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Deposition and mobilisation of body fat during sexual maturation in female trout (*Salmo gairdneri* Richardson)

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Abstract

The body components of female rainbow trout (*Salmo gairdneri*) reared in an experimental fish farm were studied during sexual maturation. For a period of 13 months, carcass, liver, gut and ovaries were sampled every month and their fat and non fat constituents analysed. In the trout, lipids are mainly stored in the carcass and to a less extent in the gut and ovaries. The distribution ranged from 68 to 73%, 31 to 6% and 0.1 to 20%, respectively from the beginning to the end of sexual maturation (March-November). Whatever the stage, carcass and visceral total lipids (TL) were mainly composed of neutral lipids (NL). The ovarian NL relative to TL content decreased from 90% during the first gonadal slow growth phase (SG. I from March to June) to 60% at ovulation in October or November while the proportion of phospholipids (PL) increased during the second slow growth phase (SG. II from July to August) and the rapid growth phase (RG from September until ovulation in October or November). This observation suggests that trout preferentially incorporate fatty acids into phospholipids in the oocyte during late ovarian growth.

During sexual maturation there was a large mobilisation of carcass and visceral lipid reserves, but non fat dry matter (NFDM) was only slightly changed. In our experimental fish (mean weight 1 200 g) the loss of lipids averaged 76 g (42 and 34 g, respectively from the carcass and gut) versus 33 g for NFDM (from the carcass mainly). Loss of visceral lipids began during SG. I and ceased at ovulation in November, whereas loss of carcass lipids seemed to start during the RG phase and finished 1 month after ovulation.

Keywords : Trout, lipids, reproduction.

Dépot et mobilisation des lipides corporels au cours du cycle sexuel chez la truite femelle (Salmo gairdneri *Richardson*).

Résumé

L'évolution des constituants corporels au cours du cycle sexuel est étudiée chez des truites femelles *Salmo gairdneri* élevées en pisciculture expérimentale. Durant une période de 13 mois, carcasse, foie, tube digestif et ovaire sont prélevés tous les mois et leurs constituants lipidiques et non lipidiques sont déterminés. Chez la truite, les lipides sont principalement stockés dans la carcasse, et à un moindre degré, dans le tube digestif et l'ovaire, variant respectivement de 68 à 73%, de 31 à 6% et de 0 à 20% du début à la fin du cycle sexuel (mars-novembre). Quel que soit le stade considéré, les lipides totaux (TL) musculaires et viscéraux sont constitués pour l'essentiel des lipides neutres (NL). Par contre, dans l'ovaire, la teneur en NL rapportée aux TL diminue de 90% au cours de la première phase de développement lent des gonades (SG. I allant de mars à juin) à 60% à l'ovulation en octobre ou en novembre, tandis que la part des phospholipides (PL) au cours de la 2e phase de développement lent (SG. II, de juillet à août) et de la phase de développement rapide (RG, de septembre jusqu'à l'ovulation en octobre ou novembre) augmentent. L'incorporation des acides gras, sous forme phospholipidique, dans l'ovocyte aux stades terminaux de la croissance ovarienne serait donc privilégiée chez la truite.

Au cours du cycle sexuel, les lipides de réserve de la carcasse et du tube disgestif subissent une forte mobilisation, mais la matière sèche non lipidique (NFDM), en revanche, est peu modifiée. Chez les poissons étudiés (poids moyen 1 200 g), la perte de lipides est en moyenne de 67 g (42 g et 34 g respectivement à partir de la carcasse et du tube digestif) contre 32 g pour la NFDM (à partir de la carcasse essentiellement). La perte des lipides viscéraux commence au cours de SG. I et s'achève à l'ovulation en novembre, tandis que celle des lipides musculaires ne débute apparemment qu'au cours de RG et prend fin un mois après l'ovulation.

Mots-clés : Truite, lipides, reproduction.

INTRODUCTION

In fish, the ovarian growth leads biochemically to the accumulation of proteins and lipids in the growing oocytes (Love, 1970). The material deposited in ovaries is of dual origine: exogenous (dietary) and endogenous (mobilised from carcass, liver and gut). The tissular origin of these materials varies from one species to another (Love, 1970, Shulman, 1974).

The utilisation of tissue reserves during sexual maturation has been reported for several fish species in the natural environment. In Sockeye salmon, *Oncorhynchus nerka* (Idler and Bitners, 1959), American plaice, *Hippoglossoides platessoides* (Mackinnon, 1972), perch, *Perca fluviatilis* (Craig, 1977) and European plaice, *Pleuronectes platessa* (Dawson and Grimm, 1980), protein and lipid body reserves decrease during gonadal development. Conservely, in Northern pike, *Esox lucius* (Diana and Mackay, 1979) and in Anabantid, *Trichogaster pectoralis* (Hails, 1983), the body reserves are not significantly reduced during ovarian growth, but they begin to decrease during spawning.

Few data are available on changes in body reserves during sexual maturation in farmed fish in general and salmonidea in particular. Mobilisation of carcass lipids was studied in rainbow trout, *Salmo gardneri* (Tveranger, 1985) and Atlantic salmon, *Salmo salar* (Aksnes *et al.*, 1986) in sea water farming conditions. In the present study, we investigated the nature of tissue lipids in trout raised in fresh water and the contribution of each tissue to the pool of lipids mobilised during sexual maturation. An attempt was made to estimate the proportion of mobilised lipids used by the egg.

MATERIALS AND METHODS

Experimental conditions

A total of 250 rainbow trout, *Salmo gairdneri*, were raised from the age of 2 years in a 5 m wide, 6 m long and 1 m deep experimental pond at Gournay (Oise, France). During the experiment, the temperature ranged from 7 to 15°C. Immediately after yolk sac resorption and until the end of the experiment, the animals received a commercial diet (Aqualim, Roullet, Saint-Estephe, France) containing 8% lipids of which the fatty acid composition has been reported previously (Léger *et al.*, 1981). The experiments lasted 13 months (from March to March) and the mean weight gain during this period was 785 to 1 622 g (*table* 1). During ovarian growth (March to October), 6 females were sampled each month (except in May). At ovulation (October-November), 6 ovulated females were sampled per month. The rest of the ovulated females were stripped and studied for a period of 4 months during which 6, 6, 3 and 4 fish, respectively were sacrificed in December, January, February and March. At slaughter, fish were individually weighed and the different body compartments: carcass (gutted fish), liver, gut and ovaries were analysed.

Table 1. — Seasonal variations in total fish weight.

Sampling Date	Fish number and physiological stage (**)	Weight (*) (g)
March	6 SG.I	785±162
April	6 »	899±220
June	6 »	1177±235
July	6 SG.II	1338±59
August	6 »	1238±146
September	6 RG	1523±81
non OV	6 »	1552±194
October		
OV	6 »	1471±185
November	6 »	1510±248
December	6 POP	1249±219
January	6 »	1622±125
February	3 »	1446±580
March	4 »	1543±219

(*) Mean±standard deviation.
(**) SG.I and SG.II: the first and second slow growth phases of gonads respectively, RG: Rapid growth phase, OV: Ovulation, POP: Postovulatory period.

Sample analysis and mode of data expression

Each fish was analysed separately. The carcass, liver, gut and ovaries were lyophilised and the dry matter (DM) content determined. The lyophilisates were stored at −30°C until analysis. Total lipids (TL)

were extracted according to the method of Maxwell *et al.* (1980). The fact extract was dried, weighed at constant weight and TL contents and weights were determined. The fat extract of each tissue was dissolved in chloroform (1% w/v). Lipid phosphorus was evaluated according to the method of Bartlett (1959) for determining the phospholipid content (PL). The weight of non phosphorus lipids or neutral lipids (NL) was obtained by difference between the weight of tissue TL and that of PL. The NFDM value was obtained by difference between tissue dry weight and TL weight.

All parameters are expressed as means ± standard deviations in both text and tables. In the figures, parameters are expressed as means with 95% confidence limits.

Statistical analysis

The homogeneity of variances was checked by Fisher's F-test (Snedecor and Cochran, 1957). Comparisons of means were made by Student's *t*-test.

RESULTS

Changes in body reserves during sexual maturation

Ovaries

Fresh weight as well as DM and TL weights increased significantly ($p<0.01$) during SG.I (first phase of gonadal slow growth, March-June), then again during SG.II (second phase of slow growth, September-November) and RG (rapid growth phase, September-November). During the postovulatory period (POP, December-March), the fresh weight as well as DM and TL weights decreased and thereafter increased, reaching in March a value exceeding ($p<0.05$) that observed in March the year before (*fig.* 1 A).

Whatever the phase considered, the NFDM weight widely exceeded that of TL. The NFDM/TL ratio was 2.9 in March, 1.3 in June, 1.7 in August, 2.6 in October and 2.9 in November.

Lipid weight increased from 1.08±0.44 in June to 4.25±1.88 in August, and then to 28.23±6.77 in October in non ovulated animals. TL weights of animals ovulated in October and November were 23.05±3.48 and 25.11±7.48, respectively. The weight of lipids deposited in the oocytes was 1.1 g during SG.I, 3.1 g during SG.II and 19-24 g during RG.

Whatever the growth phase considered, NL remained the major lipid fraction but its relative importance in ovarian TL varied considerably. The NL/PL ratio was very high during SG.I, decreased already markedly during SG.II and even more during RG (*fig.* 1 B).

Carcass

Fresh weight as well as dry matter (DM), non fat dry matter (NFDM) and total lipid (TL) weights

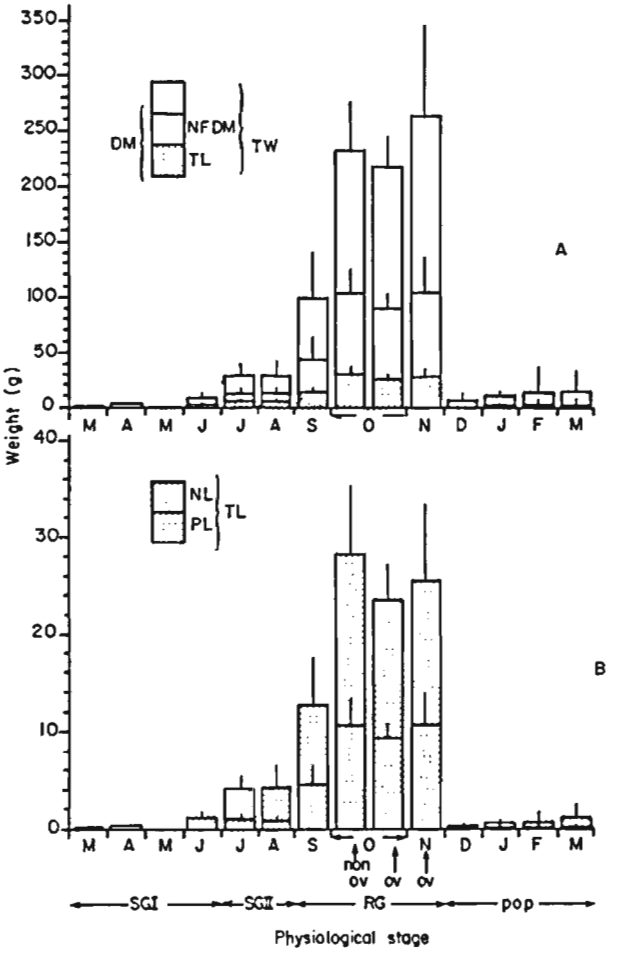
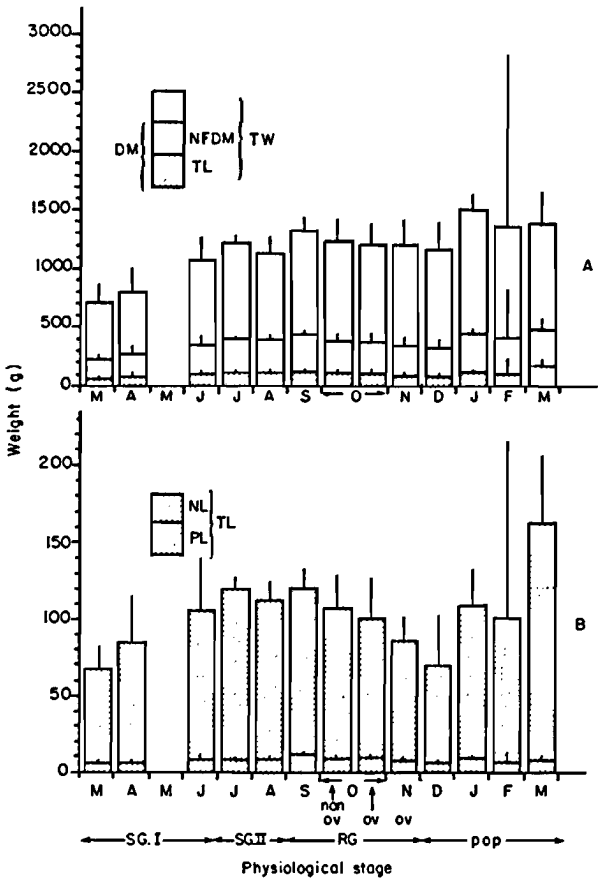


Figure 1. — Changes of lipidic and non lipidic weights of ovaries during sexual maturation of rainbow trout (*Salmo gairdneri*). A. Total weight (TW), dry matter (DM) non fat dry matter (NFDM) and total lipids (TL). B. Neutral lipids (NL) and phospholipids (PL).

increased markedly between March and June and slightly between July and August (*fig.* 2 A, *table* 2). From September to December, carcass fresh weight increased only slightly, while weights of lipid and non lipid components decreased. Compared with the values recorded in August, the NFDM weight decreased by 2.4 and 2.5% in October in non ovulated and ovulated animals, respectively, by 9.2% in November and 10.8% in December, *i.e.* 6.8, 7, 25.5 and 29.9 g, respectively. During the same period, the TL weight decreased by 5.1 and 10.7% in October, by 23% in November ($p<0.01$) and by 37.5% in December ($p<0.01$), *i.e.* 5.7, 12, 25.9 and 42.2 g, respectively. This decrease was mainly due to that of neutral lipid (NL), the phospholipid (PL) weight varying slightly during this period, 8.59±1.18 in August, 6.71±1.25 in December (*fig.* 2 B).



CARCASS

Figure 2. — Changes of lipidic and non lipidic weights of carcass during sexual maturation of rainbow trout (*Salmo gairdneri*). A. Total weight (TW), dry matter (DM), non fat dry matter (NFDM) and total lipids (TL). B. Neutral lipids (NL) and phospholipids (PL).

the different growth phases corresponded to a TL loss of 4.2 g during SG.I, 9.8 g during SG.II, 10.4, 17.9 and 19.6 g during RG, respectively for animals non ovulated in October, ovulated in October or in November. During the latter growth phase a significant difference ($p<0.05$) was observed between TL weight of non ovulated animals in October and that of ovulated animals in November. Whatever the phase considered, loss of total lipid mainly reflected that of neutral lipid (NL), the phospholipid (PL) weight remaining low, less than 0.8 g (*fig.* 3 B).

Liver

Fresh weight as well as DM and TL weights significantly increased ($p<0.05$) between March and June and remained unchanged until August (*fig.* 4 A). From September these parameters increased again ($p<0.01$), reaching maximum values in October in non ovulated animals, then significantly decreased ($p<0.01$) at ovulation in October or November. Between October and November, the NFDM weight decreased from 5.50 ± 0.63 to 2.64 ± 0.66 and the TL weight from 1.30 ± 0.17 to 0.53 ± 0.13 g. Decreases in hepatic total lipid reflected changes in neutral lipid and phospholipid (*fig.* 4 B).

DISCUSSION

For a large number of fish species, the skeletal muscle and the liver are major sites of lipid storage (Love, 1970, Shulman, 1974). In so-called lean fish, lipids are mainly stored in the liver, while in the other species it is in the muscle. According to our findings and in keeping with results obtained by other authors in trout (Vuorela *et al.*, 1979), the liver does not seem to play an important role in lipid storage. The latter

Table 2. — Carcass composition of rainbow trout, *Salmo gairdneri* (expressed as increase + or — decrease rate% between months) at different physiological stages (*) during sexual maturation (**).

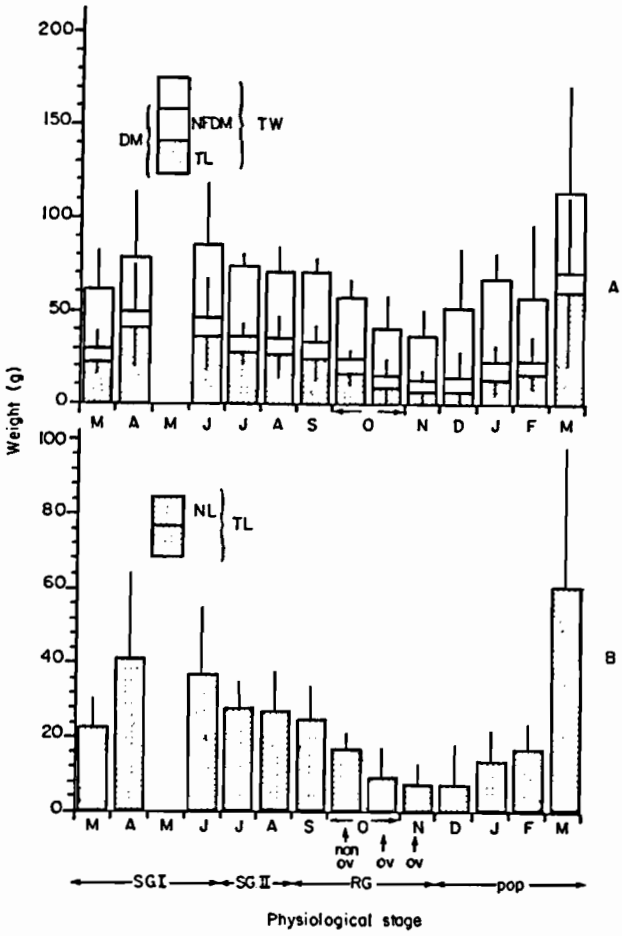
	SG.I Mar.-Jun.	SG.II Jul.-Aug.	RG Sep.-Oct. (°)	RG Sep.-Oct. (°°)	RG Sep.-Nov. (°°)	RG-POP Sep.-Dec.
Fresh tissue	+49.4	+ 5.4	+9.8	+ 6.6	+ 6.5	+ 3.8
Dry matter	+53.7	+11.4	−3.2	− 4.9	−13.2	−18.6
Non fat dry matter	+52.8	+13.7	−2.4	− 2.5	− 9.2	−10.8
Total lipids	+55.6	+ 6.3	−5.1	−10.7	−23.0	−37.5

(*) For abbreviations *see* table 1.
(**) July-Aug., value% March value, Sept. Oct., Sept.-Nov., Sept.-Dec., value % Aug. value.
(°) and (°°) Non-ovulated and ovulated animals, respectively.

Gut

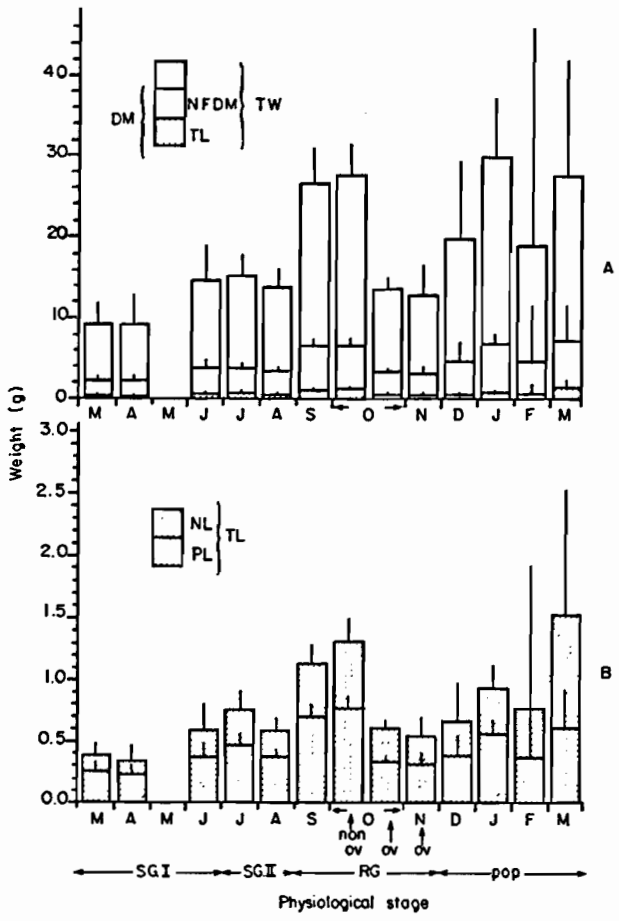
Fresh and dry matter (DM) weights reached maximum values in June and April, respectively, and thereafter progressively decreased until November. During this period, the NFDM weight varied only slightly (8.29 ± 3.24 in April, 5.62 ± 1.92 in November), whereas the TL weight decreased considerably ($p<0.001$) from 41.33 ± 21.99 in April to 7.68 ± 5.03 in November (*fig.* 3 A). The decrease recorded during were mainly deposited in the carcass (68-73%) and to a less extent in the gut (31-6%) and ovary (0.1-20%). The distribution of TL in these three deposition sites was closely related to the reproductive cycle.

The present study shows that carcass and visceral fat was mainly (>92%) composed of NL (lipid reserves). This fraction represented only 60% of the ovarian lipids at ovulation. Increase in the PL/NL ratio in the ovary during SG.II, and especially during



DIGESTIVE TRACT

Figure 3. — Changes of lipidic and non lipidic weights of digestive tract during sexual maturation of rainbow trout (*Salmo gairdneri*). A. Total weight (TW), dry matter (DM), non fat dry matter (NFDM) and total lipids (TL). B. Neutral lipids (NL) and phospholipids (PL).



LIVER

Figure 4. — Changes of lipidic and non lipidic weights of liver during sexual maturation of rainbow trout (*Salmo gairdneri*). A. Total weight (TW), dry matter (DM), non fat dry matter (NFDM) and total lipids (TL). B. Neutral lipids (NL) and phospholipids (PL).

RG, suggests that during late ovarian growth the trout preferentially incorporates fatty acids in the oocyte in the form of PL. In rainbow trout (Nakagawa and Tsuchiya, 1971), brook trout, *Salvelinus fontinalis* (Atchison, 1975) and Atlantic salmon, *Salmo salar* (Cowey *et al.*, 1985), the PL rich yolk globules are preferentially used during embryonic and larval development. This might explain the storage of PL in the oocyte. Unlike other body cells, the phospholipids do not only play a role in the membrane structure of this type of cell. They also have an important physiological role as reserves of essential fatty acids which are known to be necessary for building the structures of the embryo (Léger *et al.*, 1981, Leray *et al.*, 1985).

In trout, sexual maturation leads to large changes in body NFDM and especially TL. According to the results of this study the TL content decreased by 42 g (37.5%) in the carcass and by 34 g (81%) in the gut; the NFDM content decreased by 30 g (10.8%) in the carcass and by 3 g (52%) in the liver.

These results are in agreement with other studies on rainbow trout (Tveranger, 1985) and Atlantic salmon (Aksnes *et al.*, 1986) in seawater farming conditions. These studies show that body fat and NFDM are mobilised during the reproductive cycle and that this mobilisation is higher for the former than for the latter. Our results are partly contrary to those obtained in Northern pike (Medford and Mackay, 1978), American plaice (Mackinnon, 1972), European plaice (Dawson and Grimm, 1980) and Sockeye salmon (Idler and Bitners, 1959) in which sexual maturation leads to a decrease of the same magnitude in protein and fat content. Such differences might be due to the relative amount of body protein and body fat when these reserves are largely mobilised towards the

Table 3. – Lipid balance of rainbow trout (*Salmo gairdneri*) at different physiological stages(*) during sexual maturation.

		SG.I Mar.-Jun.	SG.II Jul.-Aug.	RG Sep.-Oct (°)	RG Sep.-Oct. (°°)	RG Sep.-Nov (°°)	POP Dec.	Total lipids (**)
Mobilised lipids (g)	Digestive tract	4.2	9.8	10.4	18.1	19.8	–	33.8
	Carcass	–	–	5.7	12.0	25.9	16.3	42.2
Deposited lipids (g)	Ovaries	1.1	3.1	24.0	19.2	21.3	–	28.2 to Oct.° 23.4 to Oct.°° 25.5 to Nov.°°
Lipid balance (°°°) (%)		26.2	31.6	149.1	63.8	46.6	–	

(*) For abbreviations *see* table 1.
(**) Total lipids, *i.e.* 28.2 (to Oct., for non ovulated) = 1.1 + 3.1 + 24.
(°) and (°°) Non-ovulated and ovulated animals, respectively.
(°°°) Deposited lipids/Mobilised lipids x 100.

oocyte. In fish with a fatty carcass, lipids are preferentially mobilised; in fish with a carcass which is naturally lean or has become so because of sexual maturation behaviour (fasting-migration), the loss of proteins is very large.

It seems that the lipolytic activity of the gut was higher than that of the carcass tissue. As compared to the lipid volume present in each tissue, the TL content decreased by 23 and 81%, respectively in the carcass and gut between March and November (ovarian growth period) and by 37 and 81%, respectively between March and December. The lipid metabolism seems to be particularly active in trout adipose tissue both in terms of lipogenesis and lipolysis involving lipoprotein-lipase and extrahepatic triglyceride-lipase, respectively (Sheridan and Allen, 1984). These results are similar to those of other authors (Skinner and Youssef, 1982, Black *et al.*, 1983) showing that the highest specific lipoprotein-lipase activity is that of the perivisceral adipose tissue.

The values of figures 2 and 3 show that during sexual maturation the metabolism in the two reserve tissues changed. The loss of visceral fat started during SG.I and ceased at ovulation, while that of carcass fat began during RG and finished one month after ovulation.

The lipid balance (*table* 3): TL deposited in oocytes/mobilised lipids reflects a trend towards: 1. a preferential utilisation of mobilised lipids for energy purposes during SG.I and SG.II, 2. a preferential utilisation in the form of storage in the oocytes during RG. However, these lipid balance values are only indicative, since the direct supply of dietary fat to the egg was not taken into account. These values can be estimated indirectly on the basis of variations in the level of *n*-3 essential fatty acids in animals of the same age subjected to a deficiency in these acids throughout the reproductive cycle (unpublished data). According to our results, a maximum of 50% of the total lipids deposited in the oocytes were of dietary origin. It may thus be deduced that 18% of the lipids mobilised during sexual maturation are directed towards the ovaries. This value is similar to that reported in European plaice (Dawson and Grimm, 1980), *i.e.* 22%, but much higher than that reported in Sockeye salmon (Idler and Bitners, 1959), ranging around 8%.

During the RG phase, the additional loss of lipids in ovulated animals, without concomittant deposition in the egg, indicates that specific ovulation events represent large energy expenditures, as shown in different species (Love, 1970, Shulman, 1974). Our results show that the flux of lipids towards the egg was interrupted during late oocyte maturation and consequencely that a mobilisation of the latter for energy purposes only took place during the same period.

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REFERENCES

Aksnes A., B. Gjerde, S. O. Roald, 1986. Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon (Salmo salar). *Aquaculture*, **53**, 7-20.

Atchison G. J., 1975. Fatty acid levels in developing brook trout (*Salvelinus fontinalis*) eggs and fry. *J. Fish. Res. Board. Can.*, **32**, 2513-2515.

Bartlett G. R., 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.*, **234**, 466-468.

Black D., A. M. Youssef, E. R. Skinner, 1983. The mechanism of lipid uptake by tissues in the rainbow trout, *Salmo gairdneri*. *Biochem. Soc. Trans.*, **11**, 93-94.

Cowey C. B., Bell J. G., Knox D., Fraser A., Youngson A., 1985. Lipids and lipid antioxidant systems in developing eggs of salmon (*Salmo salar*). *Lipids*, **20**, 567-572.

Craig J. F., 1977. The body composition of adult perch *Perca fluviatilis* in Windermere, with reference to seasonal changes and reproduction. *J. Anim. Ecol.*, **46**, 617-632.

Dawson A. S., A. S. Grimm., 1980. Quantitative seasonal changes in the protein, lipid and energy content of the carcass, ovaries and liver of adult female plaice, *Pleuronectes platessa* L. *J. Fish Biol.*, **16**, 493-504.

Diana J. S., W. C. Mackay, 1979. Timing and magnitude of energy deposition and loss in the body, liver and gonads of northern pike (*Esox lucius*). *J. Fish. Res. Board Can.*, **36**, 481-487.

Hails A. J., 1983. Temporal changes in fat and protein levels in the tropical anabantid *Trichogaster pectoralis* (Regan). *J. Fish Biol.*, **22**, 203-213.

Idler D. R., I. Bitners, 1959. Biochemical studies on sockeye salmon during spawning migration. V. Cholesterol, fat, protein and water in the body of the standard fish. *J. Fish. Res. Board Can.*, **16**, 235-241.

Léger C., L. Frémont, D. Marion, I. Nassour, M. F. Desfarges, 1981. Essential fatty acids in trout serum, lipoproteins, vitellogenin and egg lipids. *Lipids*, **16**, 593-600.

Leray C., G. Nonnotte, P. Roubaud, C. Léger, 1985. Incidence of (*n*-3) essential fatty acid deficiency on trout reproductive processes. *Reprod. Nutr. Dévelop.*, **25**, 567-581.

Love R. M., 1970. Depletion in the chemical biology of fishes. Academic Press, London and New York, 222-257.

Mackinnon J. C., 1972. Summer storage of energy and its use for winter metabolism and gonad maturation in the American plaice (*Hippoglossoides platessoides*). *J. Fish. Res. Board Can.*, **29**, 1749-1759.

Maxwell R. J., W. N. Marmer, M. P. Zubillaga, G. A. Dalikas, 1980. Determination of total fat in meat and meat products by a rapid, dry column method. *J. Assoc. Off. Anal. Chem.*, **63**, 600-603.

Medford B. A., W. C. Mackay, 1978. Protein and lipid content of gonads, liver and muscle of northern pike (*Esox lucius*) in relation to gonad growth. *J. Fis Res. Board Can.*, **35**, 213-219.

Nakagawa H., Y. Tsuchiya, 1971. Studies on rainbow trout egg (*Salmo gairdnerii irideus*). III. Determination of lipid composition of oil globule and lipoprotein. *J. Fac. Fish. Anim. Husb.*, Hiroshima Univ., **11**, 111-118.

Sheridan M. A., W. V. Allen, 1984. Partial purification of a triacylglycerol lipase isolated from steelhead trout (*Salmo gairdneri*) adipose tissue. *Lipids*, **19**, 347-352.

Shulman G. E., 1974. Life cycles of fish. J. Wiley and Sons, New York, Toronto, 25-147.

Skinner E. R., A. M. Youssef, 1982. The characterization of lipoportein lipase isolated from the post-heparin plasma of rainbow trout, *Salmo gairdneri* Rich. *Biochem. J.*, **203**, 727-734.

Snedecor G. W., W. C. Cochran, 1957. Methodes statistiques. *A.C.T.A.*, Paris, 100-128.

Tveranger B., 1985. Variation in growth rate, liver weight and body composition at first sexual maturity in rainbow trout. *Aquaculture*, **49**, 89-99.

Vuorela R., J. Kaitaranta, R. R. Linko, 1979. Proximate composition of fish roe in relation to maturity. *Can. Inst. Food Sci. Technol. J.*, **12**, 196-188.