# Lipid Class Composition during Embryonic and Early Larval Development in Atlantic Herring (*Clupea harengus*, L.)

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**ABSTRACT**

The lipid class compositions of Atlantic herring eggs and larvae were determined immediately before fertilization, after fertilization and at various times during subsequent embryonic and early larval development. Total lipid constituted 15% of the dry wt of ripe eggs, 70% of the total lipid being polar lipid with phosphatidylcholine (PC) accounting for almost 90% of the polar lipid. In general, the total lipid content decreased gradually during embryogenesis and in particular during larval development. Within 3 hr after fertilization the relative percentage of neutral lipid decreased slightly. This was followed by a general decrease in polar lipid which, by the stage of yolk sac absorption, was reduced to 52% of the total lipid. The decreased percentage of polar lipid was due entirely to a decrease in PC, which was reduced to 66% of the polar lipids at the stage of yolk sac absorption. The accompanying increase in the percentage of neutral lipids was mainly due to increased percentages of triacylglycerols (TAG) up to yolk sac absorption and cholesterol esters in the larval stages. During the first 4 days after hatching, phospholipids and to a lesser extent cholesterol were preferentially depleted in the yolk sacs, which also had higher levels of free fatty acids. The results are discussed in relation to possible roles of different lipids during embryonic and early larval development.
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## INTRODUCTION

The lipids in many marine teleost eggs are rich in phospholipids (1-4). In a recent survey we found that in teleost eggs with relatively short incubation periods (up to 20 days), e.g. cod *(Gadus morhua)*, haddock *(Melanogrammus aeglefinus)*, whiting *(Merlangus merlangus)*, saithe *(Pollachius virens)*, and herring, phospholipid accounted for 62-72% of the total lipid (4). PC was the major phospholipid class in each case, ranging from 63-83% of the total phospholipid (4). However, marine teleost eggs with relatively longer incubation periods (over 20 days), e.g. sand eel *(Ammodytes lancea)* and capelin *(Mallotus villosus)*, had higher lipid levels, with neutral lipids, mainly TAG, accounting for up to 77% of the total lipid (4). These findings suggest that in marine teleost eggs with relatively short incubation periods the majority of the lipid may be destined for biomembrane formation rather than energy production during development.

Studies of energy metabolism in developing fish eggs have been concerned mainly with measuring levels of potential energy reserves, metabolites and relevant metabolic enzyme systems (5-8). Deuchar (5) concluded that the order of utilization of energy reserves in teleost fish eggs was carbohydrate, then protein and finally lipid. This was questioned by Terner (6) on the basis of testing the ability of various externally added substrates to stimulate respiration in developing trout eggs. Acetate, a product of fatty acid catabolism, stimulated respiration to a greater extent than glycolytic substrates, including glucose (9). Radioactive acetate also was incorporated into polar lipid and subsequently into neutral lipid (10). Furthermore, the glycogenolytic and gluconeogenic pathways, although apparently operative in developing trout eggs, could not account for all of the endogenous respiration (11). More recently Vetter et al. (12) studied energy metabolism including lipid utilization in the rapidly developing egg of a marine teleost, the red drum *(Sciaenops ocellata)*, and established that lipid was utilized from both polar and neutral fractions throughout the developmental period.

The present study was undertaken to determine changes in the lipid class composition during embryonic and early larval development in a typical marine teleost, Atlantic herring, which has a high content of phospholipid (70% of total lipid) in its egg. The aim was to elucidate possible different roles of the various lipid classes during this period.

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## MATERIALS AND METHODS

*Eggs.* Roe was excised from ripe Atlantic herring *(Clupea harengus)* caught at the end of March 1983 on the Ballantrae Bank in the lower Clyde Estuary, Scotland. A batch of

unfertilized eggs was retained and the remainder fertilized with milt taken from 5 ripe males. The fertilized eggs were allowed to attach to glass plates and were maintained in outside tanks supplied with running sea water at an ambient temperature of approximately 8-10 C. Samples of 200-400 eggs were taken at time intervals during embryonic development, frozen at -15 C for 1-2 weeks and finally stored at -70 C for 6 mo prior to analyses.

*Larvae.* Roe was collected and eggs fertilized and maintained as above during March and April 1984. Hatching occurred after 20-21 days, and approximately 300-500 larvae were collected at time intervals and immediately frozen and stored in liquid nitrogen before transfer to the -70 C freezer. Yolk sacs were separated from the bodies of further samples of approximately 250-300 larvae before freezing in liquid nitrogen. Liquid nitrogen treatment was necessary with larvae due to the presence of highly active phospholipases which may maintain residual activity at -15 C. The sea water supplied to the tanks was passed through a 64 $\mu$m filter which removed all zooplankton, although phytoplankton passed through.

*Analytical.* Moisture contents were determined after freeze drying samples in an Edwards "Speedivac" centrifugal freeze drier. Total lipid was extracted by the method of Folch et al. (13), determined gravimetrically and stored in chloroform/methanol (2:1, v/v) containing 0.05% butylated hydroxytoluene at -20 C between analytical procedures.

*Lipid Analyses.* Lipid class analyses were carried out using Chromarods-SII and an Iatroscan TH-10 Mark II analyzer (Iatron Laboratories Inc., Tokyo) coupled to a Hewlett Packard 3390A recording integrator. Samples of 0.5 $\mu$l containing approximately 50 $\mu$g of total lipid were spotted on the origins of the rods using disposable 0.5 $\mu$l micropipettes (Camlab Ltd., Cambridge, U.K.). To analyze polar lipid classes the rods were developed in chloroform/methanol/distilled water (70:35:3.5, v/v/v). To analyze neutral lipid classes the rods were developed in hexane/diethyl ether/formic acid (85:15:0.04, v/v/v). The former procedure separated individual phospholipid classes together with neutral lipids as a single unresolved zone. The latter procedure separated individual neutral lipid classes together with phospholipids as a single unresolved zone. Developed rods were dried at 100 C for 2 min before being scanned at 3.1 mm $sec^{-1}$. The flame ionization detector was operated at a hydrogen pressure of 0.71 kg $cm^{-1}$ and an air flow of 2000 ml $min^{-1}$. The rods were stored in a constant humidity chamber between analyses and were scanned twice before each development and analysis. Peak areas for each lipid class obtained from the integrator were converted into $\mu$g of lipid using calibration curves constructed from standard solutions of known concentrations and with compositions similar to those of the experimental samples analyzed. Data obtained from scanning 18 individual rods were used for each single analytical determination.

## RESULTS AND DISCUSSION

Upon release and fertilization there is an increase in the water content of the eggs (Table 1), a phenomenon noted in the past (14). The presumed uptake of water is rapid in the initial phase of development and essentially complete by 3 days. Thereafter the water content remains relatively constant up to yolk sac absorption after day 25. The initial uptake of water could reflect simply a difference in osmolarity between the sea water and the eggs on spawning (15) or be a residual trace of the hydration that occurs, during maturation of marine teleost eggs, including demersal eggs (16,17). During the first 3 days the lipid content as a per cent of the egg dry weight shows a downward trend, although the lipid content increases again at 7 days (Table 1). Thereafter the per cent total lipid in the developing eggs fluctuates, although the value immediately after hatching increases 2-fold, due to the loss of much of the dry weight in the form of the chorion and associated material. The chorion alone can account for up to 1/3 of the dry wt of unfertilized herring eggs and, presumably, an even greater proportion after 3 weeks of development (18). Throughout early larval development the lipid content falls if no food is available to the larvae, as is the case here (Table 1). Overall, and despite some fluctuations, the results indicate that lipid is utilized during embryonic development and this utilization is accelerated in the early larval stages. This is consistent with the results of Boulekbache, who detected increasing lipid metabolism as embryonic development continued (7).

The results for changes in the proportion of total polar and total neutral lipids as well as the individual lipid classes can be considered, for convenience, in 4 developmental periods. The first of these is 0.1 day (approximately 3 hr) after fertilization of the eggs. The percentage of neutral lipids decreases and the percentage of polar lipids increases during this period (Table 1). The change is small but in this single sample set is statistically significant ($p<0.001$, Student t test, $t_{(28)} = 7.01$). The change in the relative proportions of polar and neutral lipids is re-

TABLE 1

Lipid Content and Lipid Class Analysis from Developing Herring Eggs and Larvae[a]

| | Time after fertilization (days) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 0.1 | 3 | 7 | 11 | 15 | 22 | 25 | 29 | 32 | 36 |
| Moisture content (%)[b] | 74.3 | 82.1 | 87.7 | 87.7 | 85.8 | 87.8 | 85.3 | 87.3 | 91.1 | 91.2 | 89.8 |
| Lipid content (% dry wt)[b] | 14.6 | 12.8 | 9.4 | 12.3 | 10.7 | 11.4 | 23.7 | 22.0 | 19.4 | 14.2 | 10.0 |
| % Polar lipids | 68.9 ± 1.4 | 71.6 ± 0.5 | 68.3 ± 0.7 | 64.9 ± 0.9 | 63.6 ± 0.5 | 57.6 ± 0.8 | 54.9 ± 2.7 | 52.5 ± 1.8 | 57.8 ± 1.5 | 57.0 ± 2.5 | 52.0 ± 1.3 |
| % Neutral lipids | 31.1 ± 1.4 | 28.4 ± 0.5 | 31.7 ± 0.7 | 35.1 ± 0.9 | 36.4 ± 0.5 | 42.4 ± 0.8 | 45.1 ± 2.7 | 47.5 ± 1.8 | 42.2 ± 1.5 | 43.0 ± 2.5 | 48.0 ± 1.3 |
| Neutral classes (% total lipid) | | | | | | | | | | | |
| Triacylglycerol | 13.7 ± 0.3 | 12.8 ± 0.9 | 15.1 ± 0.5 | 17.0 ± 0.9 | 17.8 ± 0.7 | 20.1 ± 1.3 | 22.9 ± 1.6 | 25.0 ± 0.9 | 22.0 ± 0.7 | 17.4 ± 1.1 | 12.9 ± 0.8 |
| Cholesterol/DAG | 8.6 ± 0.2 | 8.2 ± 0.6 | 9.1 ± 0.3 | 9.3 ± 0.3 | 9.6 ± 0.5 | 10.8 ± 0.8 | 8.9 ± 1.1 | 11.2 ± 0.7 | 13.7 ± 0.6 | 14.2 ± 0.6 | 18.2 ± 1.3 |
| Free fatty acids | 6.2 ± 0.3 | 4.7 ± 0.5 | 5.2 ± 0.3 | 6.1 ± 0.4 | 6.4 ± 0.7 | 8.4 ± 0.8 | 5.7 ± 0.6 | 4.5 ± 0.7 | 1.9 ± 0.4 | 3.1 ± 0.7 | 9.3 ± 0.7 |
| Sterol esters | 2.7 ± 0.2 | 2.6 ± 0.1 | 2.3 ± 0.2 | 2.6 ± 0.5 | 2.6 ± 0.2 | 3.1 ± 0.3 | 7.2 ± 1.2 | 5.8 ± 0.4 | 4.5 ± 0.3 | 7.9 ± 1.1 | 7.2 ± 0.5 |
| Polar classes (% total lipid) | | | | | | | | | | | |
| Phosphatidylcholine (PC) | 61.9 ± 2.0 | 62.4 ± 0.5 | 60.4 ± 0.9 | 58.0 ± 0.8 | 55.8 ± 0.7 | 48.7 ± 1.0 | 40.1 ± 2.7 | 41.0 ± 2.0 | 43.4 ± 1.2 | 37.6 ± 1.8 | 34.3 ± 1.1 |
| Phosphatidylethanolamine | 3.8 ± 0.5 | 6.0 ± 0.2 | 4.7 ± 0.4 | 4.0 ± 0.3 | 4.9 ± 0.3 | 5.3 ± 0.5 | 8.7 ± 1.0 | 9.5 ± 1.5 | 12.2 ± 0.9 | 16.4 ± 1.7 | 12.0 ± 1.0 |
| Phosphatidylserine | 0.7 ± 0.1 | 0.9 ± 0.1 | 0.8 ± 0.1 | 0.7 ± 0.1 | 0.8 ± 0.2 | 1.0 ± 0.2 | 1.4 ± 0.5 | 1.1 ± 0.7 | 1.5 ± 0.6 | 2.1 ± 1.0 | 4.3 ± 1.2 |
| Phosphatidylinositol | 0.5 ± 0.3 | 0.3 ± 0.1 | 0.4 ± 0.1 | 0.3 ± 0.1 | 0.4 ± 0.2 | 0.4 ± 0.2 | 1.0 ± 0.5 | | | | |
| Sphingomyelin | 0.8 ± 0.3 | 0.8 ± 0.1 | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.9 ± 0.2 | 1.1 ± 0.2 | 1.5 ± 0.6 | 0.7 ± 0.2 | 0.8 ± 0.2 | 0.8 ± 0.2 | 1.4 ± 0.7 |
| Lyso - PC | 1.4 ± 0.4 | 1.3 ± 0.2 | 1.1 ± 0.2 | 1.1 ± 0.2 | 0.9 ± 0.2 | 1.0 ± 0.2 | 2.2 ± 0.6 | 0.2 ± 0.1 | N.D. | 0.2 ± 0.1 | N.D. |

[a]Values are means ± standard deviations from 15-18 determinations unless stated otherwise.

[b]Results from one determination.

N.D. = Not detected. Traces of fatty alcohol < 0.5% were found in most samples.

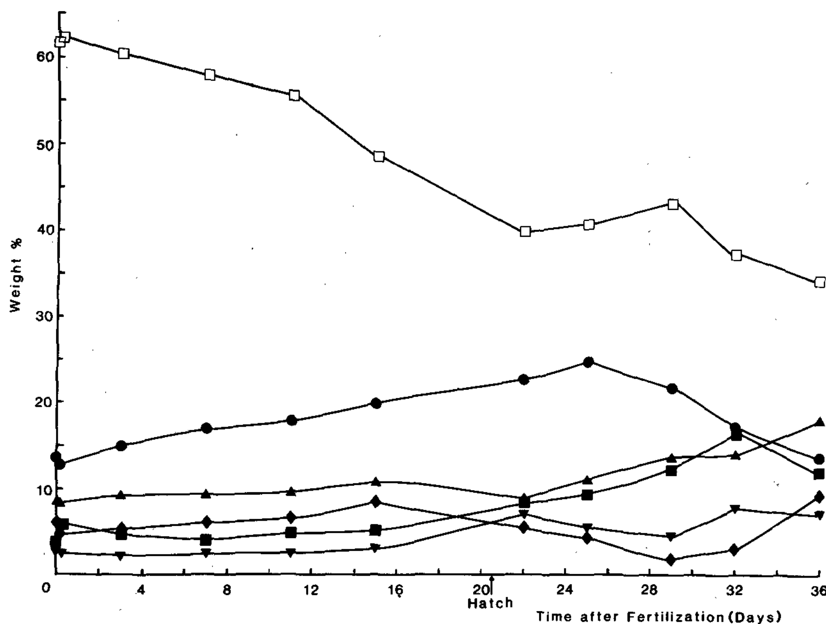


FIG. 1. Major lipid class composition of herring eggs during embryonic and subsequent early larval development. Samples of eggs and larvae were taken at the times indicated as described *in Materials and Methods*. □ = phosphatidylcholine; ■ = phophatidylethanolamine; ● = triacylglycerol; ▲ = cholesterol/diacylglycerol; ♦ = free fatty acids, and ▼ = sterol esters.

flected in the percentages of the individual neutral and major phospholipid classes (Table 1, Fig. 1).

The second developmental period is between 0.1 day and 15 days when there is a progressive decrease in the percentage of polar lipid, due entirely to a decreased percentage of PC, accompanied by a reciprocal increase in total neutral lipids, predominantly TAG (Table 1, Fig. 1). During the third developmental period, from day 15 to day 29, the proportion of polar lipids initially continues to decline but then increases due to a slight reversal in the decrease of PC and an increase in the percentage of phosphatidylethanolamine (PE) (Table 1, Fig. 1). Similarly, the increase in the percentage of TAG continues initially and then is reversed after yolk sac absorption, which had occurred in over 95% of larvae sampled on day 29 after fertilization. Hatching occurs in the middle of this period without any major changes in the lipid class composition other than the trends already noted and a 2-fold rise in sterol esters (Table 1, Fig. 1).

During the final developmental period, after yolk sac absorption, the percentage of total polar lipids decreases again due to a renewed decrease in PC and also PE in the later stage (Table 1, Fig. 1). TAG decreases sharply throughout this period and free fatty acids (FFA) increase some 4-fold from day 29 to day 36. Diacylglycerol (DAG) could not be resolved consistently from cholesterol during the analytical procedure used with the Iatroscan in the present study. However, examination of the total lipid by TLC-densitometry revealed an increase in DAG during this period, probably underlying the increased percentage of cholesterol/DAG recorded in Table 1 and Figure 1. Combined with the decreasing lipid content of the larvae (Table 1), these results suggest a general mobilization and utilization of both polar and, increasingly, neutral lipids in these starving larvae.

The lipids of the yolk sac in newly hatched (day 1) larvae show a class composition very similar to the larval bodies (Table 2). However, there are relatively high levels of lyso-PC and FFAs in the yolk sac lipids, probably reflecting the pattern of lipid mobilization at that time. At this stage the yolk sacs are large and can account for up to 50% of the dry wt of the larvae (19). By 4 days post-hatch (day 25 after fertilization) the yolk sacs are considerably depleted of polar lipids, in particular PC and PE, but of the neutral lipid classes, only cholesterol/DAG decreases (Table 2). This, combined with corresponding increases of these components in the larval bodies (Table 2) and the increase of PC, PE and cholesterol/DAG in the whole larvae (Fig. 1) during this period, suggests movement of intact biomembrane components from the yolk sac to the larval body. By this time the

TABLE 2

Lipid Class Analyses of Separated Larval Bodies and Yolk Sacs at 1 Day and 4 Days Post-Hatch[a]

| | Time after hatching (days) | | | |
|---|---|---|---|---|
| | 1 | | 4 | |
| | Larval bodies | Yolk sacs | Larval bodies | Yolk sacs |
| % Polar lipids | 53.1 ± 2.6 | 57.5 ± 2.8 | 55.0 ± 1.8 | 37.9 ± 1.7 |
| % Neutral lipids | 46.9 ± 2.6 | 42.5 ± 2.8 | 45.0 ± 1.8 | 62.1 ± 1.7 |
| Neutral classes (% total lipids) | | | | |
| Triacylglycerol | 25.6 ± 1.6 | 18.7 ± 1.6 | 23.7 ± 0.6 | 33.3 ± 2.4 |
| Cholesterol/DAG | 10.9 ± 0.7 | 6.1 ± 1.7 | 12.5 ± 0.4 | 4.3 ± 0.7 |
| Free fatty acids | 2.7 ± 0.8 | 10.2 ± 0.4 | 3.2 ± 0.5 | 12.3 ± 1.8 |
| Sterol esters | 7.0 ± 1.4 | 7.5 ± 1.0 | 5.2 ± 0.4 | 9.7 ± 1.0 |
| Fatty alcohols | 0.7 ± 0.4 | N.D. | 0.4 ± 0.2 | 2.5 ± 1.0 |
| Polar classes (% total lipid) | | | | |
| Phosphatidylcholine (PC) | 40.1 ± 3.0 | 40.1 ± 2.3 | 42.3 ± 2.1 | 33.5 ± 1.1 |
| Phosphatidylethanolamine | 9.4 ± 0.9 | 7.8 ± 1.3 | 10.8 ± 1.7 | 1.9 ± 0.8 |
| Phosphatidylserine | 1.5 ± 0.6 | ] 2.3 ± 0.8 | ] 1.2 ± 0.9 | 0.3 ± 0.1 |
| Phosphatidylinositol | 1.0 ± 0.4 | | | 0.4 ± 0.1 |
| Sphingomyelin | 1.3 ± 0.6 | 1.8 ± 0.5 | 0.7 ± 0.2 | 0.7 ± 0.3 |
| Lyso - PC | N.D. | 5.5 ± 1.4 | N.D. | 1.3 ± 0.5 |

[a]As in Table 1.
N.D. = Not detected.

yolk sac has decreased generally to only 10-20% of the total dry wt of the larvae (19), and the total lipid content of the whole larvae has decreased also (Table 1). Therefore, significant amounts of lipid are still being catabolized, reflected in the continued presence of lyso-PC and even higher levels of FFAs in the yolk sac (Table 2).

Overall the results indicate that after fertilization there is a very early, short period where some neutral lipid is utilized, predominantly FFA and TAG. Subsequent to this there is overall, a preferential net consumption of a single phospholipid class, PC, rather than neutral lipid which is conventionally regarded as more of an energy reserve than phospholipid. The major result of this process is that PC, which in the unfertilized eggs originally constituted almost 90% of the total phospholipids, is reduced by the time of yolk sac absorption to a level where the composition of the phospholipid pool is more characteristic of that found in most biological membranes. The precise reasons underlying the preponderance of PC in the released eggs are not clear at the present time. However, in addition to providing fatty acids, utilization of PC provides inorganic phosphate for intermediary metabolism including nucleic acid synthesis as well as choline for, possibly, C1 (methyl) metabolism (20) and neurotransmission. The 2-fold increase in TAG during embryonic and early larval development suggests that this lipid is preferentially retained up until yolk sac absorption, when it becomes an important energy source for the larvae until feeding commences.

The results presented here show net consumption of the polyunsaturated fatty acid-rich (4) phospholipid PC during embryonic and early larval development in Atlantic herring. One possible consequence of this strategy could be the catabolism of essential polyunsaturated fatty acids, deposited and concentrated in the eggs, that would have been expected to be conserved for biomembrane formation. The fatty acid compositions of the polar and neutral lipid fractions throughout embryonic and early larval development in Atlantic herring are the subject of a further report (21).

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