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Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). I. Respiration and nitrogen metabolism

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Abstract This paper provides the basis for a general model of catabolic metabolism for developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua* L.). Yolk-dependent routine rates of oxygen consumption, ammonia excretion, and accumulation of ammonium ions were related to quantitative changes in contents of glucose, glycogen, lactate, free amino acids, proteins and lipid classes (lipid classes published separately) in order to determine the rate and sequence of catabolic substrate oxidation that occurs with development at 6.0 °C, 34.5‰ S. The stoichiometric relation of the oxygen consumption and total ammonia production to substrate utilisation indicated that during the first 2 to 3 d of development, glycogen was the sole substrate of oxidative metabolism. After formation of the syncytium, free amino acids (75%) together with polar lipids (13%, mainly phosphatidyl choline) and neutral lipids (9%, mainly triacylglycerol) comprised the metabolic fuels of embryonic development. Following hatch (Day 16 post fertilisation), the fuels were free amino acids (32%), polar lipids (20%, mainly phosphatidyl choline), neutral lipids (17%, mainly triacylglycerol) and proteins (31%). Thus, the catabolic metabolism of endogenously feeding Atlantic cod larvae was predominantly fuelled by amino acids (67%) and lipids (32%), while glycogen only accounted for 1% of the total enthalpy dissipated. It is proposed that the above sequence of catabolic substrate oxidation is also generally applicable to other cold-water fishes which spawn eggs that do not contain oil globules.

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Introduction

Recent studies of the respiration, nitrogen, lipid and energy metabolism of yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus* L.) (Finn et al. 1995a; Rønnestad et al. 1995) proposed that the pattern of catabolic substrate oxidation that occurs with development may be typical for marine pelagic fish eggs that do not contain oil globules. The present communication reports on a similar study for developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua* L.), a species whose pelagic eggs do not contain visible oil globules either. The early life history of Atlantic cod has, over the years, been the subject of considerable research, ranging from its population biology and ecology to its detailed biochemistry (see Dahl et al. 1984). It is a species of great commercial value, whose eggs and larvae have been worked with in the laboratory for over 100 years (Solemdal et al. 1984). Like the Atlantic halibut, Atlantic cod is a multiple batch spawner yielding large numbers of homogeneous progeny (Kjesbu 1988, 1989). These traits, and the fact that rearing methods are well defined for this species, makes it well suited for a comprehensive study of physiological energetics.

Previous studies relating to the energetics of embryos and yolk-sac larvae of Atlantic cod have been restricted to limited aspects of their respiratory physiology (Davenport et al. 1983; Solberg and Tilseth 1984; Serigstad 1987) or changing biochemistry (Jørgensen 1985; Kuftina and Novikov 1986; Fyhn and Serigstad 1987; Fraser et al. 1988; Ulvund and Grahl-Nielsen 1988; Rainuzzo et al. 1992). Attempts have been made to identify the various substrates that are oxidised during development (Fyhn and Serigstad 1987; Fraser et al. 1988; Rainuzzo et al. 1992). But it remains, however, to quantify the relative importance of amino acids and lipids used to fuel metabolism, and to identify the substrates oxidised during early cellular division.

This communication therefore presents an integrated set of physiological, biochemical, gravimetric and biometric data derived from a single series of developing embryos and yolk-sac larvae of Atlantic cod. The yolk-dependent routine rates of oxygen consumption ($_{Ry}\dot{V}_{O_2}$) and ammonia production ($_{Ry}\dot{V}_{NH_{3\,TOT}}$) are stoichiometrically related to the quantitative biochemical data, and thereby reveal the sequence of catabolic substrate oxidation that occurs with development. Data for lipids, lipid classes and their associated fatty acids, together with caloric contents and the partitioning of yolk enthalpy into production, respiration and excretion are presented in a companion paper (Finn et al. 1995b).

Materials and methods

A single batch of Atlantic cod (*Gadus morhua*) eggs, artificially stripped from a single female and fertilised with the milt from a single male, was comprehensively studied. Some additional measurements of carbohydrate content were carried out on another egg batch. The eggs and larvae were incubated in static glass aquaria (5 liter) at 6.0 ± 0.02 °C, 34.5 ± 0.2‰ S, with daily exchange (80%) of water. Eggs floated in a single layer at the water surface. The light radiating at the water surface was 10 to 25 $\mu E\,s^{-1}\,m^{-2}$. The eggs and larvae initially received a photoperiod of 24 h light:0 h dark, but following hatch, were incubated under a 14 h light:10 h dark photoperiod.

Samples of 30 ind were selected every second day and measured via light microscopy (Leitz dialux 40 to 200 ×) for linear dimensions, including larval standard length (SL). Egg volume (V_E) and yolk volume (V_Y) were calculated from the measured diameters using the formula for an ellipsoid. Samples for biochemical analyses (four replicates of 15 to 100 pooled ind) were collected regularly by gently rolling eggs on soft filter paper followed by wet mass (wM) determination on a top balance (accuracy ± 1 mg). Yolk-sac larvae were individually rinsed in distilled water prior to collection. All samples were collected in a thermostatted cold room (6 ± 2 °C) and stored in cryotubes (Nunc) at −80 °C. Dry masses were determined by both dessication (dM_D) at 58 °C and lyophilisation (dM_L). Five groups of 30 ind, and eight groups of 50 to 100 ind were used for dM_D and dM_L, respectively. Dry masses were determined on a Cahn 25 Automatic Electrobalance (accuracy ± 1 µg). Individual wet or dry masses were calculated by dividing by the relevant number of individuals in each sample.

Respirometry

Routine rates of oxygen consumption ($_{Ry}\dot{V}_{O_2}$) were determined by both closed and open-flow respirometry. Closed respirometry was conducted on groups of 3 to 20 ind using spherical glass vials (Quickfit FR:S) of 10 to 30 ml calibrated volume as respirometers. Measurements were conducted in light (10 to 25 $\mu E\,s^{-1}\,m^{-2}$) and darkness using six test replicates and four blank replicates. During the embryonic phase, the respirometers were gently rotated (~1 rpm) in a specifically designed carousel as described by Serigstad (1986). The $_{Ry}\dot{V}_{O_2}$ was determined by polarographic determination of the oxygen tension (Radiometer E5046 electrode in conjunction with pHM73 pH/Blood Gas Analyser) and calculated as described by Rønnestad et al. (1992a). The polarographic oxygen sensor was calibrated regularly for the actual partial pressure of oxygen (p_{O_2}) as described by Finn et al. (1995c). The sea water for each measurement was allowed to equilibrate to air-saturation of gases at the experimental temperature, and the solubility of oxygen at 6.0 °C, 34.5‰ S, calculated after Green and Carrit (1967) and Forstner and Gnaiger (1983), was 14.730 $\mu mol\,O_2\,l^{-1}\,kPa^{-1}$. The p_{O_2} within the respirometers was never below 13 kPa (70% saturation) at the end of the respiratory experiments.

A CycloBios twin-flow microrespirometer (Gnaiger 1983) was used for the open-flow measurements between Day 10 to 15 post fertilisation (PF). These latter measurements, conducted on 71 ind in light (10 to 25 $\mu E\,s^{-1}\,m^{-2}$), were intended as a check for the closed respiratory measurements.

Routine rates of ammonia excretion ($_{Ry}\dot{V}_{NH_3}$) were determined chemically (four analytical replicates) by sampling 1 ml of the water from each test and blank respirometer and analysing after the method of Bower and Holm-Hansen (1980). The $_{Ry}\dot{V}_{NH_3}$ was calculated as described by Rønnestad et al. (1992a). The total rate of ammonia production ($_{Ry}\dot{V}_{NH_{3\,TOT}}$) was calculated by summing the $_{Ry}\dot{V}_{NH_3}$ and the routine rate of ammonium ion accumulation in the eggs ($_{Ry}\dot{V}_{NH_4^+}$). The nitrogen quotient (NQ) was calculated from the $_{Ry}\dot{V}_{NH_{3TOT}}$ and the $\dot{V}_{O_2}$.

Quantitation of nitrogenous compounds

Samples for total protein, free amino acids (FAA), ninhydrin-positive substances (NPS), proteinic amino acids (PAA) and ammonium ions (NH_4^+) were prepared for analysis in 6% trichloroacetic acid (TCA) in screw-capped polypropylene tubes (Nunc cryotubes). More than 1 d of extraction was allowed before the samples were vortexed and centrifuged at 15 000 ×g for 10 min. The supernatant was used for analyses of FAA, NPS and NH_4^+, while the precipitated egg and larval bodies were used for total and hydrolysed protein determination. Analyses for total protein, FAA, NPS and NH_4^+ were conducted on the same set of four replicates, while protein hydrolyses were performed on a separate set of four replicates.

Amino acids were analysed using an auto-analyser (Chromaspeck J180 Hilger Analytical) with fluorimetric detection (OPA reagent) and high pressure loading. The TCA extracts were appropriately diluted in 0.024 *M* suprapure HCl before analysis. An external standard solution of 20 amino acids (Pierce Chemicals, 0.5 nmol of each on the column) was run for every 8th sample. NPS were determined after the method of Moore and Stein (1948) on four sets of 15 to 50 eggs or larvae using norleucine as standard. NH_4^+ ions were determined (four analytical replicates) by neutralising 20 µl of supernatant with 980 µl 7.8 m*M* NaOH and then applying the salicylate–hyperchlorite method of Bower and Holm-Hansen (1980). Total protein was determined (four analytical replicates) by the Lowry technique (Lowry et al. 1951) and with the micro-modification of Rutter (1967) using bovine serum albumen as standard as described by Finn et al. (1991).

Hydrolysis of proteins from the eggs and yolk-sac larvae occurred in 6 *M* HCl at 110 °C over 24 h in evacuated glass vials. The HCl was then evaporated in a SpeedVac centrifuge until dry, and the PAA resolubilised in 0.024 *M* HCl and analysed as described for the FAA. Hydrolyses of crystalline standards of amino acids showed that most amino acids were fully recoverable after hydrolysis. Glutamine, however, was partly (60%) transformed to glutamate and partly degraded (40%) to a non-recoverable form. The data for glutamate therefore include an unknown fraction of proteinic glutamine, and the relative contribution of ∑(glutamine + glutamate) is slightly underestimated. However, since ∑(glutamine + glutamate) represented about 8% of the PAA, the underestimate of these amino acids was less than 3%.

Quantitation of carbohydrates

Quantitative assessment of the glucose and glycogen content of the eggs and larvae was determined on four sets of 100 ind via enzymatic

analysis (Lowry and Passoneau 1972). The lyophilised samples were precipitated in 3 *M* $HClO_4$ on ice. Following centrifugation at 3000 ×g for 10 min, 2 *M* $KHCO_3$ was added and, after 15 min at 4 °C, the samples were again centrifuged at 3000 ×g for 10 min. The supernatant was used for glucose quantitation, while the precipitate was hydrolysed in 1 *M* HCl at 100 °C for 3 h, and then used for glycogen quantitation using the same procedure as for glucose. All samples were read in duplicate on an Aminco filter fluorimeter at 340/460 nm.

Lactate was determined via the use of a Sigma Diagnostics quantitative, enzymic test kit (Procedure No. 826-UV). The frozen eggs and larvae were precipitated in 6% TCA and allowed to stand at 4 °C overnight. Following centrifugation at 3000 ×g for 10 min, the supernatant was used for lactate determination. Samples were read in triplicate on a Pye Unicam SP 8-100 recording spectrophotometer at 340 nm.

Ash contents

Ash content was determined gravimetrically (Cahn 25 Automatic Electrobalance) for each sample following combustion in a Phillipson microbomb (Ash_B) (see Finn et al. 1995b). An independent check of the ash content was made by combusting four replicates of 500 eggs on Day 6 PF at 550 °C in a muffle oven (Ash_O). The eggs were not rinsed in distilled water, and therefore the data include the salts of the perivitelline fluids.

Data handling

Statistical treatment of the data was performed following the methods of Sokal and Rohlf (1981). The 5% level was taken as the significance level, and the data were tested by one-way single classification analysis of variance (ANOVA).

Results

Physiological measurements

The routine rate of oxygen consumption ($_{Ry}\dot{V}_{O_2}$) increased gradually during embryonic development without any special deviation over the hatching period on Day 16 PF (Fig. 1). Between Day 10 and 15 PF, the technique of open-flow respirometry gave similar results to those obtained by closed respirometry. No effect of light was observed during the embryonic phase. Following hatch, however, the $_{Ry}\dot{V}_{O_2}$ of the light-adapted larvae deviated from the dark-adapted larvae after Day 19 PF, and rose to a maximum of 6.2 ± 0.43 nmol ind^{-1} h^{-1} on Day 21 PF. The $_{Ry}\dot{V}_{O_2}$ of the light-adapted larvae remained significantly higher than that of the dark-adapted larvae until Day 29 PF (Fig. 1: shaded area) at which point both rates became equivalent once more. Two 3rd order polynomial curves were fitted to each of the closed respiratory data sets in order to calculate total amounts of O_2 consumed by individual embryos and larvae (see legend to Fig. 1). As a result of an elevated $_{Ry}\dot{V}_{O_2}$, the light-adapted larvae consumed about 225 nmol ind^{-1} more O_2 than their siblings in darkness between Day 0 and 28 PF.

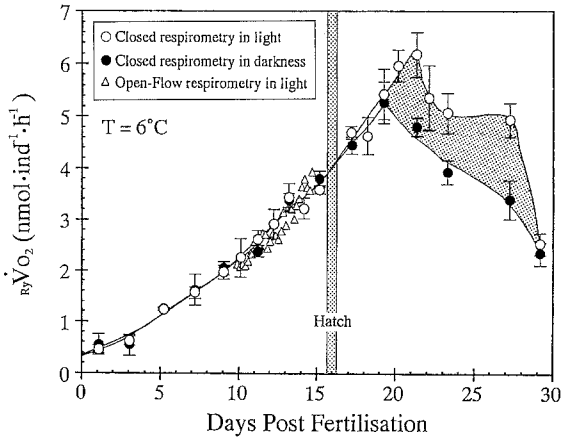

Fig. 1 *Gadus morhua* L. Routine rates of oxygen consumption ($_{Ry}\dot{V}_{O_2}$) of developing embryos and yolk-sac larvae in light or darkness. Measurements were mainly conducted by closed respirometry, but open-flow respirometry was also used between Day 10 and 15 post fertilisation. Each data point represents the mean (±SD) of six test measurements compared to four blank determinations. Polynomial curves of 3rd order were fitted to the closed respiratory data sets: Light, Day 1 to 21: $y = 0.209 + 0.169x + 0.002x^2 + 0.0002x^3$, $r^2 = 0.992$; Light, Day 21 to 29: $y = 595.585 - 71.859x + 2.908x^2 - 0.039x^3$, $r^2 = 0.999$; Darkness, Day 1 to 19: $y = 0.352 + 0.096x + 0.011x^2 - 0.0001x^3$, $r^2 = 0.994$; Darkness, Day 19 to 29: $y = 74.003 - 8.463x + 0.348x^2 - 0.005x^3$, $r^2 = 0.976$

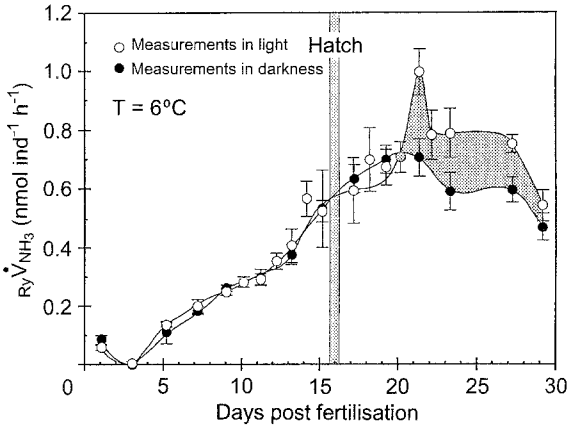

Fig. 2 *Gadus morhua* L. Routine rates of ammonia excretion ($_{Ry}\dot{V}_{NH_3}$) of developing embryos and yolk-sac larvae in light and darkness. Measurements were conducted as described for closed respirometry. Each data point represents the mean (±SD) of six test measurements compared to four blank determinations. Polynomial curves of 3rd order were fitted to the closed respiratory data sets: Light, Day 1 to 21: $y = -0.177 + 0.072x - 0.004x^2 + 0.0001x^3$, $r^2 = 0.959$; Light, Day 21 to 29: $y = 81.263 - 9.614x + 0.382x^2 - 0.005x^3$, $r^2 = 0.945$; Darkness, Day 1 to 19: $y = -0.115 + 0.044x - 0.0009x^2 + 0.00005x^3$, $r^2 = 0.988$; Darkness, Day 19 to 29: $y = 7.736 - 0.894x + 0.038x^2 - 0.0005x^3$, $r^2 = 0.860$

Shortly after fertilisation, a significant routine rate of ammonia excretion ($_{Ry}\dot{V}_{NH_3}$) was detected in both light and dark-adapted embryos (Fig. 2), but by Day 3 PF, the $_{Ry}\dot{V}_{NH_3}$ had fallen to very low levels. After Day 3 PF, the $_{Ry}\dot{V}_{NH_3}$ showed an essentially similar pattern to the

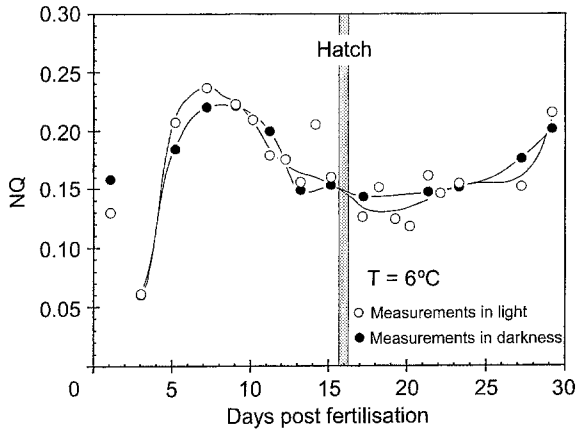

Fig. 3 *Gadus morhua* L. Nitrogen quotient (*NQ*) of developing embryos and yolk-sac larvae. *NQ* is calculated from the total rate of ammonia production $\sum({}_{Ry}\dot{V}_{NH_3} + {}_{Ry}\dot{V}_{NH_4^+})$ (Figs. 2 and 7) and the ${}_{Ry}\dot{V}_{O_2}$ (Fig. 1). Curves fitted by eye

${}_{Ry}\dot{V}_{O_2}$, where no special deviation was observed at hatch. Similar to the ${}_{Ry}\dot{V}_{O_2}$, the ${}_{Ry}\dot{V}_{NH_3}$ of the light- and dark-adapted larvae diverged following Day 19 PF. The light-adapted larvae showed a maximum ${}_{Ry}\dot{V}_{NH_3}$ of 1.0 ± 0.08 nmol ind^{-1} h^{-1} on Day 21 PF and an elevated level relative to the dark-adapted larvae (Fig. 2: shaded area) until Day 29 PF. The light-adapted larvae excreted about 35 nmol ind^{-1} more NH_3 than dark-adapted larvae between Day 0 and 28 PF.

The nitrogen quotient (*NQ*) of the developing embryos and larvae is given in Fig. 3. An average *NQ* of 0.25 or 0.23 would result from an aerobic metabolism fuelled solely by the catabolism of the FAA or PAA, that respectively, disappeared from the Atlantic cod eggs or yolk-sac larvae (see Table 5). Due to the high ${}_{Ry}\dot{V}_{NH_3}$ shortly after fertilisation, the *NQ* on Day 1 PF was also high. On Day 3 PF, however, the *NQ* was low indicating the catabolism of non-nitrogenous substrates. Following Day 3 PF, the *NQ* quickly rose to values of 0.20 to 0.24, indicating amino acid catabolism, but as hatch was approached, the *NQ* declined to intermediary values, suggesting a mixed fuel. The intermediary *NQ*s were maintained during most of the yolk-sac larval period, but showed an upward trend towards the end of the measurements.

Biometric and gravimetric measurements

The studied eggs were large for Atlantic cod (egg diameter = 1.45 ± 0.02 mm), but showed no significant changes ($p > 0.05$) in volume or wM during embryonic development (Table 1, Fig. 4). Each method of dry mass determination gave similar results thus allowing calculation of the relative water content which was stable at about 93% during the embryonic phase. For calculation of intermediate dM values, two 3rd order polynomial curves were fitted to the collated dry mass data as follows:

dM Pre-hatch (Day 0 to 16)

$$y = 114.97 + 0.1079x - 0.0059x^2 - 0.0011x^3,$$

$$r^2 = 0.652 \quad (1)$$

dM Post-hatch (Day 16 to 30)

$$y = -192.122 + 41.804x - 1.970x^2 + 0.0285x^3,$$

$$r^2 = 0.976. \quad (2)$$

Table 1 *Gadus morhua* L. Total wet mass (wM), dessicated dry mass (dM_D), lyophilised dry mass (dM_L), water, and ash content [microbomb calorimeter combusted (Ash_B) and muffle oven combusted (Ash_O)] of developing eggs and yolk-sac larvae. wM was determined on 21 groups of 15 to 100 pooled ind; dM_D was determined on 5 groups of 30 ind; dM_L was determined on 16 groups of 15 to 100 ind. Water content is the mean ($\pm$ SD) of all wM and dM measurements. Ash_B was determined on 4 groups of 100 pooled ind and Ash_O was determined on 4 groups of 500 ind (Day 6 only)

DPF	wM (µg ind^{-1})	dM_D (µg ind^{-1})	dM_L (µg ind^{-1})	H_2O (%)	Ash_B (µg ind^{-1})
0.8	1651.4 ± 78.11	116.3 ± 1.43	113.7 ± 1.31	92.9 ± 0.38	14.8 ± 0.65
2.7	1684.5 ± 70.16	116.5 ± 0.71	116.5 ± 1.05	93.1 ± 0.18	15.1 ± 1.63
4.7	1675.1 ± 64.41	114.6 ± 1.23	115.0 ± 1.67	93.1 ± 0.27	15.0 ± 1.02
6.7	1672.5 ± 59.38	113.3 ± 0.87	115.3 ± 2.07	93.1 ± 0.19	15.2 ± 1.27
6.7		113.3 ± 0.94			15.0 ± 0.53[a]
8.7	1680.8 ± 47.84	115.0 ± 0.76	114.4 ± 2.02	93.2 ± 0.11	15.4 ± 1.02
10.7	1666.1 ± 45.89	114.2 ± 0.73	113.2 ± 1.60	93.2 ± 0.18	15.2 ± 1.75
12.7	1732.6 ± 45.52	113.5 ± 1.46	115.1 ± 1.04	93.4 ± 0.16	15.1 ± 1.44
14.6	1638.4 ± 52.61	110.2 ± 0.91	111.7 ± 1.78	93.1 ± 0.16	14.6 ± 0.94
16.7			88.1 ± 3.50		
18.9			88.9 ± 1.00		
22.6			72.1 ± 2.62		
26.5			63.9 ± 1.05		
28.9			56.6 ± 0.99		17.0 ± 0.29

[a] Data from samples combusted in muffle oven (Ash_O)

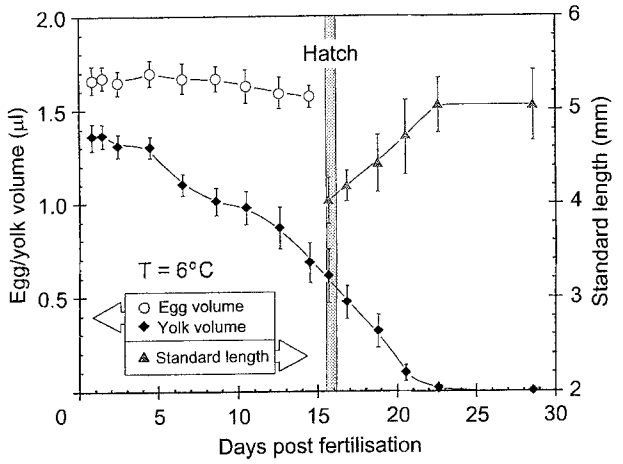

Fig. 4 *Gadus morhua* L. Biometry of developing eggs and yolk-sac larvae. Each data point represents the mean (± SD) of measurements on 30 ind. Volumes calculated from diameter measurements using the formula of an elipsoid. Curves fitted by eye

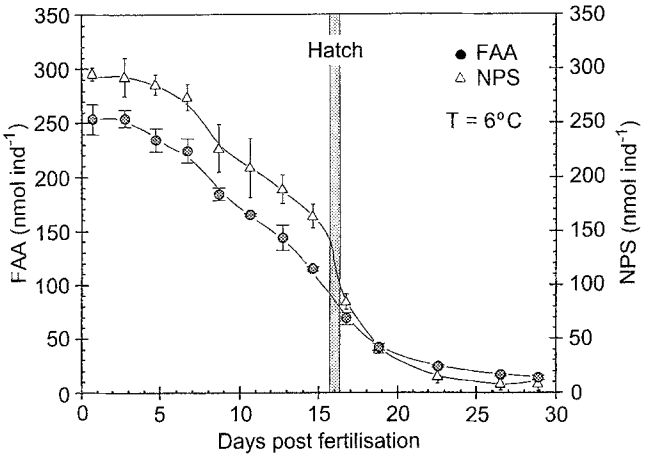

Fig. 5 *Gadus morhua* L. Total contents of free amino acids (FAA) and ninhydrin-positive substances (NPS) in developing eggs and yolk-sac larvae. Each data point represents the mean (± SD) of four separate determinations of 15 to 50 pooled ind. Curves fitted by eye

Following hatch on Day 16 PF, the larval SL increased at a linear rate of 0.15 mm d^{-1} from 4.03 ± 0.25 mm and seemed to plateau between Day 23 and 28 PF at about 5 mm. The change in dM over the hatching period suggested that the chorion and colloids associated with the perivitelline fluids had a collective dM of about 23 µg ind^{-1}, an amount equivalent to 20% of the whole egg dM. No significant decrease ($p > 0.05$) was noted in the yolk volume (V_Y) prior to Day 4.5 PF (Fig. 4). Thereafter, V_Y declined steadily during both the embryonic and larval stages. About 55% of the yolk was resorbed prior to hatch, and complete resorption had occurred by Day 23 to 25 PF.

Biochemical measurements

FAA and NPS

Shortly after fertilisation, the Atlantic cod eggs contained a total of 254 ± 14 nmol FAA ind^{-1} (Fig. 5). This amount would give a yolk concentration of about 185 m*M*. No change in the level of FAA occurred prior to Day 2.5 PF, and then a steady decline was observed during both the embryonic and larval stages until residual levels were reached after Day 25 PF. The resorption of FAA was linearly correlated to the yolk volume: [Total FAA(nmol) = $0.017 + 182.27 \times V_Y$(µl); r^2 = 0.979] which shows that the concentration of FAA in the yolk remained at about 185 m*M* throughout development. Essential amino acids [EAA: leucine, valine, isoleucine, lysine, threonine, phenylalanine, arginine, histidine, methionine (Wilson 1989) and tyrosine (Walton 1985)] and non-essential amino acids (NEAA) constituted 55 and 45% of the molar free pool, respectively (see Table 2). Leucine, valine, isoleucine and lysine quantitatively dominated in the essential FAA pool, while alanine and serine quantitatively dominated in the non-essential FAA pool. Apart from phosphoserine, aspartate and the amino acid analogue taurine, all FAA had declined to residual levels by Day 25 PF indicating that there was no preferential sparing of essential amino acids. Tyrosine and glycine declined at a slower rate than the other FAA. The total amount of NPS recorded in the Atlantic cod eggs shortly after fertilisation exceeded the total FAA by about 40 to 50 nmol ind^{-1}, and remained greater than the FAA during the embryonic phase (Fig. 5). Following hatch, the NPS became essentially equivalent to the levels of FAA.

NH_4^+ ion content

Contents of NH_4^+ ions showed no variation during the first 2.5 d of development, remaining at 36.2 ± 0.2 nmol ind^{-1} (Fig. 6). Thereafter until hatch, the eggs accumulated a further 45 nmol ind^{-1}. Upon hatching the NH_4^+ showed initial rapid depletion for the first 3 d, and thereafter continued a steady decline until measurements ceased.

Total protein

Total protein content showed an increase of about 0.5 µg ind^{-1} d^{-1} during the first 10 to 12 d of embryonic development (Fig. 7). During the last days before hatch a decrease seemed to occur. No large drop in the protein content was observed over the hatching period, showing that the chorion was only partially solubilised by the 1 *M* NaOH treatment. Indeed, specific analysis of egg proteins solubilised in 5 *M* NaOH did not significantly increase the amount of recovered protein.

Table 2 *Gadus morhua* L. Contents of individual essential (EAA) and non-essential (NEAA) free amino acids in developing eggs and yolk-sac larvae. Data represent the mean (± SD) of four determinations of 15 to 50 pooled ind. Specific analyses for the quantification of tryptophan showed that only trace amounts were present

Free amino acid (nmol ind^{-1})	Day post fertilisation												
	0.7	2.8	4.7	6.7	8.7	10.7	12.7	14.6	16.7	18.8	22.6	26.5	28.9
Essential													
leu	30.69 ± 1.73	31.14 ± 0.73	28.44 ± 0.97	28.63 ± 1.22	24.79 ± 0.93	22.98 ± 0.93	21.06 ± 1.58	18.22 ± 0.24	10.88 ± 0.89	5.46 ± 0.44	0.94 ± 0.05	0.45 ± 0.05	0.32 ± 0.04
val	21.49 ± 1.36	21.66 ± 0.78	19.63 ± 0.84	18.89 ± 0.59	16.12 ± 0.13	15.74 ± 0.50	15.23 ± 1.57	13.54 ± 0.52	9.41 ± 0.79	5.86 ± 0.57	0.85 ± 0.06	0.37 ± 0.03	0.31 ± 0.04
ile	20.12 ± 1.13	20.46 ± 0.58	17.52 ± 0.73	17.38 ± 0.61	14.82 ± 0.52	13.82 ± 0.57	12.33 ± 1.05	10.33 ± 0.15	5.36 ± 0.56	2.31 ± 0.21	0.37 ± 0.03	0.16 ± 0.02	0.11 ± 0.01
lys	18.61 ± 1.81	18.91 ± 1.21	16.77 ± 1.14	16.29 ± 0.71	13.56 ± 0.26	11.94 ± 0.12	10.38 ± 1.01	8.39 ± 0.50	5.88 ± 0.51	3.00 ± 0.38	1.48 ± 0.04	0.79 ± 0.05	0.73 ± 0.02
thr	12.18 ± 1.31	12.53 ± 0.85	10.85 ± 0.53	10.47 ± 0.97	8.17 ± 0.64	7.59 ± 0.25	6.78 ± 0.53	5.59 ± 0.70	2.98 ± 0.23	1.30 ± 0.15	0.64 ± 0.06	0.35 ± 0.04	0.31 ± 0.02
arg	10.48 ± 0.86	10.66 ± 0.33	9.12 ± 0.54	8.18 ± 0.22	6.65 ± 0.37	5.24 ± 0.21	4.33 ± 0.34	3.20 ± 0.15	1.31 ± 0.14	0.64 ± 0.07	0.68 ± 0.07	0.36 ± 0.02	0.28 ± 0.03
phe	7.47 ± 0.45	7.65 ± 0.20	6.80 ± 0.29	6.83 ± 0.25	6.02 ± 0.22	5.80 ± 0.06	5.72 ± 0.47	5.20 ± 0.09	3.68 ± 0.24	1.72 ± 0.11	0.46 ± 0.06	0.25 ± 0.04	0.17 ± 0.02
his	6.50 ± 0.40	6.74 ± 0.11	6.28 ± 0.14	6.50 ± 0.20	5.33 ± 0.15	4.84 ± 0.12	4.35 ± 0.32	3.38 ± 0.24	2.03 ± 0.12	1.35 ± 0.15	0.47 ± 0.15	0.35 ± 0.04	0.33 ± 0.03
tyr	5.66 ± 0.40	5.83 ± 0.20	5.10 ± 0.25	5.01 ± 0.25	4.33 ± 0.22	4.23 ± 0.07	4.29 ± 0.34	3.77 ± 0.04	2.43 ± 0.18	1.13 ± 0.11	0.33 ± 0.06	0.18 ± 0.04	0.13 ± 0.02
met	5.43 ± 0.27	5.01 ± 0.24	5.46 ± 0.29	4.75 ± 0.55	3.88 ± 0.30	3.23 ± 0.08	2.41 ± 0.20	1.84 ± 0.17	1.19 ± 0.19	0.48 ± 0.03	0.16 ± 0.02	0.13 ± 0.02	0.07 ± 0.02
Non-essential													
ala	47.91 ± 2.77	46.59 ± 1.08	46.17 ± 3.15	44.82 ± 2.20	35.76 ± 0.39	30.48 ± 1.05	23.42 ± 2.53	15.28 ± 0.12	4.45 ± 0.88	1.65 ± 0.12	1.61 ± 0.10	0.98 ± 0.09	0.84 ± 0.08
ser	21.06 ± 2.49	20.71 ± 1.42	16.28 ± 0.90	12.84 ± 1.69	8.83 ± 0.49	7.08 ± 0.15	6.19 ± 0.53	4.88 ± 0.17	2.57 ± 0.25	1.35 ± 0.16	1.23 ± 0.08	0.80 ± 0.05	0.74 ± 0.05
gln	16.11 ± 0.39	15.92 ± 0.66	15.88 ± 1.09	14.10 ± 2.03	9.91 ± 0.77	7.68 ± 0.25	5.13 ± 0.68	3.83 ± 0.14	1.72 ± 0.19	0.74 ± 0.05	0.45 ± 0.06	0.33 ± 0.02	0.20 ± 0.05
glu	8.10 ± 0.36	7.99 ± 0.58	6.03 ± 0.38	4.81 ± 0.76	3.29 ± 0.15	3.14 ± 0.07	3.89 ± 0.43	2.93 ± 0.10	2.06 ± 0.19	1.71 ± 0.13	1.60 ± 0.14	0.75 ± 0.04	0.60 ± 0.07
gly	7.68 ± 0.39	7.96 ± 0.17	7.95 ± 0.59	8.42 ± 0.29	7.64 ± 0.31	6.96 ± 0.08	5.75 ± 0.48	3.94 ± 0.06	1.97 ± 0.23	1.44 ± 0.10	1.34 ± 0.04	0.93 ± 0.07	0.84 ± 0.04
tau	5.71 ± 0.56	5.69 ± 0.39	5.57 ± 0.21	5.73 ± 0.39	5.50 ± 0.21	5.53 ± 0.14	5.90 ± 0.53	5.54 ± 0.24	5.68 ± 0.31	6.09 ± 0.56	5.62 ± 0.28	4.61 ± 0.18	3.20 ± 2.48
pro	5.42 ± 0.29	5.46 ± 0.09	7.04 ± 0.33	6.78 ± 0.27	5.46 ± 0.35	3.85 ± 0.17	1.64 ± 0.20	0.57 ± 0.05	–	–	0.52 ± 0.04	0.36 ± 0.05	0.32 ± 0.05
α-aba	2.22 ± 0.15	2.21 ± 0.06	2.62 ± 0.48	2.59 ± 0.23	2.21 ± 0.04	2.12 ± 0.17	2.02 ± 0.18	1.71 ± 0.00	1.32 ± 0.12	0.73 ± 0.09	0.07 ± 0.01	–	–
asp	1.01 ± 0.11	0.97 ± 0.05	0.72 ± 0.02	0.72 ± 0.12	0.75 ± 0.03	0.87 ± 0.05	1.02 ± 0.15	1.02 ± 0.02	1.21 ± 0.11	1.23 ± 0.09	0.95 ± 0.04	0.54 ± 0.03	0.53 ± 0.04
phs	–	0.78 ± 0.05	0.75 ± 0.04	1.05 ± 0.27	1.24 ± 0.09	1.54 ± 0.15	1.90 ± 0.25	2.22 ± 0.13	2.89 ± 0.44	3.88 ± 0.22	4.30 ± 0.22	3.71 ± 0.26	3.87 ± 0.28
% EAA	55	55	54	55	56	58	60	64	65	55	26	21	20
% NEAA	45	45	46	45	44	42	40	36	35	45	74	79	80

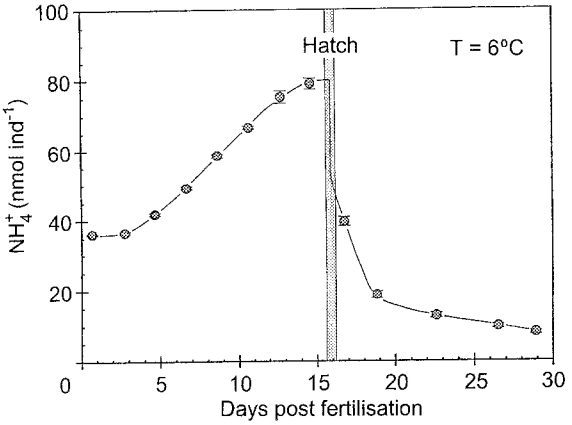

Fig. 6 *Gadus morhua* L. Ammonium ion content in developing eggs and yolk-sac larvae. Each data point represents the mean (± SD) of four separate determinations of 15 to 50 pooled ind. Curve fitted by eye

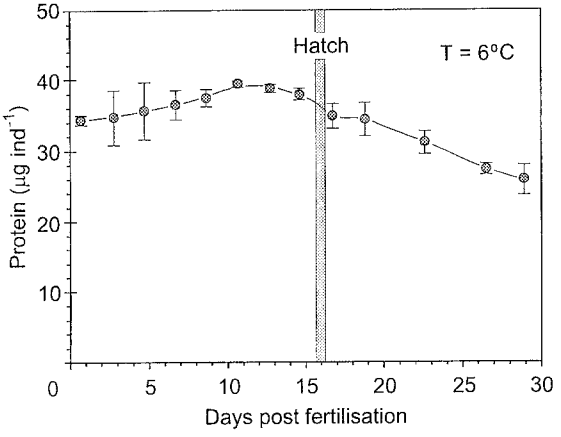

Fig. 7 *Gadus morhua* L. Protein content of developing eggs and yolk-sac larvae. Each data point represents the mean (± SD) of four separate determinations of 15 to 50 pooled ind. Curve fitted by eye

Following hatch, the protein content of the yolk-sac larvae was stable until Day 19 PF, and then declined steadily at a rate of about 1 µg ind^{-1} d^{-1} until measurements ceased.

Proteinic amino acids

The relative composition of amino acids polymerised in proteins (PAA) is given in Table 3. The PAA were equally distributed among the EAA and NEAA pools, and showed little change with development. The only notable change was a decline in the relative content of proline between unhatched eggs and hatched larvae. In general, neutral amino acids predominated in both pools, and greater amounts of sulfur-containing amino acids (methionine and cystine) were present in protein than in the free pool.

Carbohydrates and lactate

Carbohydrates in the form of free glucose and glycogen were present in low amounts (0.6% of egg dM) in the newly fertilised Atlantic cod eggs (Fig. 8). Free glucose was very low at this early stage, but a significant pool of glycogen (about 3.5 nmol ind^{-1} as glycosyl units) was detected. This pool of glycogen showed a linear decline of about 2.5 mol ind^{-1} (as glycosyl units) during the first 6 d of development. Thereafter, glycogen increased slightly during the days leading to hatch, and then declined to residual levels over the hatching period. Free glucose, although low during the first 5 d of development, rose to a maximum of about 2.7 nmol ind^{-1} on Day 12 PF. Following Day 12 PF, however, free glucose declined to low levels and essentially paralleled the content of glycogen.

Only small amounts of lactate (~1 nmol ind^{-1}) were present in the newly fertilised Atlantic cod eggs (Fig. 9). After Day 5 PF, lactate increased by 1 nmol ind^{-1}, and stabilised at a new level of about 2 nmol ind^{-1} for several days. It then declined slightly in the days leading to hatch, and showed another small increase during the yolk-sac larval phase.

Discussion

Respiratory physiology in relation to proximate composition

The two indirect methods, open-flow and closed respirometry, employed here to quantitate the routine metabolic rate (R_{Ry}), showed good agreement and suggest that the routine rate of oxygen consumption during yolk resorption ($_{Ry}\dot{V}_{O_2}$) was accurately determined. Both methods, however, only approximate that portion of R_{Ry} attributable to aerobic processes; they do not account for the possible contribution of anaerobic processes. Recently, however, Finn et al. (1995c) demonstrated, via the use of calorespirometry, that the $_{Ry}\dot{V}_{O_2}$ of developing embryos and yolk-sac larvae of turbot could be fully explained by the total rate of heat dissipation ($\dot{Q}$), and therefore approximated the true R_{Ry}. They also reported a low and relatively stable lactate content throughout development. Lactate is generally considered to be the major end product of anaerobic metabolism in fishes (Burton and Spehar 1971; Livingstone 1983; Van Waarde 1983). The low levels of lactate reported in the present study (Fig. 9) further support the notion that there is no significant anaerobic contribution to the energy dissipation during the endogenous development phase of Atlantic cod embryos and larvae.

The pattern and quantity of $_{Ry}\dot{V}_{O_2}$, commencing from low levels and steadily increasing through hatch to a maximum during the estimated time of first-feeding,

Table 3 *Gadus morhua* L. Relative composition (% total) of amino acids in the protein of developing eggs and yolk-sac larvae. Data represent the mean (± SD) of four determinations of 15 to 50 pooled ind

Proteinic amino acid (% of total)	Day post fertilisation												
	0.7	2.8	4.7	6.7	8.7	10.7	12.7	14.6	16.7	18.8	22.6	26.5	28.9
Essential													
leu	9.44 ± 1.08	9.25 ± 1.13	9.22 ± 0.66	9.50 ± 0.68	9.15 ± 1.57	8.99 ± 0.80	9.25 ± 0.24	8.66 ± 0.61	9.79 ± 1.49	9.55 ± 0.98	9.51 ± 1.95	9.58 ± 1.80	9.51 ± 1.64
val	7.78 ± 0.84	7.44 ± 0.98	7.63 ± 0.61	7.51 ± 0.36	7.51 ± 1.26	7.63 ± 0.47	7.51 ± 0.22	7.18 ± 0.54	6.88 ± 0.90	7.04 ± 0.40	6.77 ± 1.59	6.77 ± 1.63	6.39 ± 0.86
lys	6.24 ± 0.43	5.94 ± 0.48	6.11 ± 0.57	6.10 ± 0.34	6.13 ± 0.84	6.03 ± 0.27	5.98 ± 0.18	5.99 ± 0.34	7.20 ± 0.85	6.90 ± 0.82	6.91 ± 1.41	6.80 ± 1.17	6.95 ± 0.79
ile	5.50 ± 0.75	5.26 ± 0.64	5.28 ± 0.53	5.40 ± 0.27	5.20 ± 0.84	5.09 ± 0.46	5.18 ± 0.16	4.70 ± 0.37	5.06 ± 0.82	4.97 ± 0.48	4.72 ± 1.03	4.81 ± 0.97	4.68 ± 0.51
thr	5.32 ± 0.13	5.57 ± 0.83	5.46 ± 0.58	5.53 ± 0.52	5.39 ± 0.79	5.38 ± 0.34	5.23 ± 0.18	5.49 ± 0.51	5.32 ± 0.39	5.71 ± 0.62	5.30 ± 1.14	5.28 ± 0.69	5.56 ± 1.33
met	3.80 ± 0.33	4.03 ± 0.69	3.86 ± 0.34	3.57 ± 0.56	4.07 ± 0.39	3.76 ± 0.80	4.05 ± 0.21	4.15 ± 0.18	4.06 ± 0.55	4.17 ± 0.58	4.12 ± 0.97	4.22 ± 1.04	3.28 ± 0.31
arg	3.79 ± 0.34	3.60 ± 0.37	3.84 ± 0.34	3.96 ± 0.26	4.11 ± 0.61	4.27 ± 0.28	4.45 ± 0.21	4.48 ± 0.32	5.24 ± 0.65	5.03 ± 0.53	5.43 ± 1.23	5.32 ± 0.94	5.44 ± 0.49
tyr	3.51 ± 0.32	3.34 ± 0.34	3.42 ± 0.25	3.38 ± 0.16	3.43 ± 0.50	3.41 ± 0.26	3.46 ± 0.15	3.36 ± 0.22	3.05 ± 0.33	2.99 ± 0.28	3.22 ± 0.75	3.07 ± 0.59	3.12 ± 0.34
phe	3.40 ± 0.23	3.22 ± 0.35	3.29 ± 0.33	3.26 ± 0.14	3.28 ± 0.49	3.30 ± 0.27	3.30 ± 0.00	3.24 ± 0.26	3.17 ± 0.46	3.11 ± 0.41	3.15 ± 0.67	3.05 ± 0.62	3.13 ± 0.48
his	2.37 ± 0.15	2.09 ± 0.19	2.14 ± 0.13	2.05 ± 0.07	2.05 ± 0.35	2.07 ± 0.13	2.12 ± 0.02	2.12 ± 0.13	2.50 ± 0.31	2.34 ± 0.20	2.49 ± 0.54	2.36 ± 0.23	2.42 ± 0.47
Non-essential													
ala	11.07 ± 0.86	11.38 ± 2.17	11.08 ± 0.68	11.12 ± 0.95	11.20 ± 1.76	11.07 ± 0.80	10.44 ± 0.40	10.30 ± 1.13	10.46 ± 1.44	10.15 ± 1.18	9.21 ± 2.25	9.31 ± 3.04	9.44 ± 1.55
pro	9.01 ± 0.92	8.84 ± 1.14	8.97 ± 0.88	8.63 ± 0.64	8.67 ± 1.45	8.71 ± 0.63	8.81 ± 0.20	8.50 ± 0.75	5.19 ± 0.91	4.99 ± 0.46	5.06 ± 1.20	4.89 ± 1.18	5.24 ± 1.50
glu/gln	7.83 ± 0.26	8.37 ± 1.43	8.26 ± 0.63	8.20 ± 0.78	8.09 ± 1.06	8.24 ± 0.85	8.05 ± 0.33	8.41 ± 0.77	7.84 ± 1.04	7.97 ± 0.89	8.04 ± 1.75	8.34 ± 1.29	8.00 ± 0.93
asp	7.57 ± 0.14	8.00 ± 1.16	7.91 ± 0.52	7.84 ± 0.58	7.87 ± 1.02	7.60 ± 0.39	7.64 ± 0.33	8.19 ± 0.74	8.68 ± 0.96	9.25 ± 0.61	9.27 ± 1.94	9.29 ± 1.36	9.74 ± 1.18
gly	6.07 ± 0.32	6.03 ± 0.75	6.16 ± 0.25	6.26 = 0.39	6.40 ± 0.98	6.79 ± 0.39	6.99 ± 0.45	7.39 ± 0.54	8.38 ± 0.70	8.39 ± 1.09	8.99 ± 1.80	9.24 ± 1.77	9.33 ± 1.01
ser	5.93 ± 0.14	6.29 ± 0.92	6.12 ± 0.55	6.18 ± 0.34	6.12 ± 0.85	6.24 ± 0.58	6.15 ± 0.25	6.58 ± 0.33	6.22 ± 0.55	6.44 ± 0.78	6.65 ± 1.45	6.60 ± 0.95	6.71 ± 0.81
cys	1.38 ± 0.10	1.34 ± 0.16	1.26 ± 0.26	1.50 ± 0.24	1.33 ± 0.21	1.42 ± 0.06	1.39 ± 0.16	1.29 ± 0.25	0.98 ± 0.14	1.00 ± 0.02	1.17 ± 0.26	1.07 ± 0.23	1.08 ± 0.03
% EAA	51	50	50	50	50	50	51	49	52	52	52	51	50
% NEAA	49	50	50	50	50	50	49	51	48	48	48	49	50

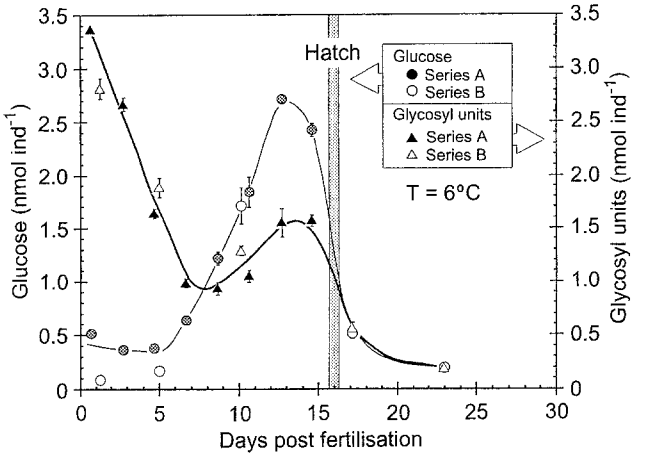

Fig. 8 *Gadus morhua* L. Contents of free glucose and glycogen (expressed as glycosyl units) in developing eggs and yolk-sac larvae. Each data point represents the mean (± SD) of four determinations of 100 pooled ind. Curves fitted by eye

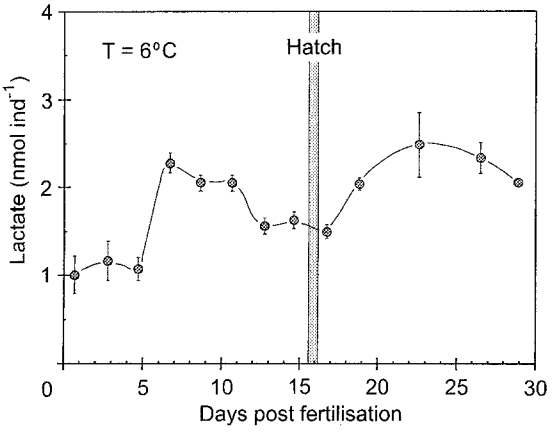

Fig. 9 *Gadus morhua* L. Lactate ion content in developing eggs and yolk-sac larvae. Each data point represents the mean (± SD) of four determinations of 15 to 50 pooled ind. Curves fitted by eye

are in good agreement with previous studies of Atlantic cod embryos and larvae (Davenport and Lønning 1980; Solberg and Tilseth 1984; Serigstad 1987; Vigrestad 1993). This general pattern is also seen in developing larvae of unfed turbot (Rønnestad et al. 1992b; Finn et al. 1995c) and Atlantic halibut (Serigstad 1987; Finn et al. 1995a). The smooth increase in $_{Ry}\dot{V}_{O_2}$ over the hatching period is also in good agreement with earlier studies of embryos and larvae of Atlantic cod (Davenport and Lønning 1980; Serigstad 1987; Vigrestad 1993), turbot (Rønnestad et al. 1992b; Finn et al. 1995c) lemon sole (Rønnestad et al. 1992b) and Atlantic halibut (Serigstad 1987; Finn et al. 1991), but contrasts the findings of Holliday et al. (1964) and Eldridge et al. (1977) investigating Atlantic and Pacific herring. These latter authors found a significant increase in $_{Ry}\dot{V}_{O_2}$ immediately following hatch, and both attributed the higher metabolic rates to increased activity associated with hatching. Both Atlantic and Pacific herring have a large perivitelline space, and the radial diffusional distance for oxygen is thus greater than for pelagic species (Berezovsky et al. 1979).

The peak $_{Ry}\dot{V}_{O_2}$, occurring on Day 21 PF, coincides closely with final stages of yolk resorption (Fig. 4) and maximum larval length. Since yolk is no longer available for fuelling growth, the larvae must sustain metabolism via autolytic processes. The larvae thus seem to enter a phase of negative energy balance where supplies of FAA are no longer sufficient to support the various catabolic and anabolic components of the developing larvae, and metabolism may be reduced accordingly. The larvae are apparently able to detect this negative energy balance, as evidenced by the reduced $_{Ry}\dot{V}_{O_2}$ of the dark-adapted larvae, and indeed the lack of FAA may act as a hunger signal for the start-feeding response. The peak $_{Ry}\dot{V}_{O_2}$ also coincides with the activity maxima of light-adapted Atlantic cod larvae (Skiftesvik and Huse 1987), suggesting that the lower $_{Ry}\dot{V}_{O_2}$ observed in dark-adapted larvae is due to reduced swimming activity. A reduced $_{Ry}\dot{V}_{O_2}$ of dark-adapted larvae has previously been observed for Atlantic cod (Serigstad 1987; Solberg and Tilseth 1987), Atlantic halibut (Finn et al. 1995a) and to a lesser extent turbot (Finn 1994), and suggests that light is required for food searching in these species.

During the first days of development, a significant $_{Ry}\dot{V}_{NH_3}$ was detected, despite the low $_{Ry}\dot{V}_{O_2}$, and this gave rise to the high NQ during this stage. This NH_3 is not thought to be derived from metabolism, but rather from the chorion hardening process (see discussion by Finn et al. 1991). Assuming, that metabolically derived NH_3 remained consistently low during the first 3 d following fertilisation, then the NQ would also have been low, indicating that non-nitrogenous fuels are being catabolised. This suggestion is further supported by the invariant levels of NH_4^+ in the egg (Fig. 6). By Day 5 PF, the $_{Ry}\dot{V}_{NH_3}$ had increased, and the embryos had begun to accumulate NH_4^+ ions, suggesting an increasing amount of NH_3 derived from catabolic processes. The ensuing $_{Ry}\dot{V}_{NH_3}$ essentially paralleled the pattern of $_{Ry}\dot{V}_{O_2}$ throughout development agreeing with previous measurements (Davenport et al. 1983), which suggests that Atlantic cod larvae predominantly utilise nitrogenous fuels for catabolic metabolism. The calculated NQs, which include the rate of NH_4^+ ion accumulation ($_{Ry}\dot{V}_{NH_4^+}$), show that a maximum dependence on nitrogenous fuels occurs shortly after gastrulation during the mid-embryonic development (i.e. during epiboly). Upon completion of epiboly, the NQ declines slightly to mixed values, suggesting that other non-nitrogenous fuels begin to be utilised. This situation continues until the last few days of experimentation when the larvae have exhausted yolk supplies and begin to utilise somatic protein stores.

At hatch, the rapid decline in NH_4^+ ion content should have resulted in a high $_{Ry}\dot{V}_{NH_3}$, on the order of

Table 4 *Gadus morhua* L. Proximate chemical composition of developing eggs and yolk-sac larvae. Each value is expressed as a percentage of the independently determined mean dry mass. (*DPF* days post fertilisation; *FAA* free amino acids; *CHO* carbohydrate)

DPF	Proximate chemical composition (% of dry mass)								
	Protein	Protein[a]	FAA	Lipid[b]	CHO	NH_4^+	Lactate	Ash	Total[c]
0.7	29.9	46.4	27.3	12.9	0.6	0.6	0.1	12.9	100.9
2.8	29.8	46.1	27.0	12.7	0.5	0.6	0.1	12.9	99.9
4.7	31.1	47.6	25.3	12.6	0.3	0.7	0.1	13.0	99.6
6.7	32.0	48.6	24.3	12.2	0.3	0.8	0.2	13.3	99.7
8.7	33.0	49.8	20.1	12.5	0.3	1.0	0.2	13.6	97.5
10.7	34.4	50.9	17.8	11.9	0.5	1.1	0.2	13.3	95.6
12.7	34.1	50.8	15.6	11.4	0.7	1.3	0.1	13.3	93.3
14.6	33.2	49.8	12.4	10.2	0.6	1.3	0.1	12.8	87.4
28.9	45.7	45.7	3.0	11.5	0.1	0.3	0.3	30.1	90.9

[a] Values calculated from the measured protein content (Column 2) with the addition of 19 µg insoluble protein ind^{-1} associated with the chorions as described in "Discussion"
[b] Data for Lipids taken from Finn et al. (1995b)
[c] Total compared to calculated protein (chorions included)

1 to 2 $nmol\,ind^{-1}\,h^{-1}$. This was not observed, and indicates that these NH_4^+ ions are not excreted in the form of NH_3. This is plausible since the pH of marine embryonic yolks is reported to be 5.3 to 5.6 (Grasdalen and Jørgensen 1985, 1987; Jørgensen and Grasdalen 1990). Thus, unless micro-basic environments exist within the embryo, the $NH_4^+ \leftrightarrow NH_3 + H^+$ equilibrium ($pK_a^s = 9.91$ at 6 °C, 0.4 Osm, Bower and Bidwell 1978) remains firmly on the left (99.998% = NH_4^+). This means that the disappearing NH_4^+ ions observed following hatch, must either be excreted in another chemical form (e.g. urea), or be incorporated in other nitrogenous molecules. A similar observation was also noted for turbot (Finn 1994), but contrasts the views of Fyhn and Serigstad (1987) and Rønnestad and Fyhn (1993) who believed the NH_4^+ ions to be excreted as NH_3.

In order to make reliable stoichiometric calculations of integrated physiological and biochemical data, it is important to ensure that both the physiological and biochemical data were accurately determined. A means of checking the validity and accuracy of the biochemical measurements made in these studies is to calculate the mass fraction of each chemical component of the eggs and larvae and compare the sum of the derived values with the independently determined dry mass. Accordingly, the proximate chemical composition of the eggs and yolk-sac larvae of Atlantic cod are given in Table 4. These values were derived from the dM and ash data presented in Table 1, and the biochemical data shown in Figs. 5, 6, 7, 8 and 9, while the data for lipids are taken from Finn et al. (1995b). The FAA data were converted from molar amounts to mass by multiplying with the relative molecular mass (M_r) of an average FAA that disappeared during development (see Table 5).

The proximate data for the proteins were underestimated relative to the dM due to the insolubility of the chorions in 1 *M* NaOH. Indeed specific analyses of eggs in 5 *M* NaOH were unable to extract further protein from the chorions, and agree with the findings of Lowry et al. (1951) who reported that increased concentrations of NaOH do not significantly increase the solubility of proteins. The protein solubility of fish egg chorions appears to vary from batch to batch (Young and Inman 1938; Fyhn and Govoni 1995). Indeed, the solubility of Atlantic cod egg chorions is reported to fall 10 to 20 fold following fertilisation due to the extensive cross-linking of isopeptide bonds associated with the hardening process (Oppen-Berntsen et al. 1990). The protein content of the Atlantic cod egg chorions can, however, be estimated from the drop in dM over the hatching period (see Table 1) and applying a relative protein content of 84% (w/w), which has been reported for relative protein content of Atlantic halibut egg chorions (Finn et al. 1991). Accordingly, the change in dM was about 23 $\mu g\,ind^{-1}$, or about 20% of egg dM, a value in good agreement with the data of Solberg and Tilseth (1984). The protein content of each chorion can thus be estimated to be about 19 $\mu g\,ind^{-1}$. When the insoluble chorion protein was added to the measured protein content, the proximate chemical composition balanced to within 3% in the first week of development. After this period, however, the proximate components appear to have been underestimated. Buckley (1981) showed that the nucleic acid contents $\sum$(RNA + DNA) of developing Atlantic cod eggs and larvae increased from about 3.5 $\mu g\,ind^{-1}$ at fertilisation to 10.5 $\mu g\,ind^{-1}$ at hatch, and according to his equations for RNA and DNA content, a larva with an SL of 5.05 mm (Day 28 PF, present study) would have a total nucleic acid content of about 7.4 $\mu g\,ind^{-1}$ (13% of dM). Such amounts of nucleic acid would explain the discrepancies in our measurements.

The contents of non-protein amino compounds (NPS) provide an independent means of estimating the total FAA pool. However, the ninhydrin used in the method also reacts with NH_3, and small peptides when present, but gives a low response to the imino acid proline (Moore and Stein 1948). The difference in the

Table 5 *Gadus morhua* L. Average molecular compositions of the component nutrients measured in the eggs at fertilisation. The specific heats of combustion ($\Delta_c U°w$) of each component are calculated from enthalpies of formation of crystalline substrate oxidised in the gas phase. The specific oxygen demands ($\Delta_k N_{O_2}$) of each component are calculated from the stoichiometric balance of oxidised substrate, assuming CO_2, H_2O, NH_3 and SO_4^{2-} to be the reaction products. (M_r relative molecular mass; *RQ* respiratory quotient; *NQ* nitrogen quotient)

Component	Formula	M_r (g mol^{-1})	$\Delta_k N_{O_2}$ (nmol O_2 g^{-1} substrate)	$\Delta_c U°w$ (kJ g^{-1})	*RQ*	*NQ*
Free amino acid	$C_{4.81}H_{10.17}O_{2.28}N_{1.31}S_{0.022}$	123.55	42.72	− 20.80	0.91	0.25
Proteinic amino acid	$C_{4.87}H_{9.83}O_{2.45}N_{1.22}S_{0.053}$	126.44[a]	41.85	− 18.22[b]	0.92	0.23
Glycosyl unit	$C_6H_{12}O_6$	180.16	33.30	− 15.61	1.00	
Lactate	$C_3H_5O_3$	89.07	30.87	− 63.33	1.09	
Phosphatidyl choline	$C_3H_{13}O_4NP(C_3H_5)$ $(C_{18.96}H_{30.76}O_2)_2$	804.60	89.23[c]	− 38.95[d]	0.74[c]	
				− 35.56[e]		
Triacylglycerol	$(C_3H_5)(C_{18.78}H_{32.27}O_2)_3$	911.31	89.75	− 38.95[d]	0.73	
				− 35.56[e]		

[a] Proteinic amino acid is given in its hydrolysed form. For computation of a polymerised amino acid, subtract 1 mol H_2O (18.02 g mol^{-1})
[b] Heat of combustion calculated for polymerised amino acid (i.e. 1 g protein)
[c] Only the esterified fatty acids and glyceryl skeleton are catabolised, the released orthophosphate and choline are assumed to be incorporated into other molecules.
[d] Values calculated from enthalpy of formation of saturated and mono-unsaturated fatty acids (Lide 1992)
[e] Values based on the specific heat of combustion of fish oils (Beamish et al. 1975)

NPS and total FAA curves shown in Fig. 5 can be fully accounted for by the NH_4^+ ion content until hatch. Upon hatch, when the NH_4^+ ions disappear, the two curves became equivalent, and the data therefore suggest that there are no significant quantities of small peptides present in the eggs or larvae. A similar conclusion was also drawn by Fyhn and Serigstad (1987).

At fertilisation, PAA (excluding chorions) and FAA essentially represent equivalent resources, which together account for 81%, on a mass-specific basis, of the nutrient pool. Lipids account for 18% of the nutrient pool (see Finn et al. 1995b), and carbohydrates 1%. In comparison, turbot (a species whose eggs contain a single oil globule) have 57 to 68%, 32 to 43%, and 1% of the egg nutrient pool as amino acids, lipids and carbohydrates, respectively (Finn 1994). This shows that Atlantic cod eggs, which do not contain an oil globule have a greater amino acid resource than lipid. It is noteworthy, however, that throughout the development of the Atlantic cod eggs and larvae, the mass-ratio of $\sum$(PAA + FAA):lipid remained constant at about 4.4:1, suggesting a proportionally constant catabolism of amino acids and lipids. A similar observation was noted for turbot eggs and larvae (Finn 1994), although the ratio was lower for this latter species (range 1.3 to 2.1:1). In terms of caloric yield, the ratio of $\sum$(PAA + FAA):lipid in the Atlantic cod eggs and larvae remained constant at about 2.4:1 (see Finn et al. 1995b) which suggests that the energetic utilisation of amino acids is two and a half times that of lipids.

The decline in the glycogen content following fertilisation suggests its use as a fuel during the early cellular stages of embryogenesis. The increase in free glucose observed after Day 5 PF coincided with gastrulation and decline in the pool of FAA, suggesting some gluconeogenesis. The decline in free glucose following its maximum on Day 12 PF coincided with the commencement of the heart beat and suggests that it may either have been used as a fuel for this purpose or was transported from a site of net production to tissues capable of net catabolism. This capacity for glucose catabolism, albeit small, is in line with the appearance of hexokinase activity following completion of gastrulation in the loach (Neyfakh and Abramova 1974).

Stoichiometry and substrate utilisation

The derived *NQ* provides an insight into the various substrates used to fuel oxidative metabolism. It is important, however, that both the O_2 consumed and the excretory nitrogen produced result from metabolic processes. An independent means of checking this is to relate, stoichiometrically, the physiologically derived O_2 consumption and total NH_3 production to the sequential quantitative changes in the biochemical substrates. For accurate comparison of the decline in substrate to the consumption of O_2, specific oxygen demands ($\Delta_k N_{O_2}$) should be used. The $\Delta_k N_{O_2}$ derived from the chemical composition of each substrate in newly fertilised Atlantic cod eggs are given in Table 5. Similarly, the amount of ammonia nitrogen produced from amino acid catabolism will also depend upon the particular molecular composition of the amino acids in question. Thus average molecular compositions of the FAA and proteinic amino acids in the newly fertilised Atlantic cod eggs are also given in Table 5, and the molar yields of NH_3 from their catabolism are 1.31 and 1.22, respectively.

During the first days of development, only glycogen showed a significant decline of about 2.5 nmol ind^{-1} (as glycosyl units). This glycogen could account for 15 nmol of the respired oxygen. Following Day 3 PF, utilisation of the FAA and lipids (see Finn et al. 1995b) commences, and by hatch, about 164 nmol FAA ind^{-1} had disappeared from the eggs. However, the total amount of ammonia produced during this period was 125 nmol ind^{-1}, which can only have resulted from the deamination of about 95 nmol FAA ind^{-1}. Thus 69 nmol FAA ind^{-1} are available for polymerisation and could generate 7 µg protein ind^{-1}. This amount is slightly larger than the measured increase of about 6 µg ind^{-1} during the egg stage. The 95 nmol FAA ind^{-1} which were catabolised could thus account for 502 nmol of the respired oxygen. Similarly, for the lipids, a net decline of 1.41 µg ind^{-1} polar lipid (mainly phosphatidyl choline; PC) and 0.75 µg ind^{-1} neutral lipid (mainly triacylglycerol; TAG) had occurred by hatch (see Finn et al. 1995b). Subtracting the phosphate, and assuming that the choline moiety of PC is not catabolised, these quantities could account for 91 and 63 nmol O_2 ind^{-1}, respectively. Thus the sum of these various oxygen equivalents would be 671 nmol ind^{-1}. The actual oxygen consumed during this phase (Day 0 to 16), estimated from the integrated polynomial curves, was 727 nmol ind^{-1} and agrees to within 92% of the biochemical data. If choline was catabolised, the agreement would be 97%.

Similar calculations for the yolk-sac larval phase (Day 16 to 28 PF) show that the net decline of 74 nmol FAA ind^{-1}, 9.0 µg protein ind^{-1}, 3.8 µg polar lipids ind^{-1} (mainly PC) and 2.5 µg neutral lipids ind^{-1} (mainly TAG) (see Finn et al. 1995b) would account for 391, 377, 244 (assuming choline is not catabolised) and 211 nmol O_2 ind^{-1}, respectively. The sum of these oxygen equivalents, being 1222 nmol ind^{-1}, is also in fairly good agreement (91%) with the actual oxygen consumption of 1350 nmol ind^{-1} for the same period. If choline was catabolised, the agreement would be 97%. Since the actual $\dot{V}_{O_2}$ during the yolk-sac larval phase depended on light conditions, the calculated total oxygen consumption is derived from a mean integral of both light and dark polynomial curves using a 14 light : 10 h dark photoperiod (see legend to Fig. 1). Similarly, the catabolism of 74 nmol FAA ind^{-1} and 9.0 µg protein ind^{-1} should have resulted in the production of about 199 nmol NH_3 ind^{-1}, which agrees precisely with the 199 nmol NH_3 ind^{-1} actually measured for this period. This exceptional agreement between the physiological and biochemical data allows the derivation of the sequence of catabolic substrate oxidation which is presented in Fig. 10. Table 6 provides a summary of the importance of each substrate during the embryonic and yolk-sac larval phases.

The initial use of glycogen as the sole fuel prior to gastrulation and epiboly agrees with other studies of fish embryos (Amberson and Armstrong 1933; Needham 1942; Hishida and Nakano 1954; Milman and Yurovitzky 1967, 1973; Terner 1968; Moroz and Luzhin 1976; Vetter et al. 1983; Santos and Vinagre 1991; Finn et al. 1995c), and lends support to the hypothesis

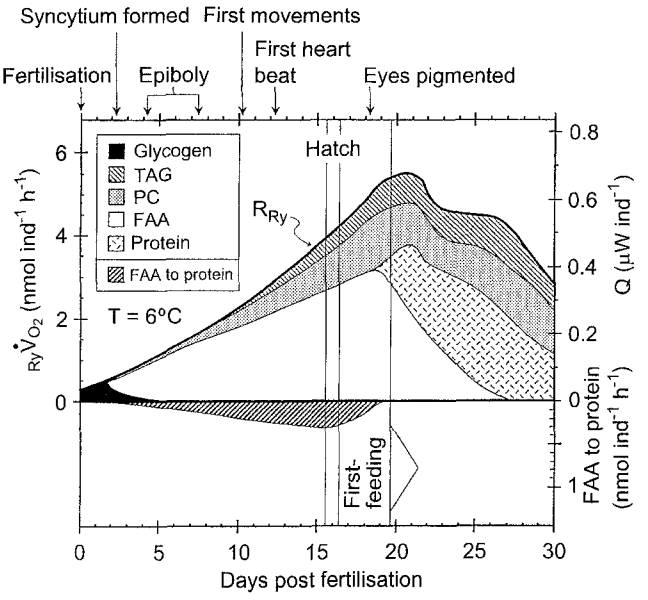

Fig. 10 *Gadus morhua* L. Proposed scheme for the sequence of catabolic oxidation of endogenous substrates by developing embryos and yolk-sac larvae. Scheme is based on stoichiometric balancing of biochemical substrates with routine rates of oxygen consumption ($_{Ry}\dot{V}_{O_2}$) and ammonia production. $_{Ry}\dot{V}_{O_2}$ is equivalent to the yolk-dependent routine metabolic rate (R_{Ry}), and the flux of each substrate is expressed as a fraction of that rate. The rate of heat dissipation ($\dot{Q}$) was calculated from $_{Ry}\dot{V}_{O_2}$ assuming a general oxycaloric equivalent of -450 kJ mol^{-1} O_2 (Gnaiger and Kemp 1990). Various developmental events were identified via light-microscopic observations and from photographs taken during experimentation. See "Discussion – Stoichiometry and substrate utilisation" for further explanation. (*FAA* free amino acids; *PC* phosphatidyl choline; *TAG* triacylglycerol)

Table 6 *Gadus morhua* L. Relative contribution (% of oxygen consumed) of the major substrates to the energy metabolism of developing embryos and yolk-sac larvae. Values are derived from the stoichiometric catabolism of oxidised substrate. The general *NQ* provides an overview of the importance of nitrogenous substrate catabolism for each phase. (*FAA* free amino acids; *AA* amino acids)

Substrate	Embryonic phase	Yolk-sac larval phase	Whole development
Glycogen	2	0	1
FAA	75	32	47
Protein	0	31	20
Polar lipid	14	20	18
Neutral lipid	9	17	14
$\sum$ Carbohydrate	2	0	1
$\sum$ Amino acids	75	63	67
$\sum$ Lipids	23	37	32
Ratio AA : Lipid	3.3	1.7	2.1
General *NQ*	0.19	0.16	0.17

that cytosolic fuel stores must sustain metabolism until the syncytium is sufficiently formed to access other potential fuels contained in the yolk (Finn et al. 1995c). Following the commencement of gastrulation, however, the strong dependence on FAA during both the embryonic and early yolk-sac larval stages, agrees with earlier studies of eggs and larvae that do not contain oil globules (Fyhn and Serigstad 1987; Fyhn 1989, 1990; Rønnestad et al. 1992a, 1993; Finn et al. 1995a). The catabolism of PC is also in agreement with earlier studies of developing fish embryos and yolk-sac larvae (Fraser et al. 1988; Rainuzzo et al. 1992; Firm 1994; Rønnestad et al. 1995) and is characteristic of eggs that do not contain oil globules. Fraser et al. (1988) further reported a catabolism of TAG in yolk-sac larvae of cod following hatch. The data in the present study suggest that catabolism of TAG may occur somewhat earlier, during the phase when the embryo first begins to flex inside the egg (see Finn et al. 1995b).

Proteins are the last to be catabolised for energy, suggesting that they are coveted for the structural and regulatory roles for which they are known (Tsuyuki 1974; Feeney and Brown 1974; Stryer 1988). The use of proteins as a last resort contrasts the proposals of Needham (1931, 1942), Deuchar (1965) and Amberson and Armstrong (1933) who all suggested that proteins were catabolised after the carbohydrate phase. However, since their conclusions were based primarily on respiratory ratios (*RQ*), uncorroborated by quantitative biochemical data, it is possible that they mistook protein for FAA despite the fact that fresh-water species contain lower amounts of FAA (Thorsen and Fyhn 1991). It is apparent that, for pelagic marine species, proteins are only recruited for energy metabolism after the exhaustion of FAA (Rønnestad and Fyhn 1993; Finn 1994; Finn et al. 1995a). The embryonic deposition of proteins from the FAA that seem to be removed for osmotic purposes (see Fyhn 1993) can be regarded as a temporary energetic reservoir.

Thus, the sequence of catabolic substrate oxidation for developing Atlantic cod embryos and larvae seems to be: (1) glycogen during blastula formation; (2) following formation of the syncytium, FAA become the main substrate but are joined by PC and later, when the embryo begins to flex, also by TAG; (3) finally, during the phase of the FAA exhaustion, amino acids derived from body proteins are catabolised together with PC and TAG.

It is suggested that this sequence of catabolic substrate oxidation, where amino acid catabolism is favoured over fatty acid catabolism (~70:30), is typical of marine pelagic eggs that do not contain visible oil globules.

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References

Amberson WR, Armstrong PB (1933) The respiratory metabolism of *Fundulus heteroclitus* during embryonic development. J cell comp Physiol 2: 387–397

Beamish FWH, Niimi AJ, Lett PFKP (1975) Bioenergetics of teleost fishes: environmental influences. In: Bolis L, Maddrell HP, Schmidt-Nielsen K (eds) Comparative physiology – functional aspects of structural materials. North Holland Publ., Amsterdam, pp 187–209

Berezovsky VA, Goida AEA, Mukalov IO, Sushko BS (1979) Experimental study of oxygen distribution in *Misgurnis fossilis* eggs. Fiz Zh 25: (4) 378–389. Can Transl Fish aquat Sci (1986) Transl No 5209

Bower CE, Bidwell JP (1978) Ionization of ammonia in seawater: effects of temperature, pH and salinity. J Fish Res Bd Can 35: 1012–1016

Bower CE, Holm-Hansen T (1980) A salicylate-hypochlorite method for determining ammonia in seawater. Can J Fish aquat Sciences 37: 794–798

Buckley LJ (1981) Biochemical changes during ontogenesis of cod (*Gadus morhua* L.) and winter flounder (*Pseudopleuronectes americanus*) larvae. Rapp P v Réun Cons int Explor Mer 178: 547–552

Burton DT, Spehar AM (1971) A re-evaluation of the anaerobic end products of freshwater fish exposed to environmental hypoxia. Comp Biochem Physiol 40A: 945–954

Dahl E, Danielssen DS, Moksness E, Solemdal P (1984) The propogation of cod, *Gadus morhua* L. 2 Vols. Institute of Marine Research, Flødevigen Biological Station, Arendal, Norway

Davenport J, Lønning S (1980) Oxygen uptake in developing eggs and larvae of the cod (*Gadus morhua*). J Fish Biol 16: 249–256

Davenport J, Lønning S, Kjørsvik E (1983) Ammonia output by the eggs and larvae of the lumpsucker (*Cyclopterus lumpus*) the cod (*Gadus morhua*) and the plaice (*Pleuronectes platessa*). J mar biol Ass UK 63: 713–723

Deuchar EM (1965) Biochemical patterns in early development stages of vertebrates. In: Weber R (ed) Biochemistry of animal development. Vol. 1. Academic Press, New York, London, pp 234–304

Eldridge MB, Echeverria T, Whipple JA (1977) Energetics of Pacific herring (*Clupea harengus pallasi*) embryos and larvae exposed to low concentrations of benzene, a monoaromatic component of crude oil. Trans Am Fish Soc 106: 452–461

Feeney RE, Brown WD (1974) Plasma proteins in fishes. In: Florkin M, Scheer BT (eds) Chemical zoology. VIII. Academic Press, London, New York, pp 307–329

Finn RN (1994) Physiological energetics of developing marine fish embryos and larvae. Dr. scient. thesis. University of Bergen, Bergen, Norway

Finn RN, Fyhn HJ, Evjen MS (1991) Respiration and nitrogen metabolism of Atlantic halibut eggs (*Hippoglossus hippoglossus L.*). Mar Biol 108: 11–19

Finn RN, Henderson RJ, Fyhn HJ, Evjen MS (1995b) Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). II. Lipid metabolism and enthalpy balance. Mar Biol 124: 371–379

Finn RN, Rønnestad I, Fyhn HJ (1995a) Respiration, nitrogen and energy metabolism of developing yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus* L.). Comp Biochem Physiol 111A: 647–671

Finn RN, Widdows J, Fyhn HJ (1995c) Calorespirometry of developing embryos and yolk-sac larvae of turbot (*Scophthalmus maximus*). Mar Biol 122: 157–163

Forstner H, Gnaiger E (1983) Calculation of equilibrium oxygen concentration. In: Gnaiger E, Forstner H (eds) Polarographic oxygen sensors. Springer-Verlag, Berlin, Heidelberg, New York, pp 321–333

Fraser AJ, Gamble JC, Sargent JR (1988) Changes in lipid content, lipid class composition of developing eggs and unfed larvae of cod (*Gadus morhua* L.). Mar Biol 99: (3) 307–314

Fyhn HJ (1989) First feeding of marine fish larvae: are free amino acids the source of energy? Aquaculture, Amsterdam 80: 111–120

Fyhn HJ (1990) Energy production in marine fish larvae with emphasis on free amino acids as a potential fuel. In: Mellinger J (ed) Animal nutrition and transport processes. 1. Nutrition in wild and domestic animals. Karger, Basel, pp 176–192

Fyhn HJ (1993) Multiple functions of free amino acids during embryogenesis in marine fishes. In: Walther BT, Fyhn HJ (eds) Physiological and biochemical aspects of fish development. University of Bergen, Bergen, Norway, pp 299–308

Fyhn HJ, Govoni JJ (1995) Endogenous nutrient mobilization during egg and larval development in two marine fishes, Atlantic menhaden and spot. In: Pittman KA, Batty RS, Verreth J (eds) The early life history of fish. ICES mar Sci Symp (in press)

Fyhn HJ, Serigstad B (1987) Free amino acids as energy substrate in developing eggs and larvae of the cod (*Gadus morhua*). Mar Biol 96: 335–341

Gnaiger E (1983) The twin-flow microrespirometer and simultaneous calorimetry. In: Gnaiger E, Forstner H (eds) Polarographic oxygen sensors. Springer-Verlag, Berlin, Heidelberg, New York, pp 134–166

Gnaiger E, Kemp RB (1990) Anerobic metabolism in aerobic mammalian cells: information from the ratio of calorimetric heat flux and respirometric oxygen flux. Biochim biophys Acta 1016: 328–332

Grasdalen H, Jørgensen L (1985) ^{31}P-NMR studies on developing eggs and larvae of plaice. Comp Biochem Physiol 81B: (2) 291–294

Grasdalen H, Jørgensen L (1987) In vivo NMR-studies of fish eggs. Monitoring of metabolite levels, intracellular pH, and the freezing and permeability of water in developing eggs of plaice, *Pleuronectes platessa* L. Sarsia 72: 359–361

Green EJ, Carritt DE (1967) New tables for oxygen saturation of seawater. J mar Res 25: 140–147

Hishida TO, Nakano E (1954) Respiratory metabolism during fish development. Embryologia 2: (8) 67–79

Holliday FGT, Blaxter JHS, Lasker R (1964) Oxygen uptake of developing eggs and larvae of the herring (*Clupea harengus*). J mar biol Ass UK 44: 711–723

Jørgensen L (1985) Carbon and nitrogen utilization in developing eggs and larvae of cod (*Gadus morhua* L.) and variation among different parents. Fish Res 3: 337–342

Jørgensen L, Grasdalen H (1990) Phosphate metabolites in larvae of halibut (*Hippoglossus hippoglossus*) studied by in vivo NMR. Program and abstracts of "Development and aquaculture of marine larvae". 12–15 August 1990. University of Bergen, Bergen, Norway

Kjesbu OS (1988) Aspects of the reproduction in cod (*Gadus morhua* L.). Dr. scient. thesis. University of Bergen, Bergen, Norway

Kjesbu OS (1989) The spawning activity of cod, *Gadus morhua* L. J Fish Biol 34: 195–206

Kuftina ND, Novikov GG (1986) Embryonic growth and utilisation of yolk storage proteins during early ontogeny of cod, *Gadus morhua*, at different temperatures. J Ichthyol (USSR) 26: 75–88

Lide DR (1992) Handbook of chemistry and physics. 73rd edn. CRC Press, Boca Raton, Florida

Livingstone DR (1983) Invertebrate and vertebrate pathways of anaerobic metabolism: evolutionary considerations. J geol Soc Lond 140: 27–37

Lowry OH, Passoneau JV (1972) A flexible system of enzymic analysis. Academic Press, London, New York

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J biol Chem 193: 265–275

Milman LS, Yurovitzky YuG (1967) The control of glycolysis in early embryogenesis. Biochim biophys Acta 148: 362–371

Milman MS, Yurovitzky YuG (1973) Regulation of glycolysis in the early development of fish embryos. In: Wolsky A et al. (eds), Monographs in developmental biology. Vol. 6. Karger, Basel

Moore S, Stein WH (1948) Photometric ninhydrin method for using chromatography of amino acids. J biol Chem 175: 376–388

Moroz IYe, Luzhin BP (1976) Dynamics of metabolism in the embryonic and early post-embryonic development of the carp *Cyprinus carpio*. J Ichthyol (USSR) 16: 964–970

Needham J (1931) Chemical embryology. 3 vols. Cambridge University Press, London, New York

Needham J (1942) Biochemistry and morphogenesis. Cambridge University Press, Cambridge

Neyfakh AA, Abravamova NB (1974) Biochemical embryology of fishes. In: Florkin M, Scheer BT (eds) Chemical zoology. Vol. VIII. Academic Press, London, New York, pp 261–286

Oppen-Berntsen DO, Helvik JV, Walther BT (1990) The major structural proteins of cod (*Gadus morhua*) eggshells and protein cross-linking during teleost egg hardening. Devl Biol 137: 258–265

Rainuzzo JR, Reitan KI, Jørgensen L (1992) Comparative study of the fatty acid and lipid composition of four marine fish larvae. Comp Biochem Physiol 103B: (1) 21–26

Rønnestad I, Fyhn HJ (1993) Metabolic aspects of free amino acids in developing marine fish eggs and larvae. Rev Fish Sci 1: 239–259

Rønnestad I, Fyhn HJ, Gravningen K (1992a) The importance of free amino acids to the energy metabolism of eggs and larvae of turbot (*Scophthalmus maximus*). Mar Biol 114: 517–525

Rønnestad I, Finn RN, Groot E, Fyhn HJ (1992b) Utilization of free amino acids related to energy metabolism of developing eggs and larvae of lemon sole *Microstomus kitt* reared in the laboratory. Mar Ecol Prog Ser 88: 195–205

Rønnestad I, Finn RN, Lein I, Lie Ø (1995) Compartmental changes in total lipid, lipid classes and their associated fatty acids in developing yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquaculture Nutrition 1: 119–130

Rutter WJ (1967) Protein determination in embryos. In: Wilt FH, Wessels NK (eds) Methods in developmental biology. Academic Press, New York, pp 671–684

Santos EA, Vinagre AS (1991) Carbohydrate metabolism during embryonic and larval development of *Odonthestes humensis* (De Buen 1953) (Pisces Atherinidae). J Fish Biol 39: 239–244

Serigstad B (1986) The effect of oil exposure on the oxygen uptake of eggs and larvae of the cod (*Gadus morhua* L.). In: Fyhn HJ (ed) Fish larval physiology and anatomy: basic research and effects of oil. University of Bergen, Bergen, Norway, pp 203–251

Serigstad B (1987) Oxygen uptake of developing fish eggs and larvae. Sarsia 72: 369–371

Skiftesvik AB, Huse I (1987) Behaviour studies of cod larvae, *Gadus morhua* L. Sarsia 72: 367–368

Sokal RR, Rohlf FJ (1981) Biometry: the principles and practices of statistics in biological research. 2nd edn. WH Freeman & Co., New York

Solberg T, Tilseth S (1984) Growth, energy consumption and prey density requirements in first feeding larvae of cod (*Gadus morhua* L.). In: Dahl E, Danielssen DS, Moksness E, Solemdal P (eds) The propagation of cod *Gadus morhua* L. Institute of Marine Research, Flødevigen Biological Station, Arendal, Norway, pp 145–166

Solberg TS, Tilseth S (1987) Variations in growth patterns among yolk-sac larvae of cod (*Gadus morhua* L.) due to differences in rearing temperature and light regime. Sarsia 72: 347–349

Solemdal P, Dahl E, Danielssen DS, Moksness E (1984) The cod hatchery in Flødevigen – background and realities. In: Dahl E, Danielssen DS, Moksness E, Solemdal P (eds) The propagation of cod *Gadus morhua* L. Institute of Marine Research, Flødevigen Biological Station, Arendal, Norway, pp 17–45

Stryer L (1988) Biochemistry. 3rd edn. WH Freeman and Company, New York

Terner C (1968) Studies of metabolism in embryonic development III. Glycogenolysis and gluconeogenesis in trout embryos. Comp Biochem Physiol 25: 989–1003

Thorsen A, Fyhn HJ (1991) Osmotic effectors during preovulatory swelling of marine fish eggs. In: Scott AP, Sumpter JP, Kime DE, Rolfe MS (eds) Proceedings on the 4th International Symposium on the Reproduction of Fish. University of East Anglia, Norwich, UK, pp 312–314

Tsuyuki H (1974) Muscle proteins of fishes. In: Florkin M, Scheer BT (eds) Chemical zoology. Vol. VIII. Academic Press, London, New York, pp 287–305

Ulvund KA, Grahl-Nielson O (1988) Fatty acid composition in eggs of Atlantic cod (*Gadus morhua*). Can J Fish aquat Sciences 45: 898–901

Van Waarde A (1983) Aerobic and anaerobic ammonia production by fish. Comp Biochem Physiol 74B: 675–684

Vetter RD, Hodson RE, Arnold C (1983) Energy metabolism in a rapidly developing marine fish egg, the red drum (*Sciaenops ocellata*). Can J Fish aquat Sciences 40: 627–634

Vigrestad A (1993) Effect of oil exposure on the metabolic rate of embryonic Atlantic cod (*Gadus morhua* L.) at different temperatures. Cand. scient. thesis, University of Bergen, Bergen, Norway

Walton MJ (1985) Aspects of amino acid metabolism in teleost fish. In: Cowey CB, Macie AM, Bell JG (eds) Nutrition and feeding in fish. Academic Press, London, pp 47–67

Wilson RP (1989) Amino acids and proteins. In: Halver JE (ed) Fish Nutrition. 2nd edn. Academic Press, London, New York, pp 111–151

Young GE, Inman WR (1938) The protein of the casing of salmon eggs. J Biol Chem 124: 189–193