with the gonad development. The GnRH-stimulated GtH I secretion was decreased in the presence of inhibin in immature fish and at the beginning of vitellogenesis, the GnRH stimulated-release of the GtH II was potentiated at the same stages. In the later stages, inhibin failed to modify the sGnRH-stimulated GtH I and GtH II secretion. Similar results were obtained using purified bovine inhibin. These results suggest that inhibins can differentially modulate the release of gonadotropins in rainbow trout, having the inhibitory influence on GtH I secretion, but stimulatory effect on that of GtH II. Our previous results demonstrated that the desteroidized rainbow trout ovarian fluid can mimic the action of inhibins in the rainbow trout after ovulation. Taken together, we suggest that endogenous inhibin-like proteins are present in the rainbow trout.

OP-63

ISOLATION AND MOLECULAR CHARACTERIZATION OF LHα AND β SUBUNITS FROM ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS* L.)

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The common gonadotropin α-subunit and the luteinizing hormone (LH, GTH II) specific β-subunit were isolated from Atlantic halibut pituitaries by extracting the glycoproteins in 40% ethanol followed by precipitation in 85% ethanol. Subsequently, LH and its subunits were purified by gel-filtration on Sephadex G-100 followed by ion-exchange chromatography on a Whatman DE-52 column. Final purification of subunits was performed on rpHPLC using a TSK gel ODS-120T column with an acetonitrile gradient (10-60%). SDS-PAGE and Western blots showed a molecular weight of 23 and 24 kD for LHα and β, respectively. Subunit identity was confirmed by N-terminal amino acid sequence analyses. Further purification and bioassay of pituitary glycoproteins are in progress. Two cDNAs encoding the Atlantic halibut LHα and β subunits were PCR cloned from reverse transcribed pituitary mRNA using degenerate primers based on conserved regions of teleost α and β sequences. 5' and 3' RACE (rapid amplification of cDNA ends) resulted in the amplification of two full-length cDNAs of 645 and 575 nucleotides, respectively. Results from Northern blot analysis of Atlantic halibut pituitary mRNA were in agreement with the length of the two transcripts. The α-cDNA encodes a protein of 124 amino acids (aa) including a signal peptide of 30 aa. The deduced LHβ subunit consists of 146 aa including a 31 aa long signal peptide. The halibut LHα and β sequences were most similar to those of striped bass giving 77 and 75% sequence identity, respectively. The cloning of the halibut follicle stimulating hormone (FSH, GTH I) β-subunit is in progress using sequence information from that of the striped bass.

OP-64

EFFECTS OF FASTING AND METABOLIC HORMONES ON THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS OF COHO SALMON, *ONCORHYNCHUS KISUTCH*

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Studies of reproductive maturation in male salmonids have found that large size, high growth rate, or high body fat level at critical times of year are associated with an earlier age of maturation than that of smaller, slower growing, or leaner cohorts. To elaborate the mechanisms by which growth and energy stores influence onset of maturation, we are examining the effects of nutritional status and hormones related to nutritional and metabolic state on activity of the hypothalamic-pituitary-gonadal axis. We tested the direct effects of insulin-like growth factor I (IGF-I) and insulin on intracellular and secreted levels of gonadotropin in dispersed coho salmon pituitary cells *in vitro*. IGF-I strongly increased both intracellular levels of follicle stimulating hormone (FSH) and gonadotropin-releasing hormone-induced FSH release, whereas insulin had moderate effects. In other experiments, we examined the effect of fasting during the autumn one year prior to the time of predicted final maturation. In fed coho salmon, pituitary content of FSH dramatically increased throughout the autumn. In fasted fish, this increase was both postponed and dampened. By evaluating gonadal development in these fish, we hope to ascertain the importance of this difference in FSH levels in pubertal development. We are also quantifying energy stores and circulating metabolic hormones in these fish to investigate the relationship between these parameters and the observed differences in the reproductive axis.

OP-65

REGULATION OF GONADOTROPINS DURING GAMETOGENESIS IN SALMON

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The synthesis and secretion of pituitary FSH (GtH I) and LH (GtH II) in fish, like other vertebrates, are temporally regulated involving the complex participation of the hypothalamus, pituitary and gonad. One objective of our research in Pacific salmon is to determine the role of gonadotropin-releasing hormone (GnRH), gonadal sex steroids and peptides, and growth factors in the regulation of synthesis and secretion of FSH. In this paper we will summarize information on changes in FSH and LH in plasma and pituitaries, and steady state levels of transcripts for α, FSHβ, and LHβ subunits that occur during the onset and progression of spermatogenesis and vitellogenesis in Pacific salmon. We will also report an overview of results from studies in salmon on factors that regulate synthesis and secretion of FSH, and highlight that with results from other species. Secretion of FSH *in vitro* is stimulated by salmon (s)GnRH and these effects of sGnRH are enhanced by pre-exposure of cells to insulin-like growth factor I (IGF I). GnRH treatment also increases steady state levels of both α and β subunit transcripts. Testicular peptides stimulate FSH secretion and synthesis, but we have not yet identified the nature of these substances. FSH release is stimulated by testicular extracts at all stages of gametogenesis tested including fully mature fish when GnRH-induced FSH release is not observed. The feedback effects of sex steroids on FSH are complex and depend on the stage of gametogenesis, sex, dose, duration and type of steroid treatment.

PP-220

THE PURIFICATION AND CHARACTERIZATION OF GONADOTROPIN, REPRESENTING THE BIOLOGICALLY ACTIVE DIMER OF TWO GTHα SUBUNITS, FROM PITUITARIES OF STURGEON (*ACIPENSER STELLATUS*)

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The reproductive cycle in fish as in tetrapod is regulated by two pituitary glycoprotein hormones LH and FSH. These hormones are composed of two chemically different subunits α and β. The existence of homodimer αα or ββ have never been demonstrated. The attempts to associate two identical subunits in a dimer *in vivo* either have not been successful. However the original hypothesis on the evolution of gonadotropins suppose that the ancestral gonadotropin was the αα-dimer, the β-subunits deriving from the α-subunit via its duplication and subsequent mutation. In the present study we purified a gonadotropin, named GTH-X, from the sturgeon pituitaries. GTH X displayed the biochemical properties very different from those of the sturgeon LH. This hormone exhibited a low level of cross-reactivity (5%) in the sturgeon LH specific RIA using the antibodies against βLH-subunit and did not cross-react with the same antibodies in the Western blot immunoassay. Contrary this GTH-X cross-reacted with sturgeon LH using the antibodies against sturgeon LH-dimer. GTH-X possessed biological activity, but it was 5 to 10 times lower than that of sturgeon LH. The GTH-X dissociated in the subunits only after reduction of cystein residues, contrary the LH, which partially dissociated after heating in guanidinium hydrochloride 6 M. The GTH-X subunit composition was studied using RP-HPLC after reduction and temperature treatment. The N-terminal sequences of two incompletely resolved RP-HPLC peaks corresponded to the α -subunits, differing by the first four aminoacids. No β-subunits were identified. These data indicate the existence of the αα-dimer in the ancestral fish, as a sturgeon, supporting the initial hypothesis of gonadotropin evolution.

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PRODUCTION OF RECOMBINANT STRIPED BASS (*MORONE SAXATILIS*) GONADOTROPINS IN CHINESE HAMSTER OVARIAN (CHO) CELL EXPRESSION SYSTEM

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Similar to higher vertebrates, fish possess two gonadotropins (GtHs), FSH (GtH-I) and LH (GtH-II). These hormones have been purified from several species of fish, allowing studies on their action and regulation. However, in perciforms, the purification of FSH met with limited success, which prevented the development of its immunoassays and physiological studies. Consequently, we choose to produce recombinant striped bass (stb) FSH and LH *in vitro* using a eukaryotic expression system. The stbGtHα, FSHβ and LHβ cDNAs were previously cloned in our laboratory. They were transfected into the mammalian expression vector pcDNA3.1/zeo+. CHO-K1 cells were transfected with the vector containing βFSH or βLH cDNAs, or cotransfected with one of this vector and the αGtH cDNA. The recombinant stbLH and LHβ production was monitored by western blot and ELISA, using antibody specific for the native striped bass LHβ. We have demonstrated that these expressed proteins were glycosylated and biology active. The recombinant stbLH induced estradiol and testosterone release from gonadal tissue incubated *in vitro*. The recombinant stb LHβ showed the same activity once reassociated with the native a subunit. As no specific antibody was available for the detection of stbFSH, the expression of FSH was tested by western blot using excess titers of the anti-stb LHβ antibody. A light band was observed at a molecular weight corresponding to the one expected for the FSHβ subunit. The stbFSH, as well as the recombinant FSHβ once reassociated with the native a subunit, were able to stimulate estradiol and testosterone release from gonadal tissue *in vitro*.

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FSHβ AND LHβ mRNA IN JUVENILE AND MATURE COMMON CARP (*CYPRINUS CARPIO*) AND THE RESPONSE TO GNRHa

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A dual gonadotropin model has been established for salmonids in which FSH predominates during puberty and vitellogenesis whereas LH surges only towards ovulation. In goldfish, the mRNA levels of both FSHβ and LHβ fluctuate in parallel, with both being low in immature fish, increasing in maturing fish, maximal in mature fish and decreasing in sexually regressed fish (Yoshiura et al., 1997). The difference between the salmonid model and the situation in goldfish was attributed to the asynchronous spawning of goldfish. The present work examined the situation in a synchronous spawner cyprinid, the common carp. Two experiments were carried out, one with juvenile females (104.6±4.1 g bw; GSI=0.36±0.02) and the other with postvitellogenic fish (1,442±33 g bw; GSI=6.1±0.02). In each of these experiments groups of 7-14 control fish or fish treated with sGnRHa (25 μgram/kg) were sampled before injection and 6, 12 and 24 h later. FSHβ and LHβ mRNA levels were determined by slot-blot hybridization with the respective DNA probes. The results are expressed as the ratio to the ribosomal RNA in the same gland. Results obtained so far indicate that mRNA levels of LHβ were already present in juvenile fish. Furthermore, mRNA levels of LHβ were always higher (about two-fold) than those of FSHβ in the same pituitaries and were similar in juvenile and mature fish. FSHβ mRNA level doubled 12 h following GnRHa injection, while that of LHβ doubled only after 24 h. Juvenile fish did not show any response to the GnRHa treatment.

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THE GENE CODING FOR βFSH OF TILAPIA - CODES FOR MULTIPLE TRANSCRIPTS

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The gene coding for βFSH of tilapia (*Oreochromis mossambicus*) was isolated from a genomic library, using the heterologous seabream βFSH cDNA sequence as probe. The isolated gene was fully sequenced and characterized. The genomic clone spans 5 kb which includes 1.7 kb and 0.6 kb of 5` flanking and 3` flanking sequences, respectively and a 2.7 kb transcriptional unit. The genomic organization of the tilapia βFSH gene appears to conform to the pattern observed for other gonadotropin beta-subunit genes, it contains three exons interrupted by two introns. The location of the first intron is within the 5` untranslated region while the second intron is located at the highly conserved position (the 38th amino acid of the mature peptide). However, an exceptional feature is the presence of markedly large introns :1256 bp for intron 1 and 957 bp for intron 2. Alternative splicing of the first intron was observed by primer extention, RT-PCR analysis and isolation of cDNA clones each representing a different splicing event. The two transcripts are expressed at different levels and differ in length by three nucleotides which do not alter the peptide sequence. The presence of two transcripts was also found in tilapia hybrids (*O.niloticus* x *O.mossambicus* and *O.niloticus* x *O. aureus*) suggesting that this feature is not species specific. The possible effect of regulatory factors, such as GnRH, steroid hormones and transcription inhibitors on the regulation of the splicing is currently being studied.

PP-224

IS THE REGULATION OF GONADOTROPIN SUBUNIT GENE EXPRESSION BY SF-1 IN TELEOSTS HOMOLOGOUS TO THE SITUATION IN MAMMALS? SETTING THE TOOLS

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The steroidogenic factor-1 (SF-1) plays a central role in the regulation of gonadotropin subunit gene expression, particularly in activating LHb synthesis in vertebrates. SF-1 shows synergy with different transcription factors, Krox-24 in mammals, and estrogen receptor (ER) in teleosts, in activation LHβ gene expression. We recently initiated a study aimed to characterize the mechanism by which SF-1 regulates the gonadotropin subunit gene expression and how its gonadotropic expression is regulated in the African catfish. For this reason we cloned the genes for the common glycoprotein hormone (GP) α-subunit, LHβ and SF-1, respectively, as well as the cDNAs for the SF-1 and Krox-24 of the catfish. The cfER cDNA has been previously cloned in our department. The GPα and LHβ gene have been completely characterized. We are currently finishing the analysis of the SF-1 clones. Two cDNAs encoding the catfish SF-1 (SF1Aa and SF1Ab) were isolated. SF1Aa is homologous to the zebrafish, zFF1A and mammalian SF-1s. The SF1Ab represents a splice variant that only differs 32 bp in the 5' flanking region of the DNA Binding Domain (DBD), resulting in a protein with 9 additional amino acids at its n-terminus. A RNAse protection assay (RPA) has been developed to analyze the SF-1 mRNA steady-state levels, during the pubertal development. The regulation of gonadotropin subunit and SF-1 gene expression will be investigated in promoter studies that are currently been set up.

PP-225

REGULATION OF FSH (GTH I) AND LH (GTH II) IN COHO SALMON (*ONCORHYNCHUS KISUTCH*): THE ACTION OF RECOMBINANT HUMAN ACTIVIN A (RHACTIVIN A) AND TESTIS EXTRACT

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It is clear that FSH (GTH I) plays an important role in the initial stages of gonadal growth of salmonids. However, it is not known how the production and release of FSH is controlled. We are studying the possible involvement of non-steroidal gonad factors, such as activins, in FSH and LH production and release. The action of rhActivin A and steroid-free testis extract on the production and release of gonadotropins has been investigated using a static dispersed pituitary cell culture system. In experiments using pituitaries from fish undergoing early gonadal development, secretion of FSH was stimulated by rhActivin A after 48 hours (e.g. a dose of 1.8 nM increased FSH levels by 37% above controls). In the same experiments the testis extract also stimulated FSH release (e.g. 1000 ug/ml increased FSH levels by 152% above controls). During later stages of maturation, rhActivin A did not stimulate FSH or LH, however, the testis extract stimulated both FSH and LH release. In spawning fish the testis extract continued to stimulate the release of FSH even when sGnRH was no longer effective. In one experiment steady state levels of α 1, α 2 and FSH β subunit mRNA (LH beta non-detectable) were measured using RNA protection assays and we found that rhActivin A, testis extract and sGnRH increased levels of these transcripts in coho salmon during the initial stages of gonadal growth. These results indicate that GnRH as well as non-steroidal factors from the testis play a role in the regulation of FSH in salmon.

PP-226

CLONING AND CHARACTERIZATION OF A GnRH-RECEPTOR FROM A PERCIFORM FISH

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A GnRH-R was cloned from the pituitaries of striped bass, *Morone saxatilis*, a perciform fish which has three distinct forms of GnRHs. The GnRH-R cDNA was isolated by 5Õ-3Õ -RACE PCR from a pool of pituitary mRNA isolated from previtellogenic, vitellogenic and postvitellogenic fish collected from their natural habitat, mature fish maintained in captivity and also from fish injected with a GnRH agonist. GnRH-R cDNA obtained by this method is 1867 bp long which includes 321 bp of 5' UTR and 410 bp of 3' UTR. An open reading frame (ORF) codes for 379 amino acids which shows 53-55% similarity when aligned with other reported GnRH-R. The cloned GnRH-R exhibits characteristics of a G-protein-coupled receptor family member, in that it has seven putative transmembrane domain, an extracellular domain and 49 amino acids long carboxy terminal tail. Similar to other GnRH-R, but unlike other G-protein-coupled receptor family members, The GnRH-R from striped bass has a substitution of Asp80 in transmembrane domain (TMD) II and Asn318 and Asp322 in TMD VII. It has a carboxy terminal tail, similar to other non-mammalian GnRH-R isolated thus far. By RT-PCR analysis, it was observed that GnRH-R is present in extrapituitary tissues, possibly implicating the three GnRHs in multiple paracrine/autocrine functions. Nevertheless, southern blot analysis indicates that GnRH-R from striped bass is encoded by a single copy gene. Experiments are in progress to understand the underlying molecular mechanisms of the interactions of GnRH-R with its three different ligands.

PP-227

FUNCTIONAL EXPRESSION OF RECOMBINANT SEABREAM LH AND FSH

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We are using the baculovirus expression system in order to produce biologically active fish recombinant LH and FSH. For that, two types of constructs have been engineered. Utilizing a vector with two independent promotors, the full length cDNAs coding for the gilthead seabream, *Sparus aurata* (sb) α and β subunits of LH and FSH have been introduced into the insect cell line Sf9, and found to be co-expressed. To determine whether the active conformation of the gonadotropin heterodimer could also be achieved when the two subunits were synthesized in tandem on a single polypeptide chain, a single chain, or yoked sb LH molecule was constructed. The cDNA coding for the full length (signal peptide and the mature protein) of the LH β subunit was co-joined to the region coding for the mature protein of the α subunit cDNA, and transfected into the Sf9 cell line. The biological activity of the different recombinant proteins will be assessed initially by their ability to induce in-vitro estradiol secretion from gonadal tissue. Subsequently, these recombinant hormones will be used for studying aspects of LH and FSH biology where absolutely pure preparations of the hormones are required, such as their biological activity during ontogeny and their biological specificity. The use of this approach partially overcomes the need to purify these hormones for their study, a requirement that greatly limited their investigation so far, as well as opens the way for the study of gonadotropins from species where limited biological material is available.

PP-228

CLONING, SEQUENCING OF β GTH-I, β GTH-II AND GROWTH HORMONE cDNAS OF THE BLUE GOURAMI (*TRICHOGASTER TRICHOPTERUS*) AND THEIR EXPRESSION DURING THE DIFFERENT STAGES OF OOGENESIS

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We cloned two cDNAs of β GtH-I, β GtH-II and growth hormone (GH) from the pituitary gland of blue gourami *Trichogaster trichopterus*. These cDNAs were cloned by RACE-PCR, sequenced and subjected to sequence analysis. Blue gourami β GTH-I and β GTH-II are most similar to the peptides of the striped-bass counterpart. The polypeptides share respectively 73% and 84% of the residues. The expression of the genes for β GTH-I and β GTH-II were evaluated by deducting the amount of specific PCR products obtained from cDNA, reverse transcripted from total RNA obtained from pituitaries of females found at different stages of the reproductive cycle. The highest intensity of β GTH-I bands was found in females classified as high vitellogenic, with less intense bands found in females that were at the stages of final oocytes maturation (FOM) and post-spawning. No PCR product was obtained from pituitaries of low vitellogenic or immature females. The β GTH-II bands with the highest intensity were those of females found in FOM. In order to quantify the results, the intensity of the bands was translated to OD values.The deduced amino acid sequence of the blue gourami GH was compared with similar sequences of GH from six different fish species. It was found to be most similar the amino acid sequence of tilapia GH (82% identical residues) and least similar to eel GH (64% identity). The expression of the blue gourami GH mRNA was measured during various stages of oogenesis: oogonia, vitellogenesis and maturation. The highest levels of GH mRNA were found during growth in juveniles, in vitellogenesis and in maturation. The lowest levels were found at the beginning of vitellogenesis.

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ONTOGENY AND REGULATION OF GONADOTROPIN-I AND -II GENE EXPRESSION IN THE FEMALE STRIPED BASS, *MORONE SAXATILIS*

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The pituitary gonadotropins (GtHs) stimulate gonadal growth and development, and the study of molecular mechanisms that underlie gonadotropin physiology is essential for the understanding of pubertal development. GtH-subunit cDNA clones were used to develop a ribonuclease protection assay (RPA) for the simultaneous quantitation of all GtH subunits in a fraction of a pituitary. Using this RPA, the expression of the GtH subunit genes was monitored in female striped bass for a period of 3 years. All females in the first and second year of life were juvenile. Pubertal development took place in 65% of 3-year-old females, whereas sexual maturation occurred in all 4-year-old females. Sexual maturity was associated with a rise in the mRNA levels of all GtH subunit genes, specifically a 218-fold increase in βGtH-I mRNA. The gene regulation of the GtH subunits in juvenile females was studied by chronic administration of testosterone (T) and/or an analog of gonadotropin-releasing hormone (G). The administration of T+G increased the mRNA levels of βGtH-II subunit to values characteristic of sexually mature fish, and also increased the plasma levels of GtH-II. However, these changes did not result in the acceleration of sexual maturation. The mRNA levels of βGtH-I subunit were slightly stimulated, but remained about 1/10 of the values characteristic of sexually mature fish. It is concluded that the stimulation of GtH-II gene expression and release does not lead to the acceleration of sexual maturity, and that the failure to sufficiently stimulate βGtH-I subunit gene expression may underlie the inability of the treatments to advance sexual maturity.

PP-230

IS LEPTIN INVOLVED IN GONADOTROPIN PRODUCTION IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)?

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In higher vertebrates, leptin, an hormone produced by adipocytes could serve as a metabolic signal to the reproductive system (induction of reproduction or puberty). In mammals, it could act at the gonad (presence of receptors) as well as the brain-pituitary (presence of receptors, induction of LHRH and basal LH and FSH releases) levels. We investigated the expression of a leptin-like gene in fish and the action of heterologous leptin at the pituitary level. Here we present preliminary data concerning the effect of increasing doses (10-11 to 10-6 M) of recombinant human leptin on basal and GnRH-induced GTH 1 and GTH 2 releases by trout pituitary cultures. Studies were conducted on immature fish of both sexes, at early gametogenesis, 2 months before ovulation and in spermiating and periovulatory animals.Whatever the sexual stage and the duration of incubation, leptin did not influence the pituitary sensitivity to GnRH. On the other hand, high doses of leptin (from 10-7 to 10-6 M) were shown to induce an effect on GTH1 and GTH 2 basal secretions which varies with the sexual status : GTH1 and GTH2 releases were increased from immaturity to 2 months before ovulation while at the time of reproduction only GTH2 was significantly modified in males. In the same cultures, no effect on GH secretion was observed. The difference of structure between human and trout leptin could explain the relative insensitivity of the pituitary cells. Works are in progress to clone a leptin like cDNA.

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GnRHs (SEA BREAM, CHICKEN-II AND SALMON) PITUITARY CONTENT AND GtH-II PLASMA LEVELS IN MALE SEA BASS (*DICENTRARCHUS LABRAX*) DURING SEX DIFFERENTIATION AND PUBERTY

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Three forms of GnRHs were analyzed in the pituitary of male sea bass from sex differentiation (11 months of age) to onset of puberty (first reproductive season): sea bream GnRH (sbGnRH), chicken GnRH-II (cGnRH-II) and salmon GnRH (sGnRH). Plasma GtH-2 levels were determined during the same time. sbGnRH levels were found to be 6-fold higher than cGnRH-II and 17-fold higher than sGnRH over the sampling time. All GnRH forms showed the highest level in November 1995, when fish were differentiating, and a weak peak appeared in March 1996, becoming significant ($P<0.05$) for cGnRH-II. Later on, levels of the three forms decreased dramatically and remained low during the second year. However, pituitary sbGnRH showed an interesting variability since one significant increase was observed in November 1996, when males attained puberty. Plasma GtH-2 levels showed the lower value in November 1995, and increased 2.5 times during the next months, in an opposite trend to levels of the GnRHs. During the first reproductive season, plasma GtH-2 levels remained low throughout October and November 1996 but there was a sudden increase in December 1996, just after the peak of sbGnRH in November 1996. These results might suggest a possible role of sb-, cII and s- GnRH in gonadal differentiation, while sbGnRH might be involved in the control of gametogenesis, and particularly in the onset of puberty in sea bass. Moreover, an important role in gonadal maturation of GtH-2 might be suggested since the highest plasma GtH-2 levels were found throughout the period of spermiation.

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REPRODUCTIVE CYCLE AND GONADOTROPIC CELLS OF SOUTH AMERICAN CHARACIDAE FISHES

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The gonadotropic cells (GtH cells) of the pituitary gland of two important economic South American freshwater migratory teleost, the curimbatá, *Prochilodus scrofa* (Steindachner, 1881), and the pacu *Piaractus mesopotamicus* (Holmberg, 1887) were studied under electron microscopy during the annual reproductive cycle. In captivity, these fishes show a pattern of gonadal maturation similar to that seen in nature, however, reproduction does not occur spontaneously without an induction to spawn. Males and females adult fish were raised in captivity in the Center of Research on Tropical Fish (CEPTA-IBAMA) in S. Paulo, Brazil. During the year the gonads of the adult fish are classified in four phases of development: resting (RT), early maturation (EM), advanced maturation (AM), and regression (RG). These phases take place in winter, spring, summer and autumn respectively. As gametogenesis and the spawning are under gonadotropin control in teleosts, the purpose of the present investigation is to report the structural modifications in the GTH cells related to the reproductive cycle. Whereas the GTH cells of pacu presented almost the same apparence during the year, the curimbata GTH cells changed greatly, showing enlarged RER vesicles mainly in the AM. Later, in RG, the cells presented a compactment of their granules, an irregular shape of the nucleous and changes in the chromathin. These feactures are not signs of degeneration or apoptosis, but a cellular rearrangement. Our results indicate the presence of a single GTH cell type in both species studied, which changes its morfological and functional characterists along the year.

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SOMATOLACTIN CELLS IN FEMALE OF PEJERREY, *ODONTESTHES BONAERENSIS* (ATHERINIDAE) PITUITARY. CHANGES RELATED WITH THE REPRODUCTIVE STATUS

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Somatolactin (SL) is a fish pituitary hormone isolated and characterised in teleost species. This protein is structurally conserved and belongs to the growth hormone/prolactin family. Although its physiological function is unknown some authors have proposed that it may be related to teleost reproduction in addition to other functions. In salmonids several evidences indicates a SL involvement in reproduction in contrast to late evolved teleost groups such as Perciformes and Pleuronectiformes. In the present study the relation between SL cells and the reproductive status of pejerrey was analysed. Females were collected monthly along a year from outdoor ponds at the Saitama Prefecture Fisheries Experimental Station (Japan). The reproductive status of each individual was determined by gonadosomatic index (GSI) and histological analyses of the gonads. Pituitary serial sections of animals with different reproductive status were identically immunostained with an antiserum raised against chum salmon SL and the cellular and nuclear area were measured with a image analyzer. The SL-immunoreactive (ir-SL) cells in immature fish were small and lightly granulated when compared with the ir-SL cells from mature females, which were large and highly granulated. The quantitative analysis showed a cellular and nuclear area correlation ($P< 0.05$) and both parameters increased with the increase of GSI. In addition, the comparative analysis showed highly significant differences in these parameters ($P< 0.01$) between reproductively active and inactive females. Our results showed a correlation in the pituitary SL activity and the reproductive cycle suggesting a role of SL in reproduction in this species.

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THE ECOLOGICAL PLASTICITY OF STURGEONS SEX DIFFERENTIATION

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The stellate sturgeons (sevruga) juveniles were kept immediately after hatching under constant water temperature regimes: optimal (24 °C, control), high (29 °C) or low (13°C). The critical hormone-dependent period of gonads sexualization (PGS) in fry under 24° began at the age of 2 months, while under 29° and 13° - at 7 months. Under 24° at the age of 1 year the ratio Females:Males (F:M) was 51:49%. According to radioimmunological analysis, under 29° in specimen of both sexes at the age of 1 year the levels of sex steroid hormones (SSH) in blood, mostly estrogens, decreased, but the relative testosterone content increased; in this case sex inversion of the F was observed and the M number increased (81%). After transduction of the fishes at the age of 7 months from 13° to 24° in specimen of both sexes the SSH levels, mainly estrogens, increased to 1 year age, the sex inversion was observed in the M and the F number increased (77%). Under the impact of food deprivation for 2 months since PGS onset in young sevruga under water temperature of 24° and 29° the character of changes of SSH levels and ratio, the gonads sex differentiation and sex ratio was the same as in the fishes that received food, correspondently, after transduction from 13° to 24° or under 29° conditions. So, plasticity of SSH sex-specific status formation (levels and ratio) in critical PGS that can be considered as the important species adaptation underlies the basis of ecological plasticity of sex differentiation in sturgeons. Perhaps it is related to the plasticity of synthesis and secretion of hypophysial sex-specific gonadotropins. As fishes are referred to poikilothermal vertebrates, then functional mobility of hypothalamus-hypophysis-gonads axis in regulation of sex differentiation in them seems to be quite reasonable in combination of effects of unfavourable (extremal) ecological factors with onset of critical hormone-dependent PGS.

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