Ann. Zool. Fennici 17: 249–254. 1980

Seasonal variations in lipid content and fatty acids in the liver, muscle and gonads of the eel-pout, Zoarces viviparus (Teleostei) in brackish water[1]

Marketta Pekkarinen

Pekkarinen, M. 1980: Seasonal variations in lipid content and fatty acids in the liver, muscle and gonads of the eel-pout, Zoarces viviparus (Teleostei) in brackish water. — Ann. Zool. Fennici 17: 249—254.

The distribution of lipids and the seasonal variations in their contents in various organs of *Zoarces viviparus* (L.) are described and related to the earlier plasma lipid analyses and to the reproductive cycle of the species. The lipid content of the liver was highest in August, at the time of mating, being 37 % in females and 44 % in males, and lowest in March, 16 % in females and 18 % in males. In females the decrease began earlier than in males because of the transfer of nourishment to the embryos during pregnancy and partly because of the flow of materials into the growing oocytes. Muscular tissue contained 5.8 % lipids in August and 2.1 % in March. The lipid content of the ovarian tissue was 2.5 % in March. The eggs contained 8.2 % lipids in August, just before ovulation. The mean lipid content of the testes was about 1.6—1.8 %.

Oleic acid was the most abundant fatty acid in liver lipids. It ranged from 34 to 39 % of the total fatty acids. Seasonal variations in the proportions of the fatty acids were greatest for 16:1, 18:1, 20:5 and 22:6. The sexes differed slightly in the proportions of 16:1, 20:5 and 22:6 in the liver. The reasons for the striking changes in the levels of plasma and liver lipids and in the proportions of liver fatty acids after ovulation and fertilization (during August — October) are discussed.

Marketta Pekkarinen, Division of Physiology, Department of Zoology, University of Helsinki, Arkadiankatu 7, SF-00100 Helsinki 10.

1. Introduction

Fishes commonly store energy reserves as lipids in various organs, e.g. in muscles or in the liver. Shul'man (1974) has given several examples of how seasonal cycles, feeding and reproduction affect lipid metabolism in various species. In many fishes the fat content declines during the prespawning period. Other fishes feed intensively during the period of gonadal maturation and their fat content increases. About the time of spawning the fat content declines and it is apparently caused by increased muscular activity and by the expenditure of energy in the act of spawning. In many species spawning coincides with an interruption of feeding.

In the females of viviparous fishes, as compared with oviparous species, the reproductive period is extended by pregnancy, which, in the case of *Zoarces viviparus* (L.) in the Gulf of Finland, lasts about 5 months in autumn — winter (Kristoffersson & Pekkarinen 1975). Brief mention has been made of the effects of reproductive stages on constituents of the blood and embryotrophe in the eel-pout by Kristoffersson et al. (1973, 1974). This work is a continuation of the article by Pekkarinen & Kristoffersson (1975) describing changes in concentrations of blood plasma lipids during the annual/sexual cycle of the eel-pout. Blood, being an active carrier of materials, reflects some aspects of lipid metabolism. This paper concerns the lipids and fatty acids present in various organs of this species, the seasonal variations in these and the relation of these variations to the stages of the reproductive cycle, particularly in the female. The findings by Korsgaard & Petersen (1979) and Korsgaard Emmersen & Petersen (1979) on the same species in the Little Belt are combined and compared with our data in the discussion.

[1] Report No. 632 from Tvärminne Zoological Station, University of Helsinki.

2. Material and methods

Eel-pouts were caught with a bottom trawl in the area of Tvärminne Zoological Station (Gulf of Finland) at depths of 25—40 m. The fish weighed 91.0 to 475.0 g, their lengths ranging from 25.0 to 45.5 cm. All fish were sexually mature. For details, see Pekkarinen & Kristoffersson (1975).

The eight sampling times throughout the year for liver analyses were (in parentheses the numbers of females and males): mid-February (6, 10), late March (11, 8), late May (21, 15), late June (7, 8), mid-August (8, 8), late September (5, 8), late October (6, 2) and mid-December (9, 5). Liver and other tissue samples for lipid analyses weighed $\leq$ 3 g. Muscle samples were taken from the right epaxonic muscle at about the middle of the body length in late March (2, 2), and in August (3, 2). The samples of ovarian tissue (5) in late March included ovarian muscles and spent *calyces nutriciae* (described in Kristoffersson et al. 1973) as well as growing oocytes with their calyces. The egg samples (2) in August were obtained by cutting off eggs with their calyces from the ovarian wall. Testes were sampled in late March (2, spermiogenesis in progress) and in August (7, breeding). The reproductive cycle is depicted in the lowest part of Fig. 1.

Histological preparations were made from liver, gonads and embryos. Pieces of tissue were fixed in Bouin's fluid and processed with the routine paraffin technique and other pieces were fixed in 10 % formalin containing 1 % $CaCl_2$ for the cryostat technique. Paraffin sections (7 μm) were stained with Masson — Gomori (chromotrope — fast green; Gray 1954) and cryostat sections (10 μm) with Fettrot and Mayer's haematoxylin (Barka & Anderson 1963).

Total lipids were extracted and purified according to the procedure of Folch et al. (1957). The tissue samples were stored in liquid nitrogen. The extracts were stored under nitrogen gas at 4°C before analysis.

The lipids were transesterified by refluxing with methanol (1 % H_2SO_4 as catalyst) at 80—85°C for 2 h (see Laukola & Suomalainen 1971). The methyl esters were extracted with n-hexane and dried overnight with a mixture of Na_2CO_3—Na_2SO_4 (1:25). Gas-liquid chromatography was carried out at 180°C in a Perkin-Elmer F11 gas chromatograph (Perkin-Elmer steel column, 2 m long, 3.2 mm in outer diameter, 20 % DEGS on Chromosorb W, 80—100 mesh) equipped with FID, and with N_2 as carrier. The triangulation method was used in calculations, with chromatograms of methyl esters of known fatty acids and their mixtures as references.

In the chromatograms of liver lipids the peaks in the regions of 20:0 and 20:1 were small and were not always sufficiently well separated, so they were excluded from the calculations. The peak 20:4 was also excluded, because it was not measurable in all samples. The peak x was more conspicuous. Its retention time was between those of 20:1 and 20:4.

3. Results

Total lipid contents and histochemistry of the organs. The lipid content of the liver (as %age of wet weight) was highest in both sexes in August,

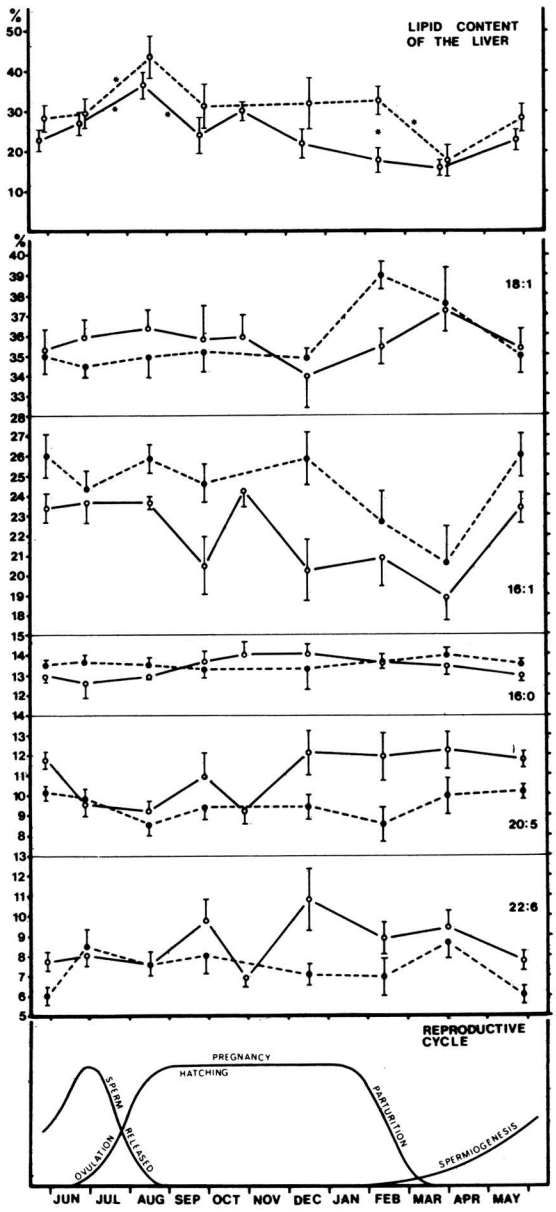

Fig. 1. At the top: seasonal changes in the lipid content of the liver of *Zoarces viviparus* expressed as percentages of wet weight (mean $\pm$ *SE*). Asterisks indicate significant differences (Student's *t* test, $P < 0.05$). Solid line = females, dotted line = males. In the middle: changes in the proportions of the major fatty acids in the liver lipids. *N* varies from 5 to 21. At the bottom: diagram representing the reproductive cycles of females and males (Kristoffersson & Pekkarinen 1975).

being 36.6 % in females and 43.6 % in males, and lowest in March, 15.8 % and 17.7 %, respectively (Fig. 1). In males it remained more or less steady in winter at a little over 30 %, and decreased from February to March. In females it decreased as early as August or September. The decline then stopped for some time and continued slowly during the winter until March. In February the lipid content of the liver was significantly ($P< 0.05$) lower in females than in males. In both sexes active deposition of liver lipids occurred from March to August. The highest liver lipid content measured was 61.5 % in a male in August.

The lipid droplets were evenly distributed throughout the liver. The proportional lipid contents in the livers could be predicted quite well from the histological preparations (Fig. 2).

Muscular tissue contained 2.1 % of lipids in March and 5.8 % in August. The increase was significant ($P < 0.01$).

The lipid content of the ovarian tissue was 2.5 % in March. The eggs contained 8.2 % lipids in August, just before ovulation. The mean lipid contents of the testes were quite small, 1.8 % in March and 1.6 % in August.

The growth of the oocytes was observed to be as described by Götting (1976). From the lipid stainings and from the "empty" vacuoles seen in paraffin sections the lipid droplets were inferred to coalesce to larger droplets as the oocyte grew, and to move towards the margin of the maturing egg. Yolk granules, staining red in Masson — Gomori and clearly visible in light microscopy, could be seen in the oocytes from October to January. These grew in size till the yolk filled the egg except for the marginal zone.

In histological preparations the blood plasma in the ovarian vessels was demonstrated with Fettrot to be hyperlipaemic during late pregnancy. Fettrot-stainable lipids were seen in the tissues of the embryos, e.g. in the liver, alimentary canal, and hindgut epithelium.

Fatty acid composition of the total lipids in the organs. In the gas chromatograms of liver lipids the areas under the minor peaks, i.e. the fatty acids 14:0, x, 18:0, and 18:2, did not vary markedly during the year. They represented about 2, 2, 2—3, and 2—3 %, respectively, of the total fatty acids. The percentage of palmitic acid (16:0) did not vary much either, being about 13—14 % (Fig. 1).

The greatest seasonal variations appeared in the fatty acids 18:1, 16:1, 20:5 and 22:6 (Fig. 1). In the liver of *Zoarces* the most abundant fatty acid was oleic acid (18:1), which varied from 34

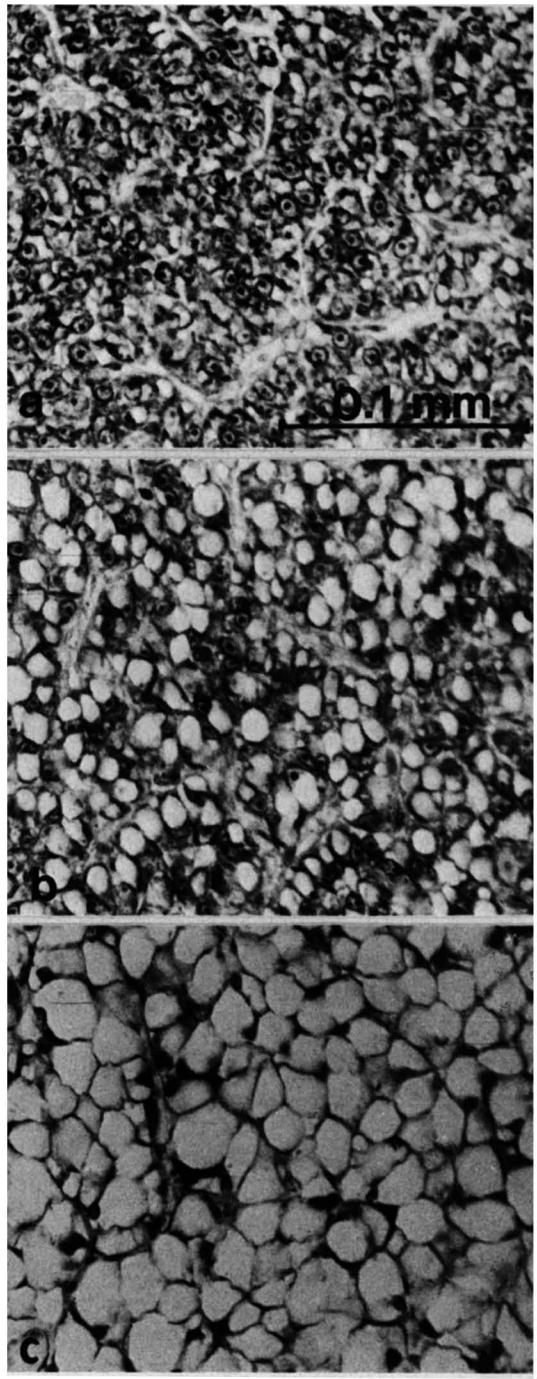

Fig. 2. Paraffin sections stained with Masson — Gomori from livers of *Zoarces viviparus*. Note the differences in size and abundance of the "empty" lipid droplets between the three samples (the droplets were shown to consist of lipids in cryostat sections stained with Fettrot). a) Liver of a female in March, lipid content 6.6 %. b) Liver of a female in February, lipid content 13.9 %. c) Liver of a male in August, lipid content 48.1 %. Magnification is the same in all micrographs.

to 39 %. In the males a striking increase took place in the proportion of 18:1 between December and February; in the females the increase was less marked. Palmitoleic acid (16:1) seemed to be more abundant in the liver lipids of males; in both sexes it was lowest in March, when the lipid content of the liver was at its lowest, and then rose steeply until May. In the liver of *Zoarces* females the proportion of 20:5 was highest in winter, being then greater in females than in males. In December liver 22:6 was also increased in females. In males liver 22:6 was decreased in May. In autumn the proportions of some fatty acids (16:1, 20:5 and 22:6) varied strikingly in the liver of females.

Table 1 shows the fatty acid composition of the liver lipids at the seasons of 'low lipid' (March) and 'high lipid' (August). The fatty acid composition of the lipids of muscular tissue, ovary/eggs and testis at the same seasons are also presented. In muscle lipids (females and males pooled) 18:1 was not so abundant as in liver lipids. In muscle lipids the proportion of 16:1 was smaller and of 22:6 larger at the time of 'low lipid' than at the time of 'high lipid'.

In the ovarian tissue in March the proportion of 16:1 was much smaller than in the liver, and 18:1 was approximately the same as in muscle (pooled females and males), whereas the percentages of 20:4, 20:5 and 22:6 were greater in the ovary than in the liver. In the eggs, too, 16:1 and 18:1 were lower than in the liver. Fatty acids 20:5 and 22:6 together represented about 34 % of the fatty acids in the eggs. A trend to more saturated C_{16} and C_{18} fatty acids was noticed in *Zoarces* testis as compared with liver. The proportions of the fatty acids 20:1, 20:4, 20:5 and 22:6 were greater in the testis than in the liver of males.

4. Discussion

All gadoids (including the eel-pout, as shown here) deposit fat almost exclusively in the liver (Lovern 1964). The oil content of cod livers varied from 15 to 75 % (Jangaard et al. 1967a). In this study the mean lipid content of the liver of the eel-pout was 16—44 %, depending on the season. While cod muscles usually contain less than 1 % fat, the lipid content of the muscle in the eel-pout is about 2—6 %. The eel-pout at Tvärminne (Gulf of Finland) has a higher lipid concentration in blood plasma (Pekkarinen & Kristoffersson 1975) and a higher lipid content in the liver than the eel-pout living in the Little Belt (Korsgaard & Petersen 1979).

In *Zoarces* spermiogenesis (see Kristoffersson & Pekkarinen 1975) and vitellogenesis take place mainly at the time when the lipid content of the liver is increasing (March — August). The increased non-esterified fatty acids (NEFA) in *Zoarces* blood plasma (Pekkarinen & Kristoffersson 1975) in June, before the breeding season in July—August, indicate very active lipid metabolism. In females the lipid content of the liver decreased after mating, from August to late September. There was also a transient fall in plasma total lipids and NEFA in September (Pekkarinen & Kristoffersson 1975). In the *Zoarces* of the Little Belt, serum and liver lipids also

Table 1. The lipid content (% wet weight) and fatty acid composition (%) of the liver, muscle, ovary/eggs and testis of *Zoarces viviparus* at the times of 'low lipid' (March) and 'high lipid' (August).

	Liver				Muscle		Ovary	Eggs	Testis	
	low		high		low	high	low	high	low	high
N	11 ♀	8♂	8♀	8♂	2♀,2♂	3♀,2♂	5	2	2	7,4[1]
Lipid	15.8	17.7	36.6	43.6	2.10	5.77	2.50	8.17	1.79	1.63
Fatty acid										
14:0	2.06	2.34	2.54	2.46	4.34	4.28	2.16	2.96	1.82	1.82
16:0	13.41	13.94	12.93	13.49	12.87	13.94	13.48	13.80	16.78	19.83
16:1	18.88	20.60	23.64	25.83	15.75	22.07	9.74	14.26	6.33	10.22
18:0	2.56	2.69	2.28	2.55	3.22	3.19	3.36	3.82	5.76	7.00
18:1	37.21	37.53	36.37	34.92	30.63	28.80	29.70	22.98	24.45	23.84
18:2	2.37	2.56	2.66	2.68	2.45	1.74	1.22	1.18	1.37	1.30
20:1					2.77	2.13	3.24?	1.73	6.27	2.47
x	1.86	1.71	2.77	1.95	2.00	3.18	1.91	2.15		
20:4					2.20	1.58	4.32	3.06	7.36	5.72
20:5	12.22	9.95	9.22	8.54	10.92	10.40	14.52	19.09	13.52	11.60
22:6	9.43	8.68	7.59	7.58	12.85	8.50	16.35	14.97	16.34	16.20

[1] Lipid and fatty acid, respectively.

decreased in mid-September (Korsgaard & Petersen 1979). The decline in liver lipids stopped about October (also in Korsgaard & Petersen's data) and then continued again. The embryos hatch in September. Why did the lipid content of the liver decrease immediately after mating? In the swordtail, *Xiphophorus helleri*, Wegmann & Götting (1971) have traced the transport of nutritive material from the maternal body into the oocytes and even into embryonic tissues. In *Clinus superciliosus* the intrafollicular embryos absorb nutrients through the epidermis during early and mid-pregnancy until absorption from the gut begins during late pregnancy (Veith 1978). The egg membrane of *Zoarces viviparus* is thin, and may allow transport of material from maternal tissues to the embryos even before they hatch (Götting 1976).

Changes in activity and feeding habits in autumn may also contribute to variations in the lipid contents of the plasma and liver and the varying proportions of fatty acids in the liver at that time. A change of habitat is suggested, because in the last few years attempts to catch eel-pouts (both females and males) in autumn at the usual locality have been unsuccessful.

Zoarces plasma is clearly hyperlipaemic from October to December, and maybe still in February (Pekkarinen & Kristoffersson 1975). This indicates very active transport of lipids. At the same time, from October to February, the liver lipid content of females decreases. Korsgaard & Petersen (1979) have presented closely similar results. The mother's metabolism is readjusted to meet the demands of the embryos. Hogarth (1976) gives data on some viviparous elasmobranchs and the relationships between their uterine milk composition, nutrient uptake by the foetuses, and the effect on the maternal liver. The greater the percentage of organic material in the uterine secretion, the greater the decline in the relative size of the liver in the pregnant female. The embryotrophe in the ovarian cavity of the eel-pout is rich in lipids (Kristoffersson et al. 1973 and unpublished data by the author). The lipid concentration in the embryotrophe seems to be greater in early and late pregnancy than in mid-pregnancy. This and the lipids demonstrated in the epithelium of the hindguts and other tissues of embryos suggest that the embryos absorb materials effectively from the embryotrophe.

The growing oocytes may also contribute to the depletion of the liver lipids and other substances during pregnancy. In the pregnant female poeciliid the liver undergoes changes very similar to those seen in starvation. However, the changes are probably not due to the metabolic strain of supplying nutrients to the foetuses, but rather to the rapid synthesis of yolk for the subsequent batch of maturing oocytes (Hogarth 1976). In *Zoarces* the growth phase of the oocytes is long (about a year, according to Götting 1976 and this study). But Shul'man (1974) has pointed out that differentiation processes in the gonads require much more energy than growth processes, at least in Atlantic sardine and Azov anchovy. According to Götting (1976), the oocytes of the eel-pout that are to be ovulated the next summer differentiate to become fully ripe about July. How much energy is needed at the beginning of this phase, in what form it is supplied and whether this is reflected in plasma and liver metabolites in August—September is not known. There is still some uncertainty about the chemical constituents of yolk in fish oocytes and confusion concerning the term vitellogenesis (cf. Khoo 1979).

It is not known whether eel-pouts feed regularly during pregnancy. In January 1979 I observed that a female which had just delivered her young in an aquarium had eaten many of the young, but the stomachs of the other females (still pregnant) in the same aquarium were empty. Natural fasting, at least in late pregnancy, would partly explain why Korsgaard Emmersen & Petersen (1979) did not notice any significant differences in the amounts of liver GOT, GPT or lipid between starved pregnant *Zoarces* females and freshly caught pregnant females. The embryos developed normally and the gonadosomatic index was found to be normal. These authors supposed that the metabolites transported from the mother to the growing embryos probably originated from muscles rather than from the liver.

In *Zoarces* males the plasma total lipid concentration also rose in autumn until December, although not so much as in females (Pekkarinen & Kristoffersson 1975), but the concentration of NEFA remained low. At the same time the testes were filled with spermatogonia. In contrast to females, however, the liver lipid content of males remained fairly high until February. The decrease in liver lipids between February and March coincided with the early stages of speriogenesis.

Large quantities of the fatty acids 20:5 and 22:6 are present in the developing eggs of fishes (e.g. Ackman & Burgher 1964, Saddler 1969). In cod roe they represent about 40 % of the fatty acids (Jangaard et al. 1967b), and in the eggs of *Zoarces* about 34 % in August. The proportions of 20:5 and 22:6 in the liver of *Zoarces* females were high

during late pregnancy and 20:5 remained high during the spring. But between May and June (during late vitellogenesis) 20:5 clearly decreased. In the liver of *Zoarces* males 20:5 and 22:6 did not vary much during the year, except for a decrease in 22:6 in May.

The proportion of palmitoleic acid seemed to correlate positively with the lipid content in the liver (and maybe also in the muscle) of *Zoarces*. The fatty acid composition of aquatic animals depends on many factors, such as diet and the proportions of different lipid classes in the tissues (e.g. Farkas 1971) and the temperature (e.g. Farkas & Csengeri 1976). The water temperature at the depth where the eel-pouts were caught is lowest during February—April, but the seasonal temperature differences at that depth do not usually exceed 10°C. Further investigations into the environmental biology and physiology of this species near Tvärminne Zoological Station are in progress.

Acknowledgements. My thanks are due to Dr. Rolf Kristoffersson, Associate Professor, Head of Tvärminne Zoological Station. The work was supported by a grant from the National Research Council for Sciences and the Emil Aaltonen Foundation. I am grateful to Mrs Jean Margaret Perttunen, B.Sc. (Hons.), for revising the English manuscript.

References

Ackman, R. G. & Burgher, R. D. 1964: Cod roe: component fatty acids as determined by gas-liquid chromatography. — J. Fisheries Res. Board Canada 21: 469—476.

Barka, T. & Anderson, P. J. 1963: Histochemistry. Theory, practice and bibliography. — 660 pp. Harper & Row, Publishers, Inc., New York.

Farkas, T. 1971: A possible explanation for the differences in the fatty acid composition of fresh-water and marine fishes. — Ann. Biol. Tihany 38: 143—152.

Farkas, T. & Csengeri, I. 1976: Biosynthesis of fatty acids by the carp, Cyprinus carpio L., in relation to environmental temperature. — Lipids 11: 401—407.

Folch, J., Lees, M. & Sloane Stanley, G. H. 1957: A simple method for the isolation and purification of total lipids from animal tissues. — J. Biol. Chem. 226: 497—509.

Gray, P. 1954: The microtomist's formulary and guide. — 794 pp. Blakiston Company, Inc., New York.

Götting, K. J. 1976: Fortpflanzung und Oocyten-Entwicklung bei der Aalmutter (Zoarces viviparus) (Pisces, Osteichthyes). — Helgoländer Wiss. Meeresunters. 28: 71—89.

Hogarth, P. J. 1976: Viviparity. The Institute of Biology's Studies in Biology no. 75. — 68 pp. Edward Arnold, London.

Jangaard, P. M., Brockerhoff, H., Burgher, R. D. & Hoyle, R. J. 1967a: Seasonal changes in general condition and lipid content of cod from inshore waters. — J. Fisheries Res. Board Canada 24: 607—612.

Jangaard, P. M., Ackman, R. G. & Sipos, J. C. 1967b: Seasonal changes in fatty acid composition of cod liver, flesh, roe and milt lipids. — J. Fisheries Res. Board Canada 24: 613—627.

Khoo, K. H. 1979: The histochemistry and endocrine control of vitellogenesis in goldfish ovaries. — Can. J. Zool. 57: 617—626.

Korsgaard, B. & Petersen, I. 1979: Vitellogenin, lipid and carbohydrate metabolism durig vitellogenesis and pregnancy, and after hormonal induction in the blenny Zoarces viviparus (L.). — Comp. Biochem. Physiol. 63B: 245—251.

Korsgaard Emmersen, B. & Petersen, I. 1979: Metabolic effects of food deprivation during vitellogenesis and pregnancy in Zoarces viviparus. — Abstract from the 2nd Symposium on Fish Physiology, Göteborg, June 8—10, 1979.

Kristoffersson, R., Broberg, S. & Pekkarinen M. 1973: Histology and physiology of embryotrophe formation, embryonic nutrition and growth in the eel-pout, Zoarces viviparus (L.). — Ann. Zool. Fennici 10: 467—477.

Kristoffersson, R., Broberg, S. & Oikari, A. 1974: Annual changes in some blood constituents of female brackish-water Zoarces viviparus (L.), Teleostei, with special reference to the reproductive cycle and the embryotrophe. — Hydrobiol. Bull. 8: 117—124.

Kristoffersson, R. & Pekkarinen, M. 1975: Histological changes in the testes of brackish-water Zoarces viviparus (L.) in relation to the reproductive cycle. — Ann. Zool. Fennici 12: 205—210.

Laukola, S. & Suomalainen, P. 1971: Studies on the physiology of the hibernating hedgehog. 13. Circum-annual changes of triglyceride fatty acids in white and brown adipose tissue of the hedgehog. — Ann. Acad. Scient. Fennicae (A IV) 180:1—11.

Lovern, J. A. 1964: The lipids of marine organisms. — Oceanogr. Mar. Biol. Ann. Rev. 2: 169—191.

Pekkarinen, M. & Kristoffersson, R. 1975: Seasonal changes in concentrations of plasma lipids in the brackish-water eel-pout, Zoarces viviparus (L.). — Ann. Zool. Fennici 12: 260—262.

Saddler, J. B. 1969: Salmon lipid studies. — Contr. Univ. Wash. College (Sch.) Fish 300: 43—44.

Shul'man, G. E. 1974: Life cycles of fish. Physiology and Biochemistry. — 258 pp. Keter Publishing House, Jerusalem (translated from Russian).

Veith, V. J. 1979: The chemical composition of the follicular fluid of the viviparous teleost Clinus superciliosus. — Comp. Biochem. Physiol. 63A: 37—40.

Wegmann, I. & Götting, K.-J. 1971: Untersuchungen zur Dotterbildung in den Oocyten von Xiphophorus helleri (Heckel, 1848) (Teleostei, Poeciliidae). — Zeitschr. Zellforsch. Mikrosk. Anat. 119: 405—433.

Received 13. III. 80
Printed 31. XII. 80