Dana, vol. 9, pp. 1-14, 1991

Studies on lipid synthesis by incorporation of ^{14}C-acetate during experimental maturation of silver eels, *Anguilla anguilla*

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Abstract

Migrating silver eels were matured with HCG and salmon pituitary extracts. At successive stages during their sexual development ^{14}C-acetate was injected. Synthesis of lipids was studied in the gonads, livers and gills.

Sexually immature and spent males show evident similarities in their gonad metabolism. Gonad lipid metabolism is apparently independent of liver metabolism in the males, in contrast to the females, where oocyte development also seems to involve phospholipid and sterol synthesis in the liver. The fatty acids of the neutral lipid fraction of egg yolk are synthesized predominantly *in situ* in the female gonads. This also applies to a further esterification into triglycerides and sterol esters. In contrast to what is known from other fishes, we have not been able to show the synthesis of wax alcohols and esters in the ovaries. Male gills synthesize predominantly wax esters, while the female gills also show a synthesis of free sterols which seems related to their maturation.

Keywords: Anguilla anguilla, sexual maturation, lipid synthesis, gonads, liver, gills.

Introduction

European silver eels are well fit for long distance migration. Their lipid reserves make up 25-35% of their total body weight. Alterations in their intestinal system indicate that the eels starve during migration. When kept in tanks, silver eels are known to endure long-termed starvation. The record is held by a female eel which survived for about 1600 days without food at a temperature of 14 °C (Boëtius & Boëtius, 1985). Silver eels are sexually immature (spermatocytes and oocytes). The size of the the gonads increases only slightly during metamorphosis from the yellow to the silver eel stage.

Boëtius & Boëtius (1967) have studied the experimentally induced, gonadal development in male silver eels. A complete cycle was characterized by the stages *1-7*. We have adopted this registration for our present purpose. From the paper cited above we present as Fig. 1 the rough morphological changes through the stages *1*, *3*, *5*, and *7*, which are the ones used in the present study. In female eels, the degree

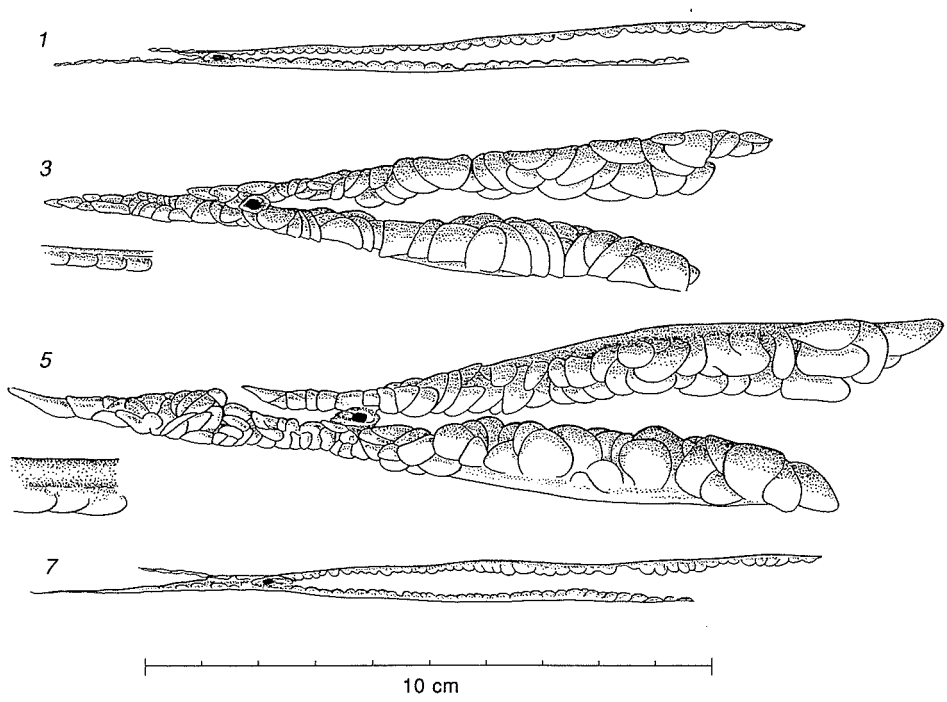


Fig. 1. Developmental stages *1*, *3*, *5*, and *7* of silver eel testes. Ventral view, cranial ends pointing to the right.The ventral lobes partly cover the dorsal *vas deferens*, which is shown in lateral views in stages *3* and *5*. The black spots indicate the area of the integument where the gut and the uro-genital vesica open. Stage *1* is the immature, so called Syrski organ. The first live spermatozoa occur in stage *3*. Stage *5* is fully matured and spawning takes place shortly after. Stage *7* is the spent gonad. (From Boëtius & Boëtius, 1967).

of maturity is indicated by their gonadosomatic index, GSI (ovary weight in per cent of the total body weight). A rough outline of the maturation processes in the two sexes is given in Table 1.

The induction of sexual maturation involves major metabolic changes. As much as 18% of the total energy reserves found before spawning migration in sexually immature female silver eels are transferred to the ovaries of the sexually mature ani-

Table 1. Overview of maturation stages.

Male eels		Female eels		Both sexes
Stage	Condition	GSI	Condition	Process
1	immature	*2*	immature	maturation
3	first spermatozoa	*10*		continued maturation and water uptake
5	mature	*45*	mature	spawning
7	spent	?	(no material)	

mals (Boëtius & Boëtius, 1980). The eel retains its ability to synthesize fatty acids even during a period of prolonged starvation (Abraham *et al.*, 1984). Endogenous lipids are broken down and re-synthesized to fit new patterns as a result of sexual maturation.

Apart from their role in energy storage, mainly as triglycerides, lipids are also vital structural constituents of cellular membranes. Especially phospholipids, but also sterols, act as 'modifiers' of the biologically active and specific membrane proteins. Within the individual lipid classes, a change in for example the type of esterified fatty acid will result in a different biophysical characteristic of the membrane lipid moiety, and thereby also in a change in the configuration of the proteins (enzymes) that are naturally enclosed in that moiety (Weissmann & Claiborne, 1975). While the chemical structure of proteins is difficult to alter, the structure of membrane lipids can be altered more readily and induce an indirect protein modification.

The role of lipids as energy reserves is especially seen in the sexual maturation of the females. Various neutral lipids, stored in the egg yolk, serve as nutrients for the developing embryo.

In the present investigation we study lipid synthesis in eel liver, gonads and gills. We have assayed the incorporation *in vivo* of (1-^{14}C)acetate into various lipid classes and their individual fatty acids. In this way we have determined the rate and pattern of lipid biosynthesis during 24 hrs intervals at different stages of the maturation process.

Material and methods

The eels used in the present study were caught in Danish waters on their way to the ocean. Catches took place in the month of October in 1977, 1982, 1985 and 1987, the years referring to the experimental series I, II, III and IV, respectively.

Shortly after capture, the eels were acclimatized in concrete tanks with circulating sea water, 31-35‰ S, 16±2°C. During hormonal treatment the male eels remained in this environment; the females, however, were matured at 23±1°C. No food was offered. The sexes were distinguished according to total length. Silver eels less than 42 cm long are males; eels surpassing 50 cm are females.

The gonadotrophic substances used for experimental maturation were human chorionic gonadotrophin (HCG) for the males and acetone dried salmon pituitaries (SP) and HCG for the females. Appropriate combinations of the two substances were dissolved (suspended) in a 0.9% NaCl solution and centrifuged at 4000 RPM for 20 minutes. The water phase was used for intramuscular injections.

The hormone treatment and the corresponding gonadal response are given in detail in Table 2. There is a wide variation in the administration of hormones in this table, because the present investigation also was designed for a study (unpublished) of the effect of varied dosages upon the maturation process.

Eels were finally injected with 250 µCi ^{14}C-acetate dissolved in 0.5 ml 0.9% NaCl. They stayed for 24 hrs in sea water in a separate glass tank before they were killed. Pieces of gonad, liver and gill tissues were removed and pooled for each organ in precisely weighed portions from either one, three, or four similarly treated

Table 2. Outline of hormonal treatments

Series	Sex	No. of eels	Init. body weight, g	Day of first injection	Day of sacri-ficing	Days	Dose/eel	Total no. of doses	Gonads Stage[1]/ GSI[2]
I	♂	2	c.120		78.04.14	0	control	0	*1*
	♂	2	»	78.02.16	78.04.14	57	250 IU HCG[3]/week	8	*3*
II	♂	8	c.110		83.02.01	0	control	0	*1*
	♂	6	»	83.02.10	83.03.14	32	500 IU HCG/week	3	*3*
	♂	6	»	83.02.10	83.04.28	77	500 IU HCG/week	3	*5*
	♂	6	»	83.02.10	83.11.14	277	500 IU HCG/week	3	*7*
III	♀	1	640		85.11.04	0	control	0	1.9
	♀	1	650		85.11.04	0	control	0	2.5
	♀	1	550	85.11.11	85.11.18	7	15 mg SP[4] +500 IU HCG/ twice a week	3	2.6
	♀	1	400	85.11.11	85.11.18	7	»	3	2.6
	♀	1	710	85.11.04	85.11.25	21	»	7	4.8
	♀	1	520	85.11.04	85.11.25	21	»	7	4.8
	♀	1	520	85.11.11	85.12.19	38	»	12	9.2
IV	♀	1	420		87.11.09	0	control	0	1.9
	♀	1	480		87.11.09	0	control	0	2.5
	♀	1	460	87.11.04	87.12.16	42	4.3 mg SP +143 IU HCG/day	15•13[5]	12.7
	♀	1	460	87.11.04	87.12.16	42	a: 0.43 mg Sp +14.3 IU HCG/day	15	22.8
							b: 0.86 mg SP +28.6 IU HCG/day	15	
							c: 4.3 mg SP +143 IU HCG/day	12	
	♀	1	390	87.11.04	88.01.04	61	4.3 mg SP +143 IU HCG/day	15•15•1[5]	34.7
	♀	1	380	87.11.04	88.01.11	68	a: 0.43 mg SP +14.3 IU HCG/day	15	53.5
							b: 0.86 mg SP +28.6 IU HCG/day	15	
							c: 4.3 mg SP +143 IU HCG/day	38	

1. Male maturation stages according to Boëtius & Boëtius, 1967.
2. GSI = gonadosomatic index = weight of ovaries in per cent of total body weight.
3. HCG = human chorionic gonadotropin. Physex, LEO, Copenhagen.
4. SP = acetone dried Pacific Salmon pituitaries. ADP, Syndel, Vancouver.
5. The symbol • indicates an interval of 15 days without treatment.

animals. Tissue lipids and fatty acids were isolated and assayed as previously described (Hansen & Abraham, 1979; Hansen, 1987). This involved thin-layer chromatography of both phospholipids and neutral lipids, and subsequent paper chromatography of the individual ^{14}C-labelled fatty acids in phosphatidylcholine (PC), phosphatidylethanolamine (PE) and triglycerides (TG), after extraction and sapo-

nification of the corresponding spots on the thin-layer foils. The chromatograms were assayed for radioactivity with an automatic scanning device (Bøtter-Jensen & Hansen, 1977). Total incorporations were furthermore assayed by liquid scintillation counting after direct saponification of tissue samples. The dominating incorporation of ^{14}C-activity into wax alcohols and esters in gill tissues shown in Fig. 2 was checked by co-chromatography with ^{14}C-stearyl alcohol and ^{14}C-stearyl alcohol esters.

Results

Total incorporation

Table 3 shows the total incorporation of ^{14}C-acetate into tissue lipids. We distinguish between an acidic extract (^{14}C-fatty acids + unsaponifiable lipids) and a water soluble fraction (mainly ^{14}C-glycerol) after saponification. To express the liver values as much as possible independent of changes in total lipid metabolism, results in liver tissue are also shown divided by the corresponding incorporation into the gills; i.e. the gills are used as a metabolic reference.

Male lipids present the most clear-cut picture. Control eels (stage *1*) and the eels in stages *5* and *7* are alike regarding the synthesis of both liver and gonad lipids. Stage *3* differs from the others and shows an enhanced lipid synthesis.

Table 3. ^{14}C-lipids, total incorporation nCi/100 mg tissue/24 h. Mean or single values, cf. Table 2. Male and female eels.

				Acidic extract (Ae)			Water phase (W)		
Series	Sex	Stage	GSI	Liver (÷gills Ae)	Gonads	Gills	Liver (÷gills W)	Gonads	Gills
II	♂	*1*	–	6.2 (0.27)	2.8	22.8	5.6 (7.0)	0.2	0.8
II	♂	*3*	–	17.3 (0.59)	13.8	29.4	5.3 (5.9)	1.3	0.9
II	♂	*5*	–	13.0 (0.20)	1.4	64.4	4.2 (2.5)	0.1	1.7
II	♂	*7*	–	14.1 (0.19)	2.9	74.5	15.5 (6.0)	0.5	2.6
Mean			–	(0.22)*			(5.4)		
III	♀	Control	2.2	8.0 (0.22)	0.5	36.4	0.7 (1.0)	0.0	0.7
IV	♀		2.2	20.3 (1.10)	0.8	18.9	5.1 (8.5)	0.1	0.6
III	♀	Treated	2.6	36.2 (1.40)	12.9	25.8	4.4 (6.3)	0.4	0.7
III	♀		4.8	29.6 (0.96)	27.6	30.8	3.9 (3.9)	0.9	1.0
III	♀		9.2	26.2 (1.77)	24.9	14.8	4.2 (5.3)	0.8	0.8
IV	♀		12.7	28.7 (1.29)	14.4	22.2	5.8 (8.3)	0.7	0.7
IV	♀		22.8	19.3 (1.14)	13.7	16.9	3.0 (7.5)	0.5	0.4
IV	♀		34.7	21.7 (1.55)	4.0	14.0	2.4 (4.8)	0.1	0.5
IV	♀		53.5	10.9 (1.09)	3.0	10.0	1.5 (15.0)	0.1	0.1
Mean of treated				24.7 (1.31)	14.4		3.6 (7.3)	0.5	
SE				3.1 (0.11)	3.5		0.5 (1.4)	0.12	

* apart from stage *3*.

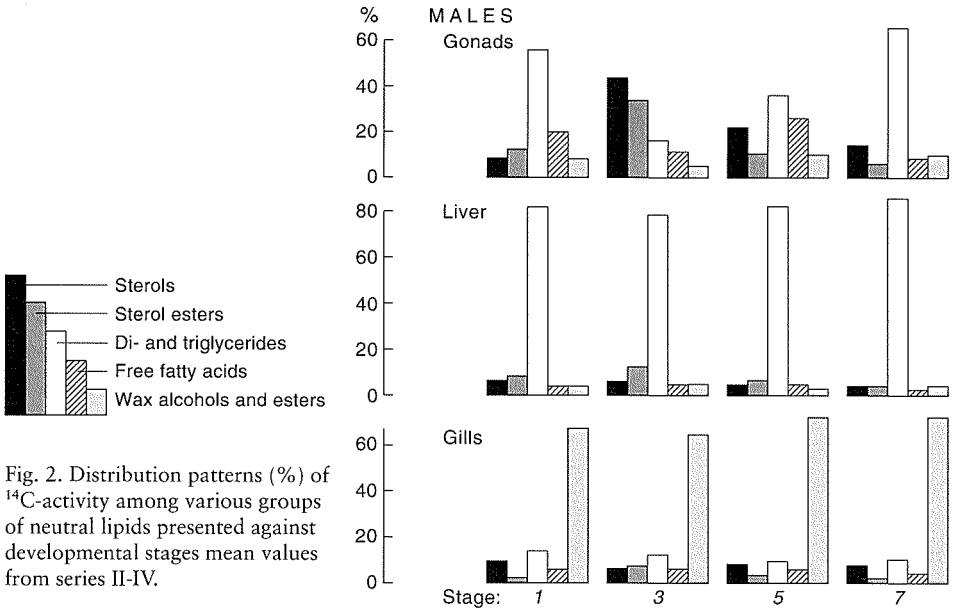


Fig. 2. Distribution patterns (%) of ^{14}C-activity among various groups of neutral lipids presented against developmental stages mean values from series II-IV.

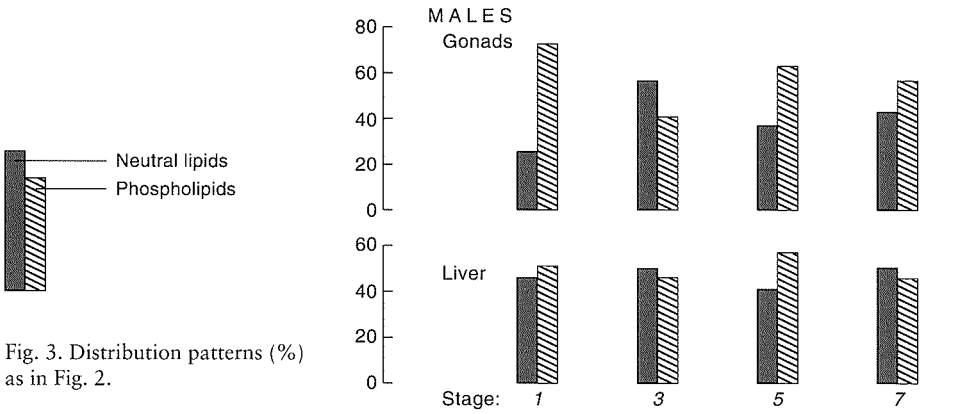
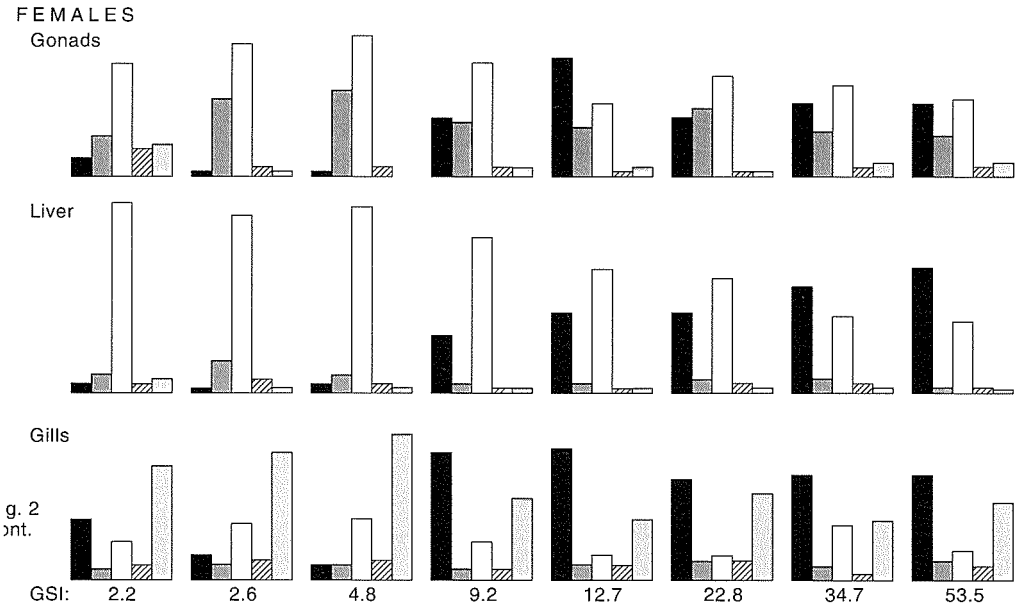
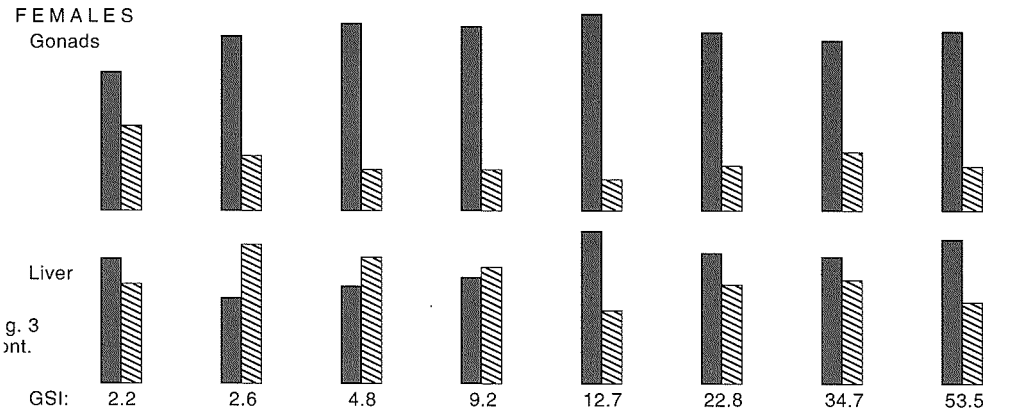


Fig. 3. Distribution patterns (%) as in Fig. 2.

The synthesis of fatty acids + unsaponifiable lipids (Ae) in the two female control animals in series III resembles the corresponding synthesis in the male animals in series II. However, in series IV the female controls show an enhanced incorporation of ^{14}C-acetate into liver Ae relative to gonad Ae. Such a relatively enhanced synthesis in the liver is also seen in all the treated female eels. It means that the average liver/gill Ae synthesis of the treated female eels becomes 1.31±0.11 which is more than 0.59 found in the male eels in stage 3. The corresponding average gonad synthesis is 14.4±3.5 and thus similar to the male value 13.8 in stage 3. There is furthermore a somewhat reduced gonad Ae synthesis in females at GSI above 22.8 and in males after stage 3, equivalent to a marked gonadal water uptake at these stages.





Lipid classes

Fig. 2 shows the relative incorporation (%) of ^{14}C-acetate into various neutral lipids in the gonads, the liver and the gills of both sexes. The similarity between the treated females and the males in stage 3 shown in Table 3 is here seen to apply also to the gonad ^{14}C-neutral lipid patterns. Both the males in stage 3 and the treated females at GSI above 4.8 show a relatively enhanced incorporation of ^{14}C-acetate into as well sterols as sterol esters in the gonads, with an apparent maximum at GSI 12.7. At the beginning of the hormone treatment (GSI 2.6-4.8), the females started with a relatively enhanced incorporation into just sterol esters in the gonads. At stage 7

Table 4. ^{14}C-fatty acids, % of total incorporation. Mean values (±SE). Male eels.

			Liver					Gonads				
Series	Lipid*	Stage	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{16:1}$+ $C_{18:1}$	$>C_{18}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{16:1}$+ $C_{18:1}$	$>C_{18}$
I	PC	*1*	2	40	32	5	21	–	–	–	–	–
II		–	3	47	33	4	13	8	37	15	13±4	27
I		*3*	1	61	23	4	11	3	43	15	18±3	21
II		–	1	60	27	1	11	3	28	22	31±2	16
II		*5*	3	63	26	1	7	11	57	12	6	14
II		*7*	2	36	32	5	25	4	33	7	18	38
II	PE	*1*	1	15	69	0	15	2	13	47	9±1	29
II		*3*	1	23	54	0	22	0	15	31	23±5	31
II		*5*	0	28	57	0	15	3	20	42	8	27
II		*7*	0	12	70	0	18	2	19	39	11	29
I	TG	*1*	13	38	13	10	26	–	–	–	–	–
II		–	10	35	16	11	28	8	24	11	14±6	43
I		*3*	17	52	8	7	16	11	25	11	16±1	37
II		–	14	54	7	9	16	6	20	13	28±1	33
II		*5*	14	52	13	13	8	18	40	20	4	18
II		*7*	10	36	19	9	26	6	30	23	9	32
II	PC+PE+TG	*1*	5	32	39	5	19	6	25	24	12	33
II		*3*	5	46	29	3	17	3	21	22	27	27
II		*5*	6	48	32	4	10	11	39	24	6	20
II		*7*	4	28	40	5	23	4	29	23	13	33

* PC = phosphatidylcholine; PE = phosphatidylethanolamine; TG = triglycerides.

the male gonad pattern is again the same as in the controls (stage *1*); we have no equivalent females.

Male and female eels differ significantly regarding the liver ^{14}C-neutral lipid patterns. In male liver tissue ^{14}C-acetate is incorporated nearly altogether into glycerides. This applies also to the female livers from GSI 2.2 to 4.8. From GSI 9.2 and upwards there is a steadily increasing relative incorporation of ^{14}C-acetate into the ovary liver sterols, equivalent to the incorporation of ^{14}C-activity into the ovary sterols and sterol esters.

Fig. 2 shows furthermore the corresponding results in gill tissue. In male gill tissue, a dominating incorporation of ^{14}C-acetate into wax alcohols and esters is seen to be independent of the induced sexual maturation. The equivalent incorporation patterns in female gill tissue start showing substantial ^{14}C-activity in free sterols at GSI above 4.8, just as in the liver and gonads.

The relative distribution (%) between incorporation of ^{14}C-acetate into total neutral lipids and total phospholipids is shown in Fig. 3 in the gonads and in the liver, equivalent to Fig. 2. While male and female livers are seen to be alike, the female gonads show a relatively low synthesis of phospholipids, not to be seen in the males.

Table 5. ^{14}C-fatty acids, % of total incorporation. Mean or single values, cf. Table 2. Female eels.

				Liver					Gonads				
Series	Lipid*	Stage	GSI	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{16:1}$+ $C_{18:1}$	$>C_{18}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{16:1}$+ $C_{18:1}$	$>C_{18}$
III	PC	Control	2.2	5	47	40	2	6	–	–	–	–	–
IV			2.2	4	42	40	6	8	7	60	18	8	7
III		Treated	2.6	1	24	58	4	13	6	47	29	0	18
III			4.8	1	39	45	3	12	2	53	23	3	19
III			9.2	1	22	34	4	39	0	38	12	7	43
IV			12.7	1	25	47	7	20	0	44	21	10	25
IV			22.8	2	35	35	9	19	1	65	17	2	15
IV			34.7	4	44	27	10	15	4	59	13	8	16
IV			53.5	2	35	35	9	19	3	64	19	5	9
III	PE	Control	2.2	1	17	72	0	10	–	–	–	–	–
IV			2.2	0	12	71	5	12	–	–	–	–	–
III		Treated	2.6	0	6	72	2	20	1	21	47	4	27
III			4.8	0	17	64	0	19	2	16	37	0	45
III			9.2	0	10	51	0	39	0	15	41	0	44
IV			12.7	0	16	61	6	17	1	28	42	4	25
IV			22.8	1	19	58	4	18	2	38	36	2	22
IV			34.7	1	20	50	8	21	0	38	31	16	15
IV			53.5	0	12	53	8	27	–	–	–	–	–
III	TG	Control	2.2	12	37	23	8	20	3	49	20	5	23
IV			2.2	9	32	8	36	15	12	44	10	24	10
III		Treated	2.6	7	30	25	18	20	11	25	19	4	41
III			4.8	9	51	9	15	16	13	22	23	9	33
III			9.2	5	43	12	21	19	5	26	19	15	35
IV			12.7	5	38	11	18	28	9	23	29	4	35
IV			22.8	10	50	10	12	18	9	33	29	4	25
IV			34.7	11	54	5	13	17	8	34	19	9	30
IV			53.5	9	52	7	11	21	1	18	40	3	38

* PC = phosphatidylcholine; PE = phosphatidylethanolamine; TG = triglycerides.

Individual fatty acids

Tables 4 and 5 present the individual ^{14}C-labelled fatty acids in the lipids given in Table 3 together with those from the male eels in series I. It is again the male lipids (Table 4) that show the most clear-cut picture. The distribution patterns of liver ^{14}C-fatty acids in all three lipid classes PC, PE, and TG are the same in the male control eels (stage *1*) and in the eels that have reached stage *7*; stages *3* and *5* show a common relatively increased synthesis of palmitic acid ($C_{16:0}$) and a relatively decreased synthesis of stearic acid ($C_{18:0}$) in liver tissue. In gonad tissue the similarity between males in stage *1* and *7* appears clearly from the mean values of PC+PE+TG (Table 4). Stages *3* and *5* are not the same in the male gonads. Furthermore, within stage *3* there is a difference between the series I and II.

While the males (Table 4) show no evidence of any direct coupling between liver and gonad ^{14}C-fatty acid patterns, such a tendency is present in the females (Table 5). This is especially seen in the TG fraction of the female control animals. It is partly seen in the PC fraction of the treated animals, where both liver and gonad fatty acids show a relatively reduced incorporation of ^{14}C-acetate into palmitic acid ($C_{16:0}$) together with a relatively enhanced incorporation into stearic acid ($C_{18:0}$) and long chain fatty acids > C_{18}; an apparent maximum is seen at GSI 9-13.

The above suggested coupling between the fatty acids of liver and gonad TG in the female controls is shown to be abolished by the hormone treatment (Table 5).

Discussion

Substantial amounts of lipids accumulate in the gonads during experimental maturation in spite of the eels' starving condition. Data from Boëtius & Boëtius (1967, 1980, and some unpublished material) has enabled us to estimate an average accumulation of total lipids during the maturation from untreated controls to maximum development. The estimate is valid for temperatures about 23 °C, much the same as those used during the ^{14}C-incorporations in the present study. Expressing the accumulation as g total lipid per kg initial body weight per 24 hrs we arrive at the figures 0.3 and 0.8 for males and females, respectively.

We are able to distinguish in the present investigation between lipid synthesis at fixed points of the maturation process and previous equivalent lipid metabolism in the eel in question. The measured ^{14}C-labelled lipids represent only the period from the injection of ^{14}C-acetate to the time of sacrifice. This is in contrast to most other investigations where an assay of total lipid content and composition covers *all* lipid metabolism up to the time of sacrifice.

During sexual maturation, males and females are in the same nutritional (non-trophic) state. The relatively enhance synthesis of fatty acids and unsaponifiable lipids in the stage *3* males and in the treated females (Table 3) thus reflects a specific increase of enzyme activity, not correlated with any difference in nutritional state. A relatively enhanced synthesis of ^{14}C-labelled palmitic acid in male livers in stages *3* and *5* (Table 4) points to an increased activity of the basic enzyme fatty acid synthetase in those tissues; the liver makes more fatty acids in general, which the gonads then transform to their need, depending on whether it is stage *3* or stage *5*. However, in stage *3* (Table 4) the gonads in series I still show the same pattern of ^{14}C-fatty acids as in the controls in stage *1*; fatty acid synthesis in the gonads is thus not fully correlated with the morphological development.

Before further commenting the two sexes separately, we should point out that in the males a complete sexual cycle was induced by only 3 HCG-injections, while in the females the maturation had to be sustained by a continuous supply of gonadotrophic substances. The females died after having obtained maximum GSI; only occasionally did they spawn. The spent males survived.

Males

In the male gonads it is evident from Fig. 1 that the developmental stages *1* and *7* are identical as to external morphology. Boëtius & Boëtius (1967) have furthermore shown that this applies also to the histological structure. Actually, the original Syrski-organ (stage *1*) is reformed at the end of the sexual cycle.

We have several reasons to believe that the European eel after arrival at its breeding area (the Sargasso Sea) will spawn only once and die soon after. However, Boëtius & Boëtius (1982) have shown that stage *7* is potentially equal to stage *1*: a full sexual cycle was experimentally induced using eels in stage *7* as a starting material. Later, Dollerup & Graver (1985) were able to provoke no less than three complete sexual cycles in individual male eels, which were fed between the cycles. Untreated silver eels normally resume feeding after having been kept in tanks for about half a year.

Thus it is very satisfying to be able to state in the present work that the stages *1* and *7* show evident similarities (identity ?) as to their lipid metabolism. Both total incorporations (Table 3) and the individual ^{14}C-fatty acid patterns (Table 4) in liver and gonads are alike in the two stages. The same applies to the liver and gonad ^{14}C-neutral lipid patterns presented in Fig. 2.

The synthesis of individual ^{14}C-neutral lipids in the liver and the gills (Fig. 2) is apparently unaffected by the induced sexual maturation in the male eels. There is no indication of any coupling between neutral lipid metabolism in the liver and the enhanced synthesis of sterols and sterol esters in the gonads in stages *3* and *5*. Neither is there any change in gill lipid metabolism during the sexual maturation of male eels. The dominant lipid synthesis in the gills is a formation of wax alcohols and esters. This seems to reflect some specific gill function, since further studies *in vitro* and a lack of ^{14}C-labelled wax alcohols and esters in the blood (Hansen, unpublished results) indicate a synthesis *in situ*.

A clear rise in gonadal synthesis of sterols and their esters takes place from stage *1* to stage *3* (Fig. 2). This no doubt reflects the production of testosterone and the formation of cell membranes in the maturing gonad. In stage *7*, the spent eel, where the interstitial cells have degenerated and cell formation has ceased, there is no need for any further sterol synthesis.

Females

In the female gonads the distribution patterns of the neutral lipids (Fig. 2) resemble those of the males up to stage *5*. Sterols and sterol esters are synthesized at rather high rates, while the formation of wax esters is relatively poor.

Eggs of *Anguilla* belong to the 'pelagic' type with a perivitelline space and an oil globule clearly separated from the remaining yolk. The globule is estimated to occupy about 12% of the yolk volume. According to Mommsen & Walsh (1988) all fish eggs harbouring oil globules have been shown to contain substantial amounts of wax and sterol esters. Eldridge *et al.* (1983) made separate analyses of oil globules and yolk in *Morone saxatilis*. The oil globules consisted entirely of lipid material; 90.4% of this was represented by sterol/wax esters. The wax esters were

considered important in the control of buoyancy. Anderson *et al.* (1990) conclude in the case of *Macquaria ambigua* that the increased level of wax esters in the pelagic eggs must be considered an adaptation to provide buoyancy to the eggs, rather than an additional energy supply for the larvae.

In the eel, the oil globule is the very last energy reserve to be ingested. Several days after hatching a highly refractive globule can still be observed in pre-larvae (about 6 mm long) caught in the sea (Schmidt, 1925). Experimentally reared pre-larvae of the Japanese eel show the same feature (Yamamoto *et al.* 1975). These observations indicate that the oil globule might also serve functions other than nutritive (buoyancy control?). We emphasize, however, that in the present investigation we have not been able to show any synthesis of ^{14}C-wax esters in the eel ovary.

It is a remarkable feature (Fig. 2) that the liver of female eels, quite opposite the males, synthesizes sterols in substantial amounts. The process starts at a GSI-value between 5 and 9 and continues at an increasing rate up to the strip-ripe condition. It seems reasonable to link up this apparently sex-specific synthesis of sterols in the female liver with the process of vitellogenesis (yolk formation). Idler & Campbell (1980) have induced a synthesis of the high-molecular lipoprotein vitellogenin in juvenile rainbow trout by treatment with estradiol. According to Norberg & Haux (1985) the lipid component of vitellogenin in rainbow and sea trout is dominated by phospholipids. Thus the sterol production demonstrated in the present study can hardly be related to the formation of vitellogenin. We would suggest that a female-specific liver synthesis of sterols plays part in vitellogenesis predominantly by supplying the oocytes with material for the formation of oil globules. The shown female-specific enhanced overall synthesis of pentane-extractable (Ae) liver lipids (Table 3) agrees with this concept.

A further female-specific pattern in gonad lipid synthesis is the high neutral lipid/phospholipid ratio seen in Fig. 3. This could indicate that the principal site of phospholipid synthesis, essential to yolk formation, should also be sought elsewhere in the eel's body. The similar ^{14}C-fatty acid patterns in gonad and liver PC (Table 5) at relatively low GSI would point to the liver as the main site of yolk phospholipid biosynthesis. Norberg & Haux (1985) working on *Salmo* and Lal & Singh (1987) and Singh & Singh (1990), working on female specimens of the catfishes *Clarias* and *Heteropneustes*, respectively, have previously suggested an enhanced synthesis of phospholipids (vitellogenin) in the liver and a subsequent transport to the ovary via plasma.

While the fatty acid patterns of the phospholipid PC in Table 5 show similarities between gonad and liver lipid metabolisms, this is not seen in the corresponding patterns in the neutral lipid TG. Our results point to an independent synthesis of TG fatty acids in the gonads of the treated eels (Table 5), similar to what we could show for as well TG as PC and PE in the males (Table 4). In contrast to phospholipid and sterol synthesis, the fatty acids of the neutral lipid fraction (oil globules) are apparently synthesized predominantly *in situ* in the female gonads. We show this only in the TG fraction but it applies probably also to the fatty acids of the sterol esters; c.f. previous results on the synthesis *in situ* of gill fatty acids (Hansen & Abraham, 1989). The formation of ^{14}C-labelled sterol esters at GSI 2.6-4.8

(Fig. 2) indicates furthermore an initial esterification *in situ* of free sterols in the gonads, following hormone treatment. Previous results of Lal & Singh (1987) and Singh & Singh (1990) have likewise suggested that sterol ester synthesis takes place locally in the ovary.

The parasitic, anadromous lampreys face a problem similar to that of the catadromous eels: they too have to build up gonads from their own body reserves during long-term starvation. Bird & Potter (1983) have studied fatty acid compositions in *Geotria australis*. The weight of ovarian lipids increases by a factor of more than 60 in this species during a 16 month upstream non-trophic spawning migration. During the same period, the relative content of the poly-unsaturated fatty acids $C_{22:5}$ and $C_{22:6}$ in the ovaries is shown to be enhanced. This agrees with our results in Table 5 regarding an enhanced relative incorporation of ^{14}C-acetate into the fatty acids $>C_{18}$ in PC (PE?) and TG of female eel gonads after hormonal treatment; we were not able to show such a relatively increased incorporation in the males (Table 4). It suggests that the long chain poly-unsaturated fatty acids play a special nutritional role in fish oocytes; in agreement also with the results of Anderson *et al.* (1990), based on the composition of egg lipids of six Australian species of teleosts.

Turning finally to the eel gills, they also seem to demonstrate a female-specific marked synthesis of sterols just as that shown in the liver at GSI above 4.8 (Fig. 2). The sterol activity found in the female gills could perhaps be the result of an 'elsewhere'-synthesis, e.g. in the liver, detected in the gills on its way to the ovaries. However, the results in Table 3 on total ^{14}C-incorporation into female gill lipids do not indicate any enhanced activity in the treated eels, relative to the controls, and show furthermore lower values than in the males. This would suggest an independent lipid synthesis in the gills, and not only a reflection of the corresponding synthesis elsewhere. An actual formation of sterols in the female gills could well be an artefact of the repeated hormone treatment, if it is not just the natural result of a metabolic 'total mobilization' prior to spawning.

Acknowledgements

We are grateful to Vibeke Jørgensen and Alice Kjølhede for their analytical and technical assistance. Our thanks are also due to LEO Pharmaceutical Products, Ballerup, Denmark, for supplying us with HCG ('Physex', LEO).

References

Abraham, S., H.J.M. Hansen & F.N. Hansen, 1984: The effect of prolonged fasting on total lipid synthesis and enzyme activities in the liver of the European eel (*Anguilla anguilla*). – Comp. Biochem. Physiol. 79B: 285-289.

Anderson, A.J., A.H. Arthington & S. Anderson, 1990: Lipid classes and fatty acid composition of the eggs of some Australian fish. – Comp. Biochem. Physiol. 96B(2): 267-270.

Bird, D.J. & I.C. Potter, 1983: Changes in the fatty acid composition of triacylglycerols and phospholipids during the life cycle of the lamprey *Geotria australis* Gray. – Comp. Biochem. Physiol. 75B(1): 31-41.

Boëtius, I. & J. Boëtius, 1967: Studies in the European eel, *Anguilla anguilla* (L.). Experimental induction of the male sexual cycle, its relation to temperature and other factors. – Meddr Danm. Fisk.- og Havunders. N.S. *4*: 339-405.

Boëtius, I. & J. Boëtius, 1980: Experimental maturation of female silver eels, *Anguilla anguilla*. Estimates of fecundity and energy reserves for migration and spawning. – Dana *1*: 1-28.

Boëtius, I. & J. Boëtius, 1982: Experimental maturation of European silver eels. – Proc. int. Symp. reproductive Physiol. Fish, Wageningen 1982: 174-176.

Boëtius, I. & J. Boëtius, 1985: Lipid and protein content in *Anguilla anguilla* during growth and starvation. – Dana *4*: 1-17.

Bøtter-Jensen, L. & H.J.M. Hansen, 1977: A multicounter system for scanning ultra-low-level radiochromatograms. – Nuclear Instrum. Meth. *144*: 529-532. North Holland Publishing Co.

Dollerup, J. & C.M. Graver, 1985: Repeated induction of testicular maturation and spermiation, alternating with periods of feeding and growth in silver eels, *Anguilla anguilla* (L). – Dana *4*: 19-39.

Eldridge, M.B., J.D. Joseph, K.M. Taberski & G.T. Seaborn, 1983: Lipid and fatty acid composition of the endogenous energy sources of Striped Bass (*Morone saxatilis*) eggs. – Lipids *18*(8): 510-513.

Hansen, H.J.M., 1987: Comparative studies on lipid metabolism in various salt transporting organs of the European eel (*Anguilla anguilla*). Mono-unsaturated phosphatidylethanolamine as a key substance. – Comp. Biochem. Physiol. *88*B: 323-332.

Hansen, H.J.M. & S. Abraham, 1979: The influence of environmental salinity, temperature, ionizing irradiation and yellow or silver stage on lipid metabolism in the gills of the European eel (*Anguilla anguilla*). – Comp. Biochem. Physiol. *63*B: 483-490.

Hansen, H.J.M. & S. Abraham, 1989: Compartmentation of gluconeogenesis in the fasted eel (*Anguilla anguilla*). – Comp. Biochem. Physiol. 92B(4): 697-703.

Idler, D.R. & C.M. Campbell, 1980: Gonadotropin stimulation of estrogen and yolk percursor synthesis in juvenile rainbow trout. – Gen. comp. Endocrinol. *41*: 384-391.

Lal, B. & T.P. Singh, 1987: Changes in tissue lipid levels in the freshwater catfish *Clarias batrachus* associated with the reproductive cycle. – Fish Physiol. Biochem. *3*(4): 191-201.

Mommsen, T.P. & P.J. Walsh, 1988: Vitellogenesis and oocyte assembly. – *In* W.S. Hoar & D.J. Randall (eds): Fish Physiology 11A(*5*), pp. 347-406. Academic Press Inc.

Norberg, B. & C. Haux, 1985: Induction, isolation and a characterization of the lipid content of plasma vitellogenin from two *Salmo* species: Rainbow trout (*Salmo gairdneri*) and sea trout (*Salmo trutta*). – Comp. Biochem. Physiol. *81*B: 869-876.

Schmidt, J., 1925: The breeding places of the eel. – Rep. Smithsonian Inst. 1924: 279-316.

Singh, P.B. & T.P. Singh, 1990: Seasonal correlative changes between sex steroids and lipid levels in the freshwater female catfish, *Heteropneustes fossilis* (Bloch). – J. Fish Biol. *37*: 793-802.

Weissmann, G. & R. Claiborne, eds, 1975: Cell membranes (biochemistry, cell biology & pathology). – HP Publishing Co., Inc. New York. 283 pp.

Yamamoto, K., K. Yamauchi & T. Morioka, 1975: Pre-leptocephalic larvae of the Japanese eel. – Bull. Jap. Soc. Sci. Fish. *41*(1): 29-34.