Ann. Zool. Fennici 34: 115–126 ISSN 0003-455X
Helsinki 27 May 1997

Seasonal changes in fish biomarkers

Tarja Nakari

Nakari, T., Finnish Environment Institute, Hakuninmaantie 4–6, FIN-00430 Helsinki, Finland

Received 1 August 1996, accepted 6 February 1997

Seasonal changes in the detoxification, hormone levels, energy metabolism and water content were measured in juvenile 2-year-old brown trout held in two environmentally different test conditions. In spite of the different test conditions, the course of seasonal changes in the measured biomarkers was quite similar in both test group fish. However, the measured values of these biomarkers differed significantly between the groups, and also between sexes. Although the fish were juveniles, seasonal changes similar to adults were observed in male gonadal growth and plasma testosterone concentration. Also, only in males, during the spawning period, the liver ethoxyresorufin-O-deethylase activity was negatively correlated with the plasma testosterone concentration, which was at its highest at that time. The liver detoxification activity reached maximum in winter. Plasma osmotic concentration was also higher in winter than summer. During the entire year, the tissue-water content of the "laboratory" fish was significantly higher than that of the fish farm group. Energy resources of the fish increased during summer, and were high in autumn and early winter. Between the groups notable differences were observed only in winter.

1. Introduction

Along with the growing chemical pollution of the aquatic environment, attention has been directed towards the development of biochemical and physiological markers suitable for the early and sensitive detection of environmental degradation caused by pollutants (Uthe *et al*. 1980 rev. in Lindström-Seppä 1990). However, there is very little information available on the normal seasonal baseline values of these biomarkers in environmentally different test conditions. This information is especially needed in northern areas such as in Finland (60°N–70°N), where the illumination and water temperature conditions are extreme. Days are long in summer as opposed to constant darkness, especially under the ice and snow, in winter. Water temperature varies between close to zero and above + 20°C. Background data on the normal values of the biomarkers and their expected variability are particularly required by managers of fishery resources and water quality, for assessment as to whether observed changes in a biomarker are caused only by contaminants, or by natural or endogenous agents.

The goal of this study was to investigate the seasonal changes and differences in the detoxification, water content, energy metabolism and hormone levels — the most generally used fish biomarkers — of juvenile brown trout held in two environmentally different test conditions.

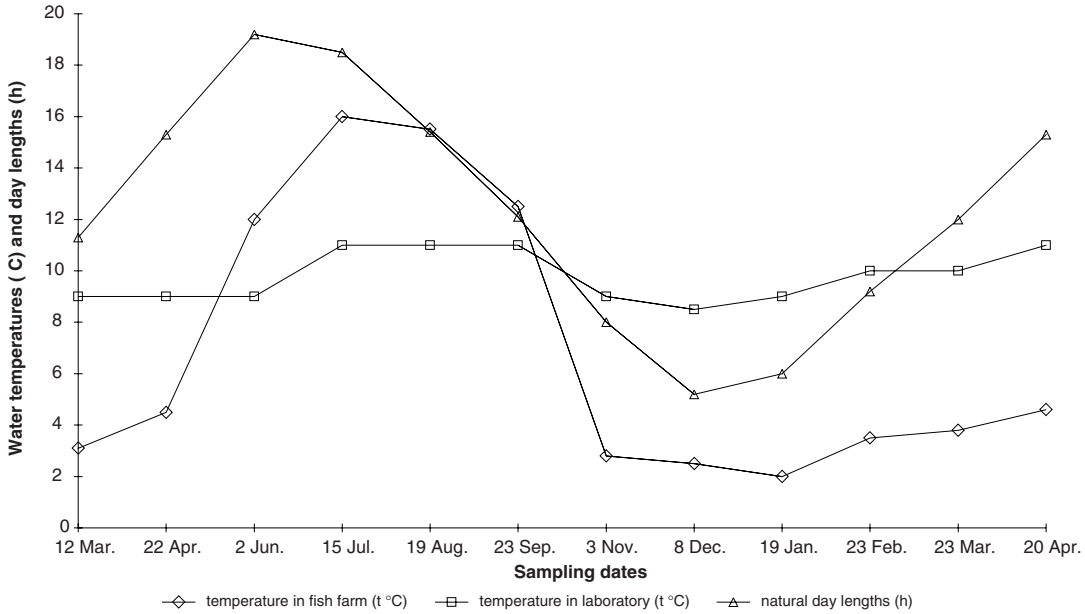

Fig. 1. Water temperatures (°C) in different test groups, and natural day lengths (h) in Central Finland.

2. Material and methods

The experiment was carried out in a fish farm of Taimen Co. in Laukaa (62°30´N, 26°E), and in the ecotoxicological laboratory of the Finnish Environment Institute. Juvenile 2-year-old lake-form brown trout (*Salmo trutta* m. *lacustris* L.) was chosen for the study. This species is native to Finnish lakes, and does not undergo smoltification (Soivio *et al*. 1989).

In the fish farm, the fish were held in an outdoor earth pond under the natural light and water temperature conditions. They were reared according to the normal routine of the fish farm and fed daily. Natural day lengths and water temperatures are shown in Fig. 1.

In the laboratory, fish from the same broodstock were placed in a fibreglass basin in untreated Helsinki City tap water (drawn from Lake Päijänne). There was constant water flow and the water oxygen level was maintained at near 100% of air saturation by means of continuous aeration. Fish were acclimated to a water temperature of 10° ± 2°C and illumination of 12L/12D. They were fed to satiety three times a week (the same pelleted food as in the fish farm). The fish density was kept at 120 indiv./m^3 throughout the experiment.

Fish were sampled monthly, for a period of 13 months, starting at the same time of the day, at noon, to avoid interference by circadian rhythms (Laidley & Leatherland 1988). The first sampling in the fish farm was in March, and in the laboratory in the beginning of June.

Fish were not fed for 24 h prior to each sampling. Each sampled fish (one at a time) was killed by a blow to the head, measured and weighed. A blood sample was taken from the dorsal vein with a heparinized syringe and centrifuged at 20 000 rpm**.** The liver and gonads were dissected and weighed for calculation of organosomatic indices (OSI = % of body weight). The isolated plasma and the organs were frozen without delay in liquid nitrogen and stored there until analyzed. Sampling of one fish took about three minutes. Each time, 20 fish per group (10 males and 10 females) were sampled.

Microsomal UDP-glucuronyltransferase (UDPGT) and ethoxyresorufin-O-deethylase (EROD) activity, and glutathione (GSH), protein, water, glycogen and lipid concentrations were assayed in the liver; water and lipid content were determined in white muscles. Liver microsomes were prepared according to Lindström-Seppä and Oikari (1989). UDPGT activity was determined by the method of Castrén and Oikari (1983) using p-nitrophenol as the aglycone, and pure ammonium salt of uridine-5-diphosphoglucuronic acid as the glucuronyl donor. EROD activity was determined by the method of Porter *et al.* (1989) using 0.1 M Tris-buffer of pH 7.8 and the incubation temperature of + 18°C. Liver GSH content was determined according to Saville (1958), and protein concentration by the method of Lowry *et al*. (1951) using bovine serum albumin as a standard. Glycogen and lipid concentrations were assayed as reviewed by Soivio and Virtanen (1980). Tissue water concentration was determined by drying the samples to their constant weights at 105°C.

Blood plasma was analyzed for osmolality (The Advanced Micro-Osmometer, model 3 MO), testosterone (T), 17β-estradiol (E_2) and total cholesterol concentration. Plas-

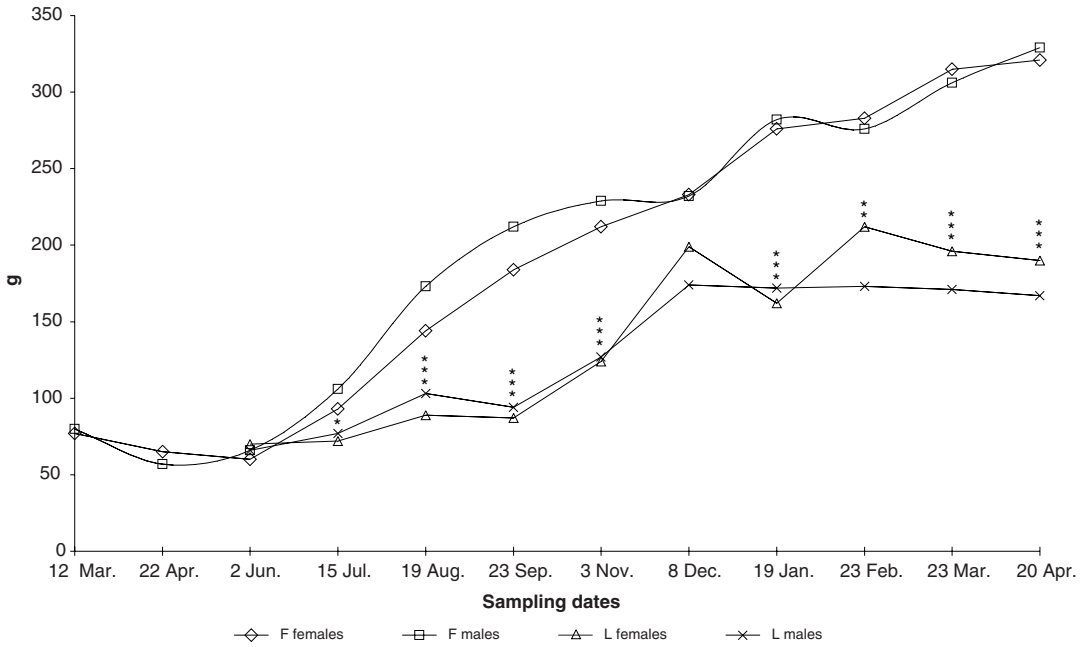

Fig. 2. Fish weights (g). F = "fish farm" group, L = "laboratory group". (♀) = differences observed only in females between the test groups, (♂) = differences observed only in males between the test groups. * $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$

ma T and E_2 concentrations were assayed by RIA as described by Scott *et al*. (1982). Tritium-labelled hormones were purchased from the Radiochemical Centre, Amersham, and antisera from the Sigma Chemical Company. Cross-reactivities of the antisera are shown in the Sigma test files (T-4276 and E-2885). Plasma total cholesterol was assayed with Boehringer test kits (Mono-test). All measurements were performed in duplicates.

The results for males and females from each sampling time are expressed in figures as group means. Differences between groups and sexes were tested for each sampling date using Student's *t*-test, which is satisfactory for this kind of experiment.

3. Results

At first, fish did not grow much, but from July onwards the weight increase of the "fish farm" fish started to be significantly faster ($p < 0.001$) than that of the "laboratory" fish (Fig. 2). However, though the "fish farm" group grew better, significant differences in the energy resources between the groups were observed only in winter. Changes in the muscle lipid content were not very distinct, while those in the liver were more perceptible (Fig. 3). The values varied between 0.5–1% and 1–6% for muscle and liver, respectively, and were at their highest in winter. Additionally in the liver the second peak was observed in summer.

In both groups, liver glycogen reserves increased towards the autumn (Fig. 4). In the "laboratory" fish, the concentrations dropped unexpectedly in September, but in the beginning of November they were high again, even higher ($p < 0.1$) than in the "fish farm" group. After December concentrations began to decrease towards the spring values.

In the "laboratory" fish, the course of changes in the liver protein concentrations was quite the opposite to that of the liver glycogen (Fig. 4), which was very distinctly observed e.g. in September. In the "fish farm" group these two variables changed quite parallelly until December. But after that, when the liver glycogen concentration started to decrease, that of the protein still showed an increasing trend.

As shown by the muscle and liver water concentrations (Fig. 5), and plasma osmolalities (Fig. 6), fish contained more water in summer than during the rest of the year. Moreover, during the

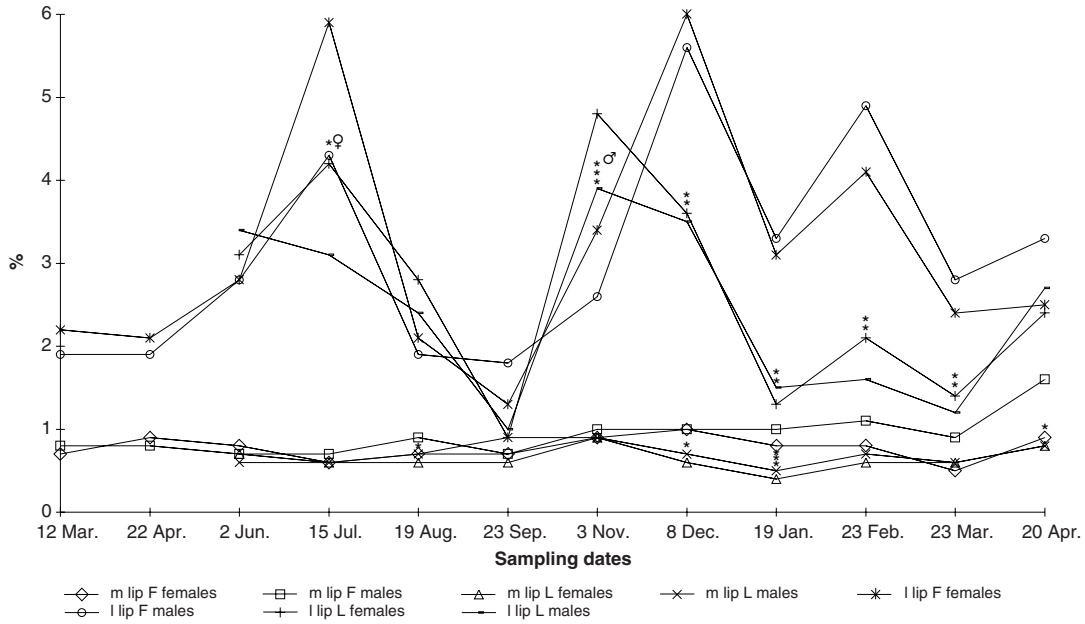

Fig. 3. Muscle (m) and liver (l) lipid concentrations (wet weight %). For more details, see Fig. 2.

entire year, the tissues of the "laboratory" fish contained significantly more water ($p < 0.001$) as compared with that of the "fish farm" fish. Changes in water content were smaller in the muscle than in the liver.

Seasonal changes in the liver EROD activity are obviously partly controlled by the water temperature (Fig. 7). In the "fish farm" fish the activity was high, when the water temperature was low, and vice versa. During summer the "laboratory"

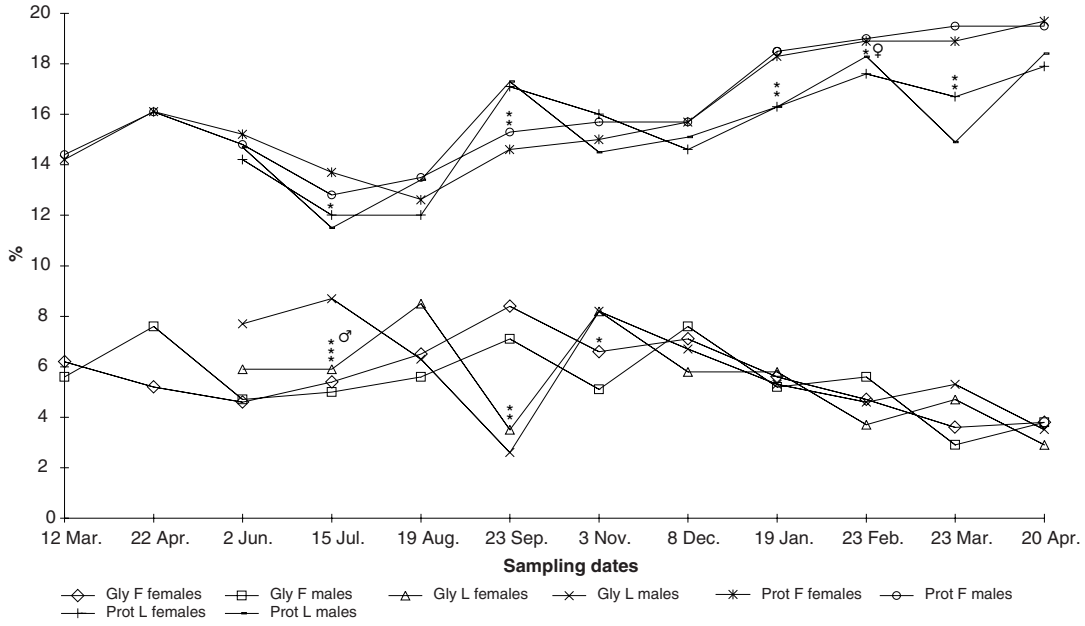

Fig. 4. Liver glycogen (Gly) and protein (Prot) concentrations (wet weight %). For more details see Fig. 2.

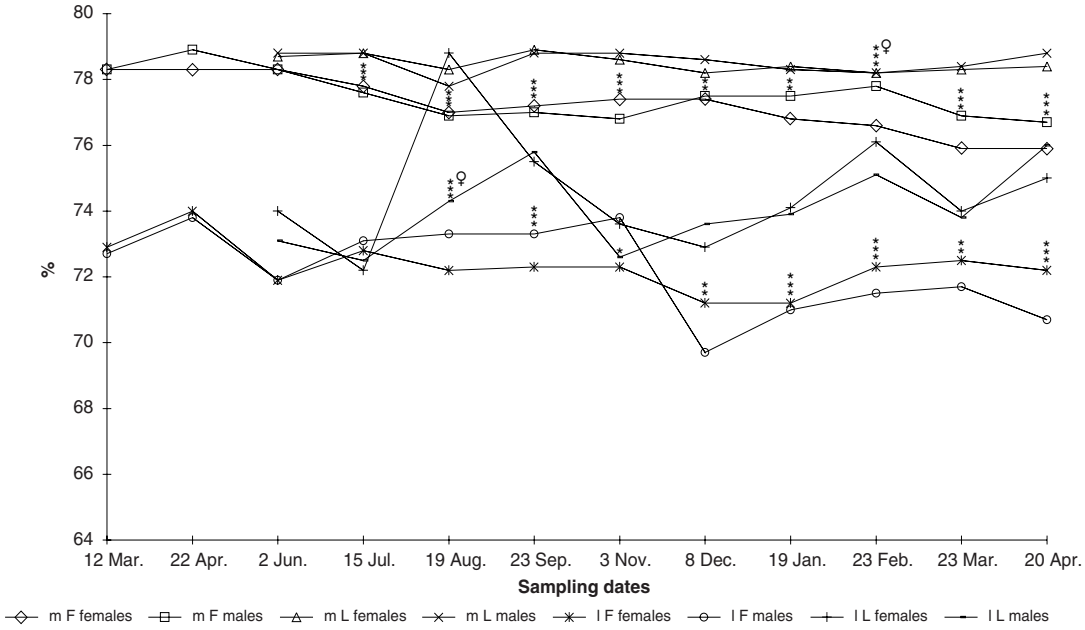

Fig. 5. Muscle (m) and liver (l) water concentrations (%). For more details, see Fig. 2.

fish were in colder water, and had significantly higher ($p < 0.01$) liver EROD activity than the "fish farm" fish. In spring, the results between the groups were opposite. In autumn, the water temperatures were similar in both places and so were the liver EROD activities.

During the spawning period, in spite of the low water temperature, the EROD activity of the "fish farm" males was significantly lower as compared with that of the females, and the "laboratory" males. The activity also remained on that low level until the beginning of December, while

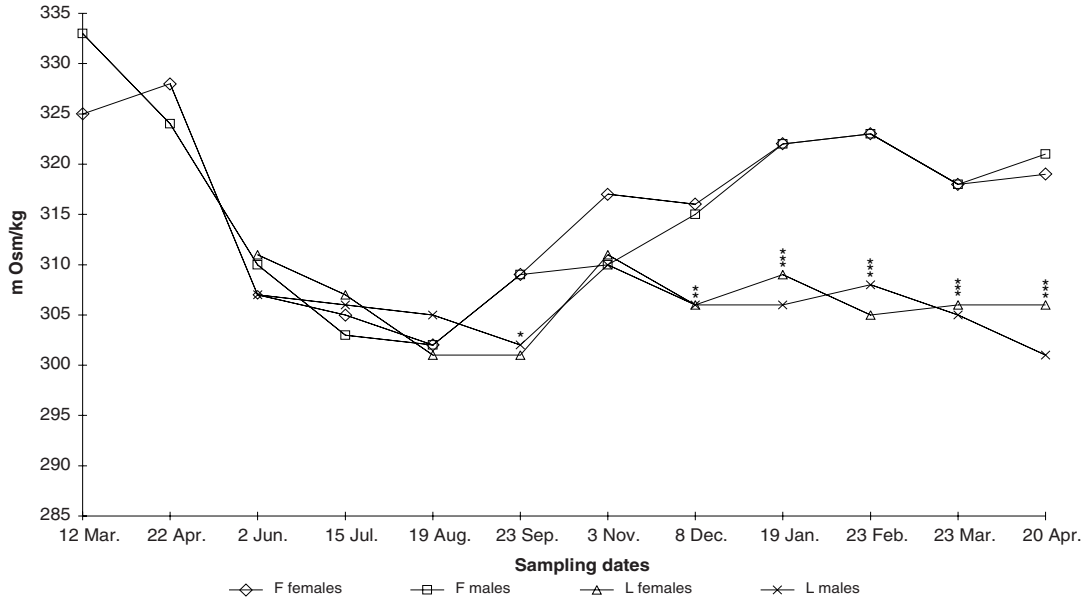

Fig. 6. Plasma osmotic concentrations (mOsm/kg). For more details, see Fig. 2.

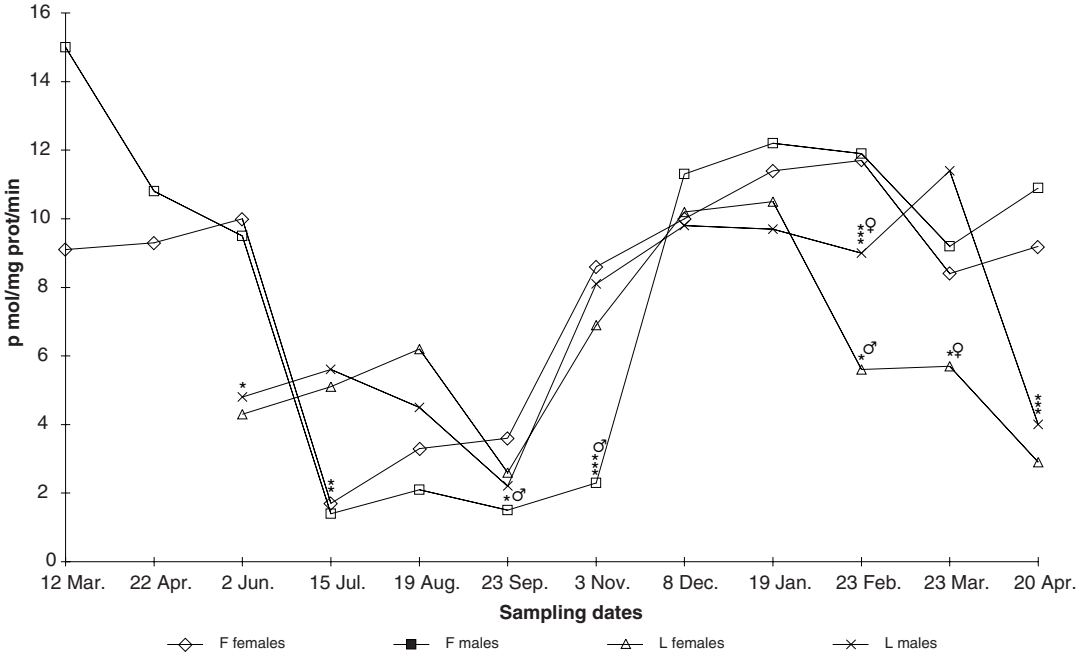

Fig. 7. Liver microsomal ethoxyresorufin-O-deethylase activities (pmol/mg prot/min). For more details, see Fig. 2.

in the females and "laboratory" males it had started to increase already after September.

There was a decreasing trend in the liver UDPGT activity of the "fish farm" fish throughout the year (Fig. 8). In the "laboratory" fish the UDPGT activity stayed on a reasonably steady level until December–January, after which it also started to decrease. During winter, the liver UDPGT activity of the "laboratory" fish, especially the females, was higher than that of the "fish farm" fish.

The highest peak in the liver glutathione concentration was measured in mid-winter, though the concentration seemed to peak every third month throughout the year (Fig. 9). During summer and spring, the GSH concentration measured in the "fish farm" fish was higher than that of the "laboratory" fish, but during winter it was lower.

Seasonal changes in the gonadal growth were distinct only in males (Fig. 10). In females GSI values stayed on a steady low level (about 0.2%) throughout the year. The difference between sexes was significant ($p < 0.001$). Testes started to grow after June. When they reached their maxima sizes late in autumn, they were significantly bigger ($p < 0.001$) in the "fish farm" males than in the "laboratory" males. During the spawning period, only in the "fish farm" group, were there males with running milt. Variations between individuals were great. In the "fish farm" fish, the testes started to decrease after October, about two months earlier than in the "laboratory" fish. A new cycle started again after February.

In all fish, during the whole experiment, plasma 17β-estradiol concentration was low (< 2 μg/l) (Fig. 11). In females, also plasma testosterone concentration stayed at a low level (about 1 μg/l) (Fig. 11). In males, the plasma testosterone concentration changed quite parallelly to the GSI values. The difference between sexes was significant ($p < 0.001$). The maximum was measured in November during the normal spawning period of the fish. There were no differences in the concentrations between the groups, although the testes of the "laboratory" males were significantly smaller than those of the "fish farm" males. The basal low level was reached after December, in accordance with the GSI values, in the "fish farm" males two months earlier than in the "laboratory" males.

Seasonal changes in the plasma cholesterol concentration were more distinct in the "fish farm"

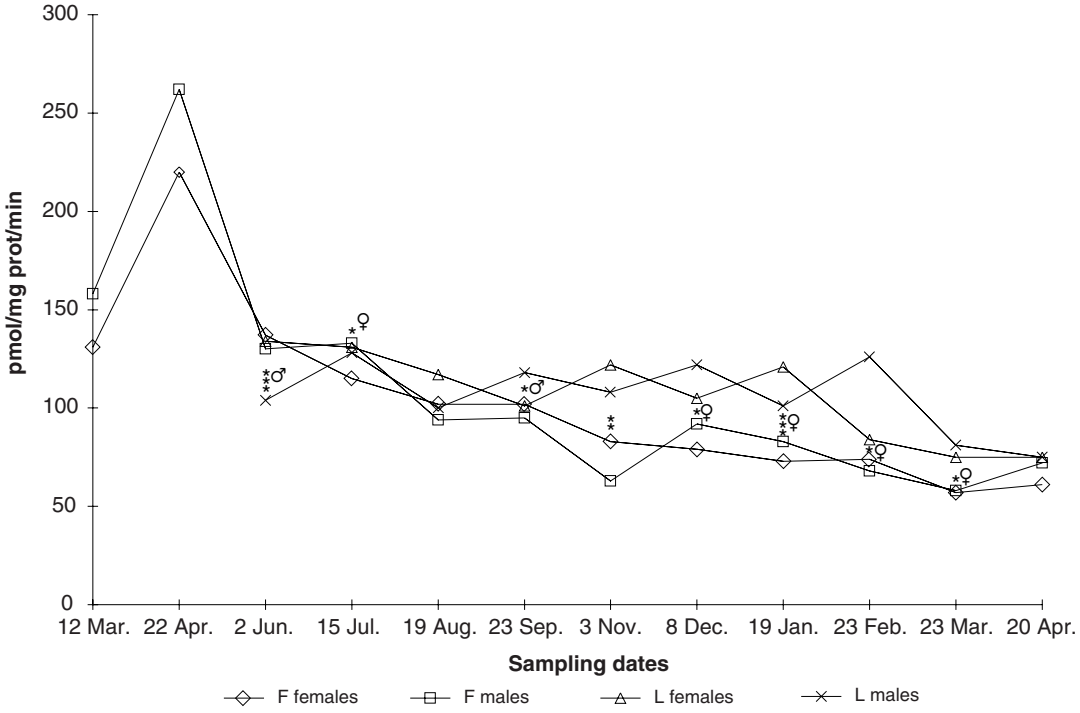

Fig. 8. Liver microsomal UDP-glucuronyltransferase activities (pmol/mg prot/min). For more details, see Fig. 2.

group than in the "laboratory" fish (Fig. 12). Also, during the entire year, cholesterol concentrations in the "fish farm" fish were significantly ($p < 0.001$) higher than those of the "laboratory" fish. The

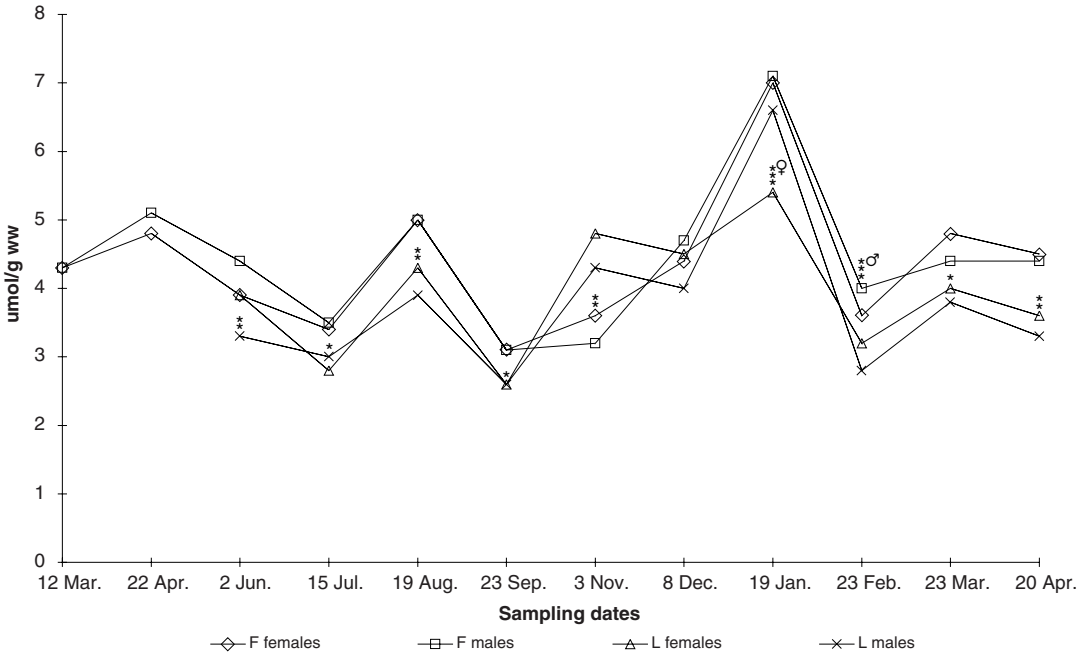

Fig. 9. Liver glutathione concentrations (μmol/g ww). For more details, see Fig. 2.

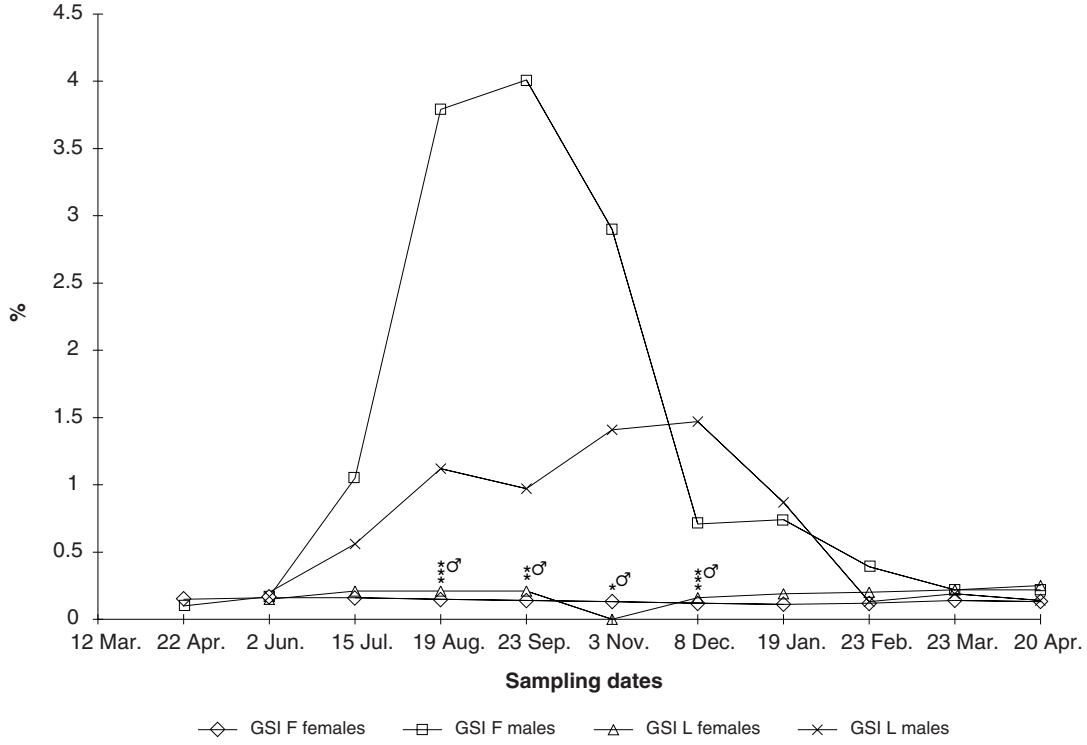

Fig. 10. Gonadal development, expressed as GSI %. For more details, see Fig. 2.

lowest values were measured during spawning, when the difference between groups were observed only between females. In the "fish farm" group the peak was measured in December.

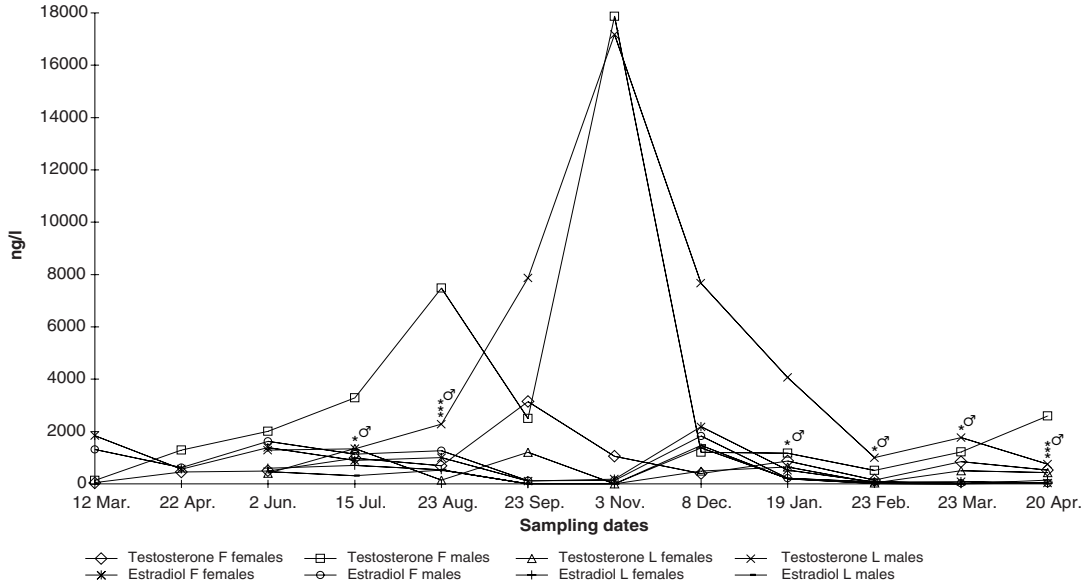

Fig. 11. Plasma testosterone and estradiol-17β concentrations (ng/l). For more details, see Fig. 2.

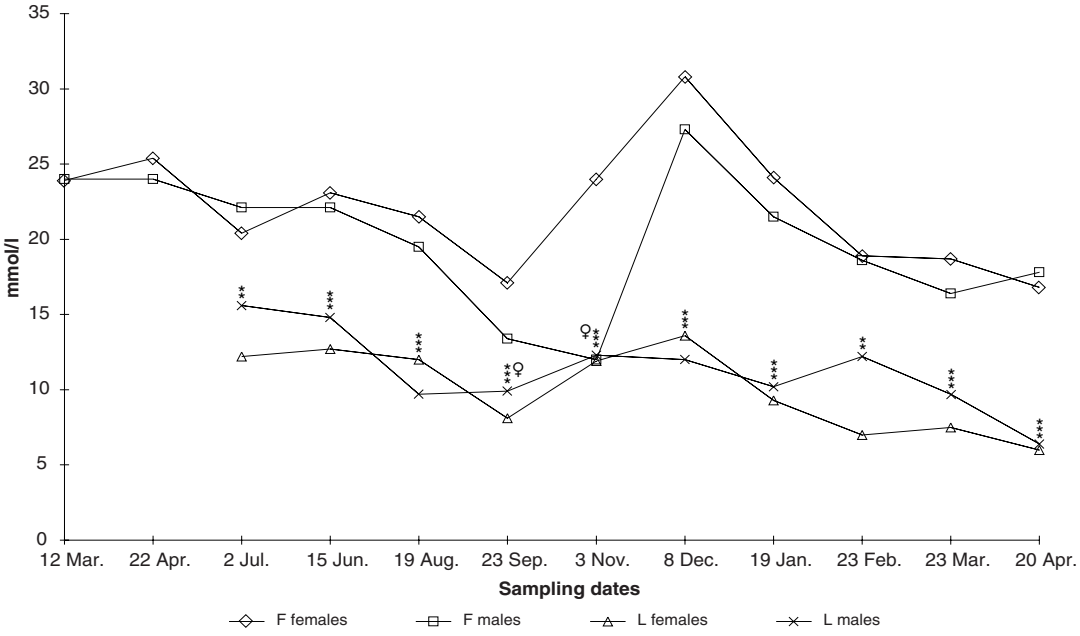

Fig. 12. Plasma total cholesterol concentrations (mmol/l). For more details, see Fig. 2.

4. Discussion

The water temperature, together with the light rhythm, are the most important factors controlling plasma sex hormones and cholesterol concentrations, and also hepatic detoxification activity in adult fish (Whitehead *et al*. 1978, Koivusaari *et al*. 1981, Koivusaari 1983, Boulier & Billard 1984, Elliot *et al*. 1984, Scott *et al*. 1984, Lindström-Seppä 1985, Nakari *et al*. 1987, Skarphedinsson *et al*. 1985). Water quality may also affect these (Nakari *et al*. 1986). Sex hormones can depress and be the inhibitors of the liver detoxification enzyme activities (Förlin & Hansson 1982, Stegeman *et al*. 1982, Pajor *et al*. 1990, Larsen *et al*. 1992). In this work, the above-mentioned may explain the changes in the liver EROD activity of males, which, in spite of the great individual differences within the groups, during spawning period, had equally high plasma testosterone concentrations as mature fish (Soivio *et al*. 1982), although the fish were juveniles. However, the similar changes in the liver EROD activity and plasma cholesterol concentration were observed also in females with low GSI values and plasma hormone concentrations. One very important factor in controlling the liver EROD activity, also in juvenile fish, seems to be the water temperature. Fish kept in warm water have lower liver EROD activities than those in colder water. The case seems to be the same with the plasma cholesterol concentration.

Van den Hurk *et al*. (1978) and Hoffmann and Wondrak (1980) have given a good description of the seasonal anatomical changes of fish testes. In fish with a proper maturation cycle, changes in the GSI values are accompanied with the changes in plasma sex hormone concentrations. However, smaller gonads do not always mean lower plasma sex hormone levels, as was stated by Munkittrick *et al*. (1991), at least not in healthy fish. This was observed in the "laboratory" males with the significantly smaller testes, but equally high plasma testosterone concentrations as in the "fish farm" males.

The nutritional state of fish can affect their liver detoxification activity (Campbell & Hayes 1974), and energy metabolism (Sheridan & Mommsen 1991). In this work, no correlation was observed between enzyme activities, energy resources or the fish weights. The liver detoxification activity was in accordance with Buhler and Rasmusson (1968), who found no changes in monooxygenase activities of rainbow trout starved

for 8 weeks, or when fed with diets high in protein or carbohydrate, and with Andersson *et al*. (1985) showing that inducibility of cytochrome P-450-dependent reactions in rainbow trout is not influenced by starvation. However, the differences observed in plasma cholesterol and tissue lipid and protein concentrations between the groups are obviously caused by the different feeding rhythms. Yet, to avoid variation in results, diet must be carefully chosen and controlled in long-term experiments, because some commercial fish pellets may contain both inducers and inhibitors of several liver microsomal enzymes (Ankley & Blazer 1985, Viganó *et al*. 1993). In this work, according to the manufacturer's guarantee, the used fish feed was free from hazardous substances. Also, there were no pollutants in the test waters, which could have affected the results.

Handling, which can cause changes e.g. in osmoregulation and water balance of fish (Eddy 1981), may be excluded from the results, because fish were handled quite similarly in both test sites. However, in spite of the similar handling, the laboratory group fish were more watery than the fish farm group fish. Pearcy (1961) observed that during winter the plasma osmolality and the water content of flounders (*Pseudopleuronectes americanus*, Walbaum) were higher than during summer. Umminger and Mahoney (1972) found a similar winter increase in the plasma osmotic pressure of flounders, but no changes in the muscle water concentration. They proposed that the watery condition of the fish flesh in winter in Pearcy's work could have been related to gonadal development, because flounders in his experiment were ripe adults. In this work, the results are in accordance with Umminger and Mahoney (1972), with the exception that the "laboratory" fish were more watery throughout the entire year than the "fish farm" fish. The higher osmotic pressure in the "fish farm" fish during winter may have some adaptive significance as a protection against freezing for fish inhabiting cold shallow waters, as has been suggested already in 1957 by Scholander *et al*. But on the other hand, Umminger (1970) and Denton and Yousef (1975) have shown that seasonal changes in the osmotic metabolism in rainbow trout and winter flounder are not related to temperature. So, what might be the effects of light or exercise?

Arising from the above, it is very difficult to compare results from different works as such, or to ascertain, if the seasonal changes in biochemical or physiological variables of fish are attributable to temperature, photoperiod, or developmental or innate circannual influence, or are caused by pollutants. In spite of the different experimental and external conditions in the fish farm and in the laboratory, the seasonal changes in the measured variables were similar, but the quantitative values between the groups differed. According to this, the seasonal changes in fish biomarkers are controlled by the endogenous factors, but the quantitative values by the exogenous ones.

In northern areas like in Finland, where the seasonal environmental changes are great, and the normal environmental conditions in northern and southern parts of the country are quite different, it would be very important that the baseline values and the background data on the normal seasonal changes in the used biomarkers in certain environmental conditions could be available, when results from different experiments are evaluated, and environmental monitoring test programs are made.

Acknowledgements. I express my warmest thanks to everybody who helped me in this work, especially to the staff of Taimen Co., for their grand collaboration, to Prof. Antti Soivio for reviewing the manuscript, and to Ms. Kaisa Heinonen, Ms. Sirpa Paattakainen, Ms. Helena Tanttu and Ms. Kirsti Erkomaa for technical help.

References

Andersson, T., Koivusaari, U. & Förlin, L. 1985: Xenobiotic biotransformation in the rainbow trout liver and kidney during starvation. — Comp. Biochem. Physiol. 82C: 221–225.

Ankley, G. T. & Blazer, V. S. 1985: Effects of diet on PCB-induced changes in xenobiotic metabolism in the liver of channel catfish (Ictalurus punctatus). — Can. J. Fish. Aquat. Sci. 45: 132–137.

Boulier, A. & Billard, R. 1984: Delay of gametogenesis and spawning by constant illumination of rainbow trout (Salmo gairdneri) during the first reproductive cycle. — Can. J. Zool. 62: 2183–2187.

Buhler, D. R. & Rasmusson, M. E. 1968: The oxidation of drugs by fishes. — Comp. Biochem. Physiol. 25: 223–239.

Campbell, T. C. & Hayes, J. R. 1974: Role of nutrition in the drug-metabolizing enzyme system. — Pharmac. Rev. 36: 171–197.

Castrén, M. & Oikari, A. 1983: Optimal assay conditions for liver UDP-glucuronosyltransferase from the rain-

bow trout Salmo gairdneri. — Comp. Biochem. Physiol. 76C: 365–369.

Denton, J. E. & Yousef, M. K. 1975: Seasonal changes in hematology of rainbow trout, Salmo gairdneri. — Comp. Biochem. Physiol. A 51: 151–153.

Eddy, F. B. 1981: Effects of stress on osmotic and ionic regulation in fish. — In: Pickering, A. D. (ed.), Stress and Fish, I ed.: 77–102. Academic Press, London.

Elliot, J. A. K., Bromage, N. R. & Springate, J. R. C. 1984: Changes in reproductive function of three strains of rainbow trout exposed to constant and seasonally changing light cycles. — Aquaculture 43: 23–34.

Förlin, L. & Hansson, T. 1982: Effects of oestradiol-17ß and hypophysectomy on hepatic mixed function oxidases in rainbow trout. — J. Endocrinol. 95: 245–252.

Hoffmann, R. & Wondrak, P. 1980: Seasonal anatomical variations in the testes of European pike, Esox lucius L. — J. Fish Biol. 16: 475–482.

Koivusaari, U. 1983: Thermal acclimatization of hepatic polysubstrate monooxygenase and UDP-glucuronosyltransferase of mature rainbow trout (Salmo gairdneri). — J. Exp. Zoology. 227: 35–42.

Koivusaari, U., Harri, M. & Hänninen, O. 1981: Seasonal variation of hepatic biotransformation in female and male rainbow trout (Salmo gairdneri). — Comp. Biochem. Physiol. 70C: 149–157.

Laidley, C. V. & Leatherland, J. F. 1988: Circadian studies of plasma cortisol, thyroid hormone, protein glucose and ion concentration, liver glycogen concentration and liver and spleen weight in rainbow trout, Salmo gairdneri Richardson. — Comp. Biochem. Physiol. 89A: 495–502.

Larsen, H. E., Celander, M. & Goksøyr, A. 1992: The cytochrome P450 system of Atlantic salmon (Salmo salar): II. Variations in hepatic catalytic activities and isozyme patterns during an annual reproductive cycle. — Fish Physiol. Biochem. 10: 291–301.

Lindström-Seppä, P. 1985: Seasonal variation of the xenobiotic metabolizing enzyme activities in the liver of male and female vendace (Coregonus albula L.). — Aquat. Toxicol. 6: 323–331.

Lindström-Seppä, P. 1990: Biotransformation in fish: Monitoring inland water pollution caused by pulp and paper mill effluent. — Ph.D.-thesis, Publications of the University of Kuopio, Original reports 8/1990.

Lindström-Seppä, P. & Oikari, A. 1989: Biotransformation and other physiological responses in whitefish caged in a lake receiving pulp and paper mill effluents. — Ecotoxicol. Environ. Saf. 18: 191–203.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. 1951: Protein measurements with the Folin phenol reagent. — J. Biol. Chem. 193: 265–275.

Munkittrick, K. R., Portt, C. B., van der Kraak, G. J., Smith, I. R. & Rokosh, D. A. 1991: Impact of bleached kraft mill effluents on population characteristics, liver MFO activity and serum steroid levels of a Lake Superior white sucker (Catostomus commersoni) population. — Can. J. Fish. Aquat. Sci. 48: 1371–1380.

Nakari, T., Ruoppa, M., Soivio, A. & Miettinen, V. 1986: Raportit Päijänteen ja Vantaanjoen veden laadun sekä lämpötilan vaikutuksista 2-vuotiaiden meritaimenten fysiologiseen tilaan. — Vesihallituksen monistesarja 422.

Nakari, T., Soivio, A. & Pesonen, S. 1987: Effects of an advanced photoperiod cycle on the gonadal development and spawning time of 2-year-old Salmo gairdneri R. reared in earth ponds under extreme annual water temperatures. — Aquaculture 67: 369–384.

Pajor, A. M., Stegeman, J. J., Thomas, P. & Woodin, B. R. 1990: Feminization of the hepatic microsomal cytochrome P-450 system in brook trout by estradiol, testosterone, and pituitary factors. — J. Exper. Zool. 253: 51–60.

Pearcy, W. G. 1961: Seasonal changes in osmotic pressure of flounder sera. — Science 134: 193–194.

Porter, E. L., Payne, J. F., Kiceniuk, J., Fancey, L. & Melvin, W. 1989: Assessment of the potential for the mixed-function oxygenase enzyme induction in the extrahepatic tissues of cunners (Tautogolabrus adspersus) during reproduction. — Mar. Environ. Res. 28: 117–121.

Saville, B. 1958: A scheme for the colorimetric determination of microgram amounts of thiols. — Analyst. 83: 670.

Scholander, P. F., van Dam, L., Kanwisher, J. W., Hammel, H. T. & Gordon, M. S. 1957: Supercooling and osmoregulation in arctic fish. — J. Cell. Comp. Physiol. 49: 5–24.

Scott, A. P., Sheldrick, E. L. & Flint, A. P. F. 1982: Measurement of 17α,20β-dihydroxy-4-pregnen-3-one in plasma of trout (Salmo gairdneri R.); seasonal changes and response to salmon pituitary extract. — Gen. Comp. Endocrinol. 46: 444–451.

Scott, A. P., Baynes, S. M., Skarphedinsson, O. & Bye, V. J. 1984: Control of spawning time in rainbow trout, Salmo gairdneri, using constant long daylengths. — Aquaculture 43: 225–233.

Sheridan, M. A. & Mommsen, T. P. 1991: Effects of nutritional state in vivo lipid and carbohydrate metabolism of coho salmon, Oncorhynchus kisutch. — Gen. Comp. Endocrinol. 81: 473–483.

Skarphedinsson, O., Bye, V. J. & Scott, A. P. 1985: The influence of photoperiod on sexual development in under-yearling rainbow trout, Salmo gairdneri Richardson. — J. Fish Biol. 27: 319–326.

Soivio, A. & Virtanen, E. 1980: Methods for physiological experiments on fish. Ekotoxikologiska metoder för akvatisk miljö. — Rapp 16, Nordforsk.

Soivio, A., Muona, M. & Virtanen, E. 1989: Smolting of two populations of Salmo trutta. — Aquaculture 82: 147–153.

Soivio, A., Pesonen, S., Teräväinen, T., Nakari, T. & Mwenesi, R. 1982: Seasonal variations in oestrogen and testosterone levels in the plasma of brown trout (Salmo trutta lacustris) and in the metabolism of testosterone in its skin. — Ann. Zool. Fennici 19: 53–59.

Stegeman, J. J., Pajor, A. M. & Thomas, P. 1982: Influence of estradiol and testosterone on cytochrome P-450 and monooxygenase activity of immature Brook trout, Salvelinus fontinalis. — Biochem. Pharmacol. 31: 3979–3989.

Umminger, B. L. 1970: Effects of subzero temperatures and

trawling stress on serum osmolality in the winter flounder Pseudopleuronectes americanus. — Biol. Bull. 139: 574–579.

Umminger, B. L. & Mahoney, J. B. 1972: Seasonal changes in the serum chemistry of the winter flounder, Pseudopleuronectes americanus. — Trans. Amer. Fish. Soc. 4: 746–748.

Uthe, J. F., Freeman, H. C. & McIntyre, A. D. 1980: The selection of sublethal testing procedures for assessment of chemical toxins in the aquatic environment and experiences in the use of steroid hormone metabolism. — Can. Tech. Rep. Fish. Aquat. Sci. 975: 231–242.

Van den Hurk, R., Peute, J. & Vermeij, J. A. J. 1978: Morphological and enzyme cytochemical aspects of the testis and vas deferens of the rainbow trout, Salmo gairdneri. — Cell Tiss. Res. 186: 309–325.

Viganõ, L., Arillo, A., Bagnasco, M., Bennicelli, C. & Melodia, F. 1993: Xenobiotic metabolizing enzymes in uninduced and induced rainbow trout (Oncorhynchus mykiss): Effects of diets and food deprivation. — Comp. Biochem. Physiol. 104C: 51–55.

Whitehead, C., Bromage, N. R., Forster, J. R. M. & Matty, A. J. 1978: The effects of alterations in photoperiod on ovarian development and spawning time in the rainbow trout (Salmo gairdneri). — Ann. Biol. Anim. Biochem. Biophys. 18: 1035–1043.