Journal of Fish Biology (2002) **60,** 1511–1528
doi:10.1006/jfbi.2002.2004, available online at http://www.idealibrary.com on IDEAL®

Energy density of marine pelagic fish eggs

J. RIIS-VESTERGAARD

Danish Institute for Fisheries Research, Department of Marine Ecology and Aquaculture, North Sea Centre, P.O. Box 101, DK-9850 Hirtshals, Denmark

(*Received 16 August 2001, Accepted 8 May 2002*)

Analysis of the literature on pelagic fish eggs enabled generalizations to be made of their energy densities, because the property of being buoyant in sea water appears to constrain the proximate composition of the eggs and thus to minimize interspecific variation. An energy density of 1·34 J μl^{-1} of total egg volume is derived for most species spawning eggs without visible oil globules. The energy density of eggs with oil globules is predicted by $\hat{\sigma} = 1{\cdot}34 + 40{\cdot}61\ x$ (J μl^{-1}) where x is the fractional volume of the oil globule.

Key words: fish eggs; pelagic; energy density; buoyancy; proximate composition; free amino acids.

INTRODUCTION

The cost of reproduction is an important issue in bioenergetics modelling of fishes, but data on the amount of energy spawned as eggs are unavailable for most species. The energy density is closely linked to the proximate composition, and research during the last three decades has revealed that neutral or positive buoyancy of fish eggs in sea water is caused by a special proximate composition. This composition has, as hypothesized by Fyhn *et al.* (1999), been evolutionary conserved because buoyancy of the eggs was an early key step when freshwater ancestors of extant teleosts conquered the oceans.

The proximate composition of pelagic fish eggs seems to be constrained due to the fact that the specific gravity of the major organic constituents of pelagic eggs, free amino acids (FAA) and amino acids polymerized into protein (PAA), is higher than the specific gravity of sea water. Pelagic teleost eggs without oil globules of low specific gravity are rendered neutrally buoyant in sea water of 32–34‰ salinity by the presence of large amounts of water (Craik & Harvey, 1984, 1987), the only quantitatively important egg substance apart from lipids having a specific gravity lower than sea water. The water reservoir is formed during final oocyte maturation by a mechanism called swelling or hydration in which an extensive hydrolysis of ovoplasmic protein into FAA (Thorsen & Fyhn, 1991, 1996; Thorsen *et al.*, 1993) creates an osmotic gradient driving the uptake of water (Østby *et al.*, 2000), thus minimizing the amount of inorganic osmolytes that otherwise would be necessary for this process. The resulting osmolarity of the ovulated oocyte is 285–350 mOsm, around three times lower than the osmolarity of oceanic sea water. FAA make up more than half of the osmolytes

Tel.: +45 33 96 32 45; fax: +45 33 96 32 60; email: jrv@dfu.min.dk

0022–1112/02/061511+18 $35.00/0

with K^+, Cl^-, Na^+ and NH_4^+ contributing most of the remaining osmolarity (Riis-Vestergaard, 1982, 1987; Mangor-Jensen, 1986). Thus, it is to be expected that the ratio of FAA to total amino acids (TAA, TAA=FAA+PAA) shows little interspecific variation in eggs without hydrostatic lift from oil globules, when the specific gravities are similar. This lends support to the assumption that the energy density is similar for species spawning pelagic eggs without oil globules.

More than half of the pelagic fish egg species contain oil globules (Russell, 1976; Ahlstrom & Moser, 1980) contributing to buoyancy. Data from the literature support the assumption that there is no general difference in buoyancy between pelagic eggs with and without oil globules, because eggs with oil globules undergo less protein hydrolysis during maturation. In this case, the effect of oil globules on both the FAA : TAA ratio and the ovoplasmic energy density can be predicted.

The purpose of the present work was to analyse data from the literature on the proximate composition of pelagic fish eggs in order to derive generalized expressions for their energy density. Information about the size of pelagic fish eggs of most species can be found in the literature (Russell, 1976; Ahlstrom & Moser, 1980), and data on fecundity exist at least for many commercially important species. Combining this with the energy density will enable estimation of the energy spawned as eggs.

MATERIALS AND METHODS

THE MARINE PELAGIC FISH EGG

The ripe, ovulated but unspawned egg consists of an egg cell surrounded by a soft proteinaceous envelope commonly called the chorion. For the purpose of energy calculations, the egg cell will be considered one homogenous compartment of ovoplasm. Visible oil globules are in the present context considered separate constituents, not being part of the ovoplasm. To avoid confusion, the term ' oil ' will be restricted to the content of visible oil globules, whereas the term ' lipid ' will be applied to lipoid substances present in the ovoplasm.

Contact with sea water at spawning triggers several processes, commonly termed activation. The chorion becomes hardened due to cross-linking of protein molecules (Young & Inman, 1938; Oppen-Berntsen *et al.*, 1990) rendering the chorion tough and resistant to tensile stress. A perivitelline space (PVS) is formed between the chorion and egg cell (Fig. 1) by a small amount of colloid molecules liberated from the egg cell, creating an osmotic turgor that distends the chorion. The fluid in the PVS is, apart from the colloids, identical to the ambient medium (normally sea water), because the chorion is highly permeable to solutes of at least the size of monosaccharides (Riis-Vestergaard, 1982, 1984, 1987; Hølleland & Fyhn, 1986; Mangor-Jensen, 1987). A protrusion is formed at the animal pole of the egg cell. In eggs successfully fertilized, cell divisions become visible in the protrusion thus initiating formation of the embryo, but chorion hardening and the protrusion occur also in unfertilized eggs of many fish species. The protrusion, later the embryo, faces downwards in eggs floating freely in sea water. Both the chorion and the activated egg cell of most species are of almost perfect spherical shape when viewed from above, but the egg cell is actually of a more irregular shape due to the protrusion (Fig. 1).

MAIN PRINCIPLES FOR GENERALIZATION OF THE ENERGY DENSITY

Thorsen *et al.* (1996) introduced a buoyancy model for eggs of cod *Gadus morhua* L. in which the buoyancy was calculated as the sum of positive and negative contributions

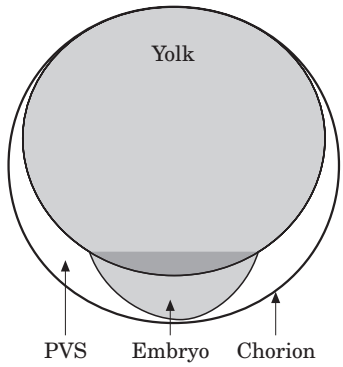

FIG. 1. Physical compartments of the fertilized, fully activated pelagic fish egg. PVS, Perivitelline space.

from four egg components: chorion, PVS, lipid and OML (ovoplasm minus lipid). In the present work, the model was expanded to include the effect of oil globules and the effect of less hydration due to less protein hydrolysis. The contribution to buoyancy from PVS was always set to zero, assuming the influence of the colloids to be insignificant (Thorsen *et al.*, 1996). Contributions from the other components were calculated by their mass, their specific gravity and the specific gravity of the ambient medium. The specific gravity of sea water was calculated from salinity and temperature according to UNESCO (1981). The specific gravity of chorion and of protein in the ovoplasm was set to 1·3 mg μl^{-1} (Mahler & Cordes, 1966). A specific gravity of 0·9 mg μl^{-1} was accepted as an approximation for the specific gravity of lipid and oil in teleost eggs, as discussed by Craik & Harvey (1987).

The energy content of fish eggs was calculated from the proximate composition of the eggs and the energy densities of the constituent classes, primarily FAA, PAA and lipid plus oil. Commonly used energy densities *c.* 39 J mg^{-1} for lipid and oil (Finn *et al.*, 1991) seem derived from mammalian fat containing mainly saturated and mono-unsaturated fatty acids. Lipids of marine origin contain high concentrations of poly-unsaturated fatty acids influencing the energy density as discussed by Finn *et al.* (1995*a*) who applied a value of 35·6 J mg^{-1} measured on extracted fish oil (Beamish *et al.*, 1975). This value was adopted in the present paper. Protein energy densities *c.* 24 J mg^{-1} are also commonly used in fish bioenergetics. Finn *et al.* (1995*a*) calculated energy densities of 20·8 and 18·2 J mg^{-1} of FAA and PAA, based on the molecular composition of newly fertilized cod eggs. These values were used here, as the relative composition of nitrogenous compounds shows little interspecific variation between species producing pelagic eggs (Thorsen *et al.*, 1993; Fyhn *et al.*, 1999). Data from the literature specifying content of FAA in units of mmol were converted to mass by the average molecular mass 123·6 g mol^{-1} of FAA in cod eggs (Finn *et al.*, 1995*b*). A similar value of 126·3 g mol^{-1} was derived for eggs of lemon sole *Microstomus kitt* (Walbaum) by Rønnestad *et al.* (1992*a*).

Generalizations concerning the energy density in the present paper were based on two assumptions. One was that the optimum specific gravity of pelagic fish eggs ensures but also minimizes positive buoyancy in sea water, so that there is no general difference between the specific gravity of eggs with and without oil globule. The other assumption was that the presence of oil globules does not influence the concentration of lipid in the ovoplasm. Based on these two assumptions, a relation between the fractional volume of the oil globule and the ovoplasmic composition, and thereby the overall energy density, was derived (Appendix) by an expansion of the buoyancy model.

PROBLEMS DUE TO DIFFERENT EXPERIMENTAL PROTOCOLS

Some differences in sampling procedures as well as difficulties with protein quantification must be considered when comparing results from various sources in the literature.

TABLE I. Cod egg compartments

	Whole egg	Chorion	PVS	Ovoplasm	OML
M_w, unwashed egg (mg)	**1·651**[*]	**0·019**	0·331[*]	1·302	1·287
M_w, washed egg (mg)	1·643	0·019	0·322	1·302	1·287
M_d, unwashed egg (mg)	**0·116**[*]	**0·019**	0·011[*]	0·086	0·071
M_d, washed egg (mg)	0·105	0·019	0·000	0·086	0·071
Ash, unwashed egg (mg)	**0·0148**[*]	—	0·0114[*]	—	—
Ash, washed egg (mg)	0·0034	—	0·0000	—	—
Ash free M_d (mg)	0·1015	—	0·0000	—	—
H_2O % of M_w, unwashed egg	93·0[*]	0·0	96·6[*]	93·4	94·5
H_2O % of M_w, washed egg	93·6	0·0	100·0	93·4	94·5
Volume, %	100·0	0·90	20·0[1]	79·1	78·1
Volume, µl	1·610	0·02	0·322	1·273	1·257
Specific gravity, mg µl^{-1}	1·026[†,2]	1·30[3]	1·026[†]	1·022	1·024

Bold figures are original data cited from Finn *et al.* (1995*a*,*b*); other figures are calculated by the present author except [1]Mangor–Jensen (1987); [2]Thorsen *et al.* (1996); [3]Mahler & Cordes (1966). [*]Incubated in 34·5‰. [†]In sea water of neutral buoyancy 33‰. M_w wet mass. M_d dry mass.

Activated eggs (and all developmental stages until the chorion starts softening prior to hatching) may be sampled free of adhering medium by placing them singly on filter paper (Riis-Vestergaard, 1982, 1984) or by rolling them on filter paper (Finn *et al.*, 1995*b*). Without precautions, some medium will adhere to the eggs influencing data on the proximate composition. As an example, Flüchter & Pandian (1968) found that ash made up 20% of the dry mass of sole *Solea solea* (L.) eggs sampled without precautions, but only 1·16% after washing the eggs with distilled water.

Inorganic solutes in the PVS contribute substantially to the dry mass of activated pelagic eggs. In the case of cod eggs, *c.* 10% of the dry mass and 75% of the ash mass come from the PVS (Table I). The solutes may be removed by washing the eggs for 3–5 min with distilled water (Riis-Vestergaard, 1982; Hølleland & Fyhn, 1986; Mangor-Jensen, 1987; Thorsen *et al.*, 1996). To facilitate comparisons between mass-related results on washed and unwashed eggs, the decrease in wet mass due to washing can be calculated as the product of perivitelline volume and the difference in specific gravity between distilled water and incubation medium, whereas the decrease in dry mass is the product of perivitelline volume and the salinity of the incubation medium. Methods for direct measurement of the perivitelline volume are available (Riis-Vestergaard, 1982, 1984; Mangor-Jensen, 1987) but are seldom used. It is common practice to measure diameters of the chorion and the egg cell and calculate volumes by the formula for a sphere. Estimation of the perivitelline volume as the difference will be of low accuracy and is omitted in the present context because the shape of the egg cell is more irregular at all stages from formation of the protrusion (Fig. 1) and onwards.

It would be desirable for the purpose of the present paper to compare ovoplasmic energy densities calculated from the concentration of organic constituents, but this also demands accurate data about ovoplasmic volumes, which are not available. Generalizations of energy densities are therefore closely linked to the assumption of invariant specific gravity, which enables the application of indirect methods.

Ovulated unactivated eggs are sampled by dissecting or stripping ripe females. The eggs are accompanied by ovarian fluid, which is difficult to remove completely. The fragile unactivated eggs tend to burst if placed on filter paper, and application of suction to samples of unactivated eggs does not remove the ovarian fluid completely (Craik & Harvey, 1987). The PVS is very small in unactivated eggs and the concentration of dry matter in the perivitelline fluid as well as in the ovarian fluid is expected to be low, so

ovarian fluid contaminating samples of unactivated eggs will influence observed dry mass less than observed wet mass. For this reason, data expressed as % dry mass of unactivated eggs and as % dry mass of washed activated eggs should be preferred when eggs from both states are to be compared.

Quantification of fish egg protein is usually performed by the method of Lowry *et al.* (1951). It is essential for the method that the proteins are soluble in NaOH. Chorion protein proved at least partly soluble in most investigations (Finn *et al.*, 1991; Rønnestad *et al.*, 1992*a*, *b*, 1994, 1998), but not always (Finn *et al.*, 1995*b*), and there may even be intraspecific differences (Fyhn & Serigstad, 1987; Finn *et al.*, 1995*b*; Fyhn & Govoni, 1995). Without verification, quantification of protein in fish eggs by the Lowry method is dubious (Thorsen & Fyhn, 1996; Thorsen *et al.*, 1996).

The Lowry method revealed a protein loss of 0·015 mg associated with shedding of the chorion from hatching cod eggs (Fyhn & Serigstad, 1987); this amounted to 83% of the dry mass of isolated cod chorions reported by Thorsen *et al.* (1996). Finn *et al.* (1991) exposed isolated chorions of halibut *Hippoglossus hippoglossus* (L.) eggs to the Lowry method and recovered 84% of the dry matter as protein. The rest was not chemically identified; protein insoluble in NaOH remains a possibility together with carbohydrates present as glycoprotein, which is known to be part of the chorion (Oppen-Berntsen *et al.*, 1990). Considering that the energy density of carbohydrate deviates little from protein, the energy content of the chorion was approximated by applying the energy density of protein to the whole dry matter of the chorion in the present paper. This approach is justified by Yúfera *et al.* (1999) who measured <1% dry mass of ash in isolated chorions from eggs of Senegal sole *Solea senegalensis* Kaup, and by Finn *et al.* (1991), who measured the energy content of isolated halibut chorions by a bomb calorimeter and found an average energy density of 18·2 J mg^{-1} dry mass, identical to the energy density of fish egg protein calculated by Finn *et al.* (1995*a*) as mentioned above.

RESULTS

PELAGIC EGGS WITHOUT VISIBLE OIL GLOBULES

A comprehensive analysis of the proximate composition of cod eggs incubated 0·8 days after fertilization and sampled without washing (Finn *et al.*, 1995*a*, *b*) was utilized to calculate data given in Tables I and II. The egg volume in Table I was calculated from the wet mass, assuming that the specific gravity of this batch of eggs was equal to sea water of 33‰ salinity, the mean value for marine cod eggs (Thorsen *et al.*, 1996). The volume calculated from the mean diameter published by Finn *et al.* (1995*b*) lead to a calculated specific gravity equivalent to sea water of 44‰ salinity, whereas the eggs were, in reality, positively buoyant in 34·5‰. Chorion protein from this batch of cod eggs could not be quantified by the Lowry method (Finn *et al.*, 1995*b*). A dry mass decrease of 0·023 mg egg^{-1} observed at hatching was ascribed solely to the loss of chorion and proteinaceous colloids in the PVS. The authors did not account for the fact that the PVS makes up *c.* 20% of the egg volume prior to hatching (Mangor-Jensen, 1987) contributing *c.* 0·011 mg of inorganic ions to the dry mass of eggs sampled without washing with distilled water. The authors assigned 84%=0·019 mg of the observed dry mass loss to chorion protein in parallel to results from isolated chorions of halibut eggs (Finn *et al.*, 1991). Other literature sources (Solberg & Tilseth, 1984; Thorsen *et al.*, 1996) reported dry masses of 0·018–0·020 mg for isolated cod chorions. In the present paper, the energy density of protein is applied to the whole dry mass of chorions as argued in the material and methods section. Therefore, the (protein) dry mass and energy contribution actually

TABLE II. Chemical composition of cod eggs

	FAA	PAA ovoplasm	PAA chorion	Lipid	Carbohydrate	Ash	SUM	Observ.
Energy density, J mg^{-1}	**20·80**	**18·22**	18·22	35·56[1]	**15·61**	0·00	—	—
Specific gravity, mg μl^{-1}	—	1·30[2]	1·30[2]	0·90[3]	—	—	—	—
Mass, mg egg^{-1}	0·0317	0·0348	0·0192	0·0150	0·0007	—	0·1162	**0·1163**
Mass, % of unwashed M_d	**27·3**	**29·9**	**16·5**	**12·9**	**0·6**	**12·9**	100·1	100
Mass, % of washed M_d	30·3	33·2	18·3	14·3	0·7	3·2	—	—
Energy, J egg^{-1}	**0·654**	**0·627**	**0·346**	**0·528**	**0·011**	0·000	2·166	**2·213**
Energy, % of observed	29·6	28·3	15·6	23·9	0·5	0·0	97·9	100
Energy density, J μl^{-1} egg	0·406	0·389	0·215	0·328	0·007	0·000	1·345	—
Energy density, J μl^{-1} ovoplasm	0·514	0·492	—	0·415	0·009	0·000	1·429	—
Energy density, J μl^{-1} OML	0·520	0·499	—	—	0·009	0·000	1·028	—
Energy density, J mg^{-1} unwashed M_d	5·62	5·39	2·98	4·54	0·09	0·00	18·62	—
Energy density, J mg^{-1} washed M_d	6·23	5·98	3·30	5·03	0·10	0·00	20·65	—
Energy density, J mg^{-1} ash free M_d	6·44	6·18	3·41	5·20	0·11	0·00	21·34	—

Bold figures are original data cited from Finn *et al.* (1995*a*,*b*); other figures are calculated by the present author except [1]Beamish *et al.* (1975); [2]Mahler & Cordes (1966); [3]Craik & Harvey (1987). M_w wet mass; M_d dry mass.

assigned to the chorion by Finn *et al.* (1995*a*, *b*) were retained uncorrected in Table I, together with the 29·9% dry mass of the ' Lowry ' protein attributed to the ovoplasm.

Masses of the PVS were calculated from a perivitelline volume of 20% in fully activated cod eggs (Mangor-Jensen, 1987) with a composition identical to the incubation medium. Masses of ovoplasm were obtained as the mass of the egg minus chorion and PVS. The incubation medium is continuous with the perivitelline fluid through the pores of the chorion which cannot be ascribed a definite water content. Therefore, the chorion enters the calculations only as dry mass. Calculation of the expected increase in water content due to washing with distilled water (Table I) affected the whole egg and the PVS but not the ovoplasm. As a result of the compartmental analysis of cod eggs in Table I, the specific gravity of the egg cell (i.e. the specific gravity of the ovoplasm) was calculated to be 1·022 mg μl^{-1}, slightly lower than sea water of neutral buoyancy for the whole egg.

An approximation of the relative water content before activation was made by assuming that formation of the PVS increases the preactivated volume by 25%, corresponding to 20% postactivated volume. This means that the relative content of dry matter, which is (100–93·6)=6·4% in activated washed cod eggs, is 25% higher=8·0% dry matter before activation, leaving 92% as water. The calculated difference in relative water content due to activation may be small but is nevertheless recognized in Table III, where three published values of the water content of unactivated cod eggs were *c.* 91% as opposed to the two values of 92·7 and 92·9% water respectively in activated eggs.

The energy contributed by FAA, PAA and lipid yields more than 97% of the measured egg energy (Table II); the contribution from carbohydrate is negligible. Assuming all lipid in cod eggs to be located in the ovoplasm, the volume of OML was calculated to be 1·26 µl, the specific gravity was 1·024 mg μl^{-1} and the energy density 1·03 J μl^{-1} [Tables I and II, Appendix (9)].

A compilation of the composition of pelagic eggs of several fish species is given in Table III. The sampling methodology influences results related to volume and mass, but not the FAA : TAA ratio. Chorion proteins were included in the calculated FAA : TAA ratios for all species except plaice *Pleuronectes platessa* L., where the status is unknown. FAA (the determinant of water uptake during oocyte maturation) comprised 30 to 40% of the total mass of amino acids in eggs without oil globules from three species, cod, lemon sole and Pacific halibut *Hippoglossus stenolepis* Schmidt. The FAA : TAA ratio was lower in eggs of plaice and Atlantic halibut, showing that FAA contribute less to hydration and buoyancy. The properties of Atlantic halibut eggs are untypical in several respects, as judged from the available evidence. In nature, Atlantic halibut eggs seem to be bathypelagic distributed, corresponding to neutral buoyancy at the lower end of the pycnocline, where the salinity is >34‰ (Haug *et al.*, 1984, 1986). Eggs stripped from wild or domesticated females and developing normally after fertilization are often negatively buoyant in sea water of 34‰ salinity (Riis-Vestergaard, 1982; Mangor-Jensen & Waiwood, 1995). It was calculated from data in Riis-Vestergaard (1982) that 95% of Atlantic halibut egg volume was yolk, as opposed to 80% in cod eggs, and that the concentration of the inorganic osmolytes Na^+, K^+ and Cl^- made up only 130 mmol l^{-1} of ovoplasmic water,

TABLE III. Composition of pelagic fish eggs

Species	Oil globule % egg volume	Lipid + Oil % dry mass	FAA/TAA %	H_2O % wet mass	Sampling method*
Gadus morhua L.[1]	0	12·9	37·4	92·9	c
Gadus morhua L.[2,3]	0	12·2	38·7	92·1	a
Gadus morhua L.[4]	0	—	38·4	92·7	b
Gadus morhua L.[5]	0	10–12	—	90–91	a
Merlangius merlangus (L.)[3]	0	12·5	—	92·2	a
Pollachius virens (L.)[3]	0	14·6	—	91·9	a
Melanogrammus aeglefinus (L.)[3]	0	13·1	—	93·0	a
Microstomus kitt (Walbaum)[6]	0	—	31·0	—	?
Microstomus kitt (Walbaum)[7]	0	—	34·6	—	?
Limanda limanda (L.)[3]	0	10·5	—	92·2	a
Platichthys flesus (L.)[5]	0	10–11	—	89–91	a
Pseudopleuronectes americanus (Walbaum)[8]	0	14·3	—	—	b
Hippoglossoides platessoides (Fabricius)[3]	0	10·5	—	86·4	a
Hippoglossoides platessoides (Fabricius)[5]	0	11–13	—	90–91	a
Hippoglossus stenolepis Schmidt[10]	0	15·8	39·0	91·2	c
Hippoglossus hippoglossus (L.)[11]	0	—	23·5	90·1	c
Hippoglossus hippoglossus (L.)[3]	0	17·2	—	90·4	c
Hippoglossus hippoglossus (L.)[12]	0	—	—	89·8	c
Hippoglossus hippoglossus (L.)[5]	0	11–15	—	87–89	a
Hippoglossus hippoglossus (L.)[13]	0	12	—	—	a
Pleuronectes platessa L.[3,6]	0	15·3	25·2	91·8	a
Pleuronectes platessa L.[3]	0	10·7	—	—	a
Pleuronectes platessa L.[5]	0	8–9	—	88–89	a
Glyptocephalus cynoglossus (L.)[3]	0	9·8	—	88·5	a
Scophthalmus maximus (L.)[14]	0·78	—	21·4	—	c
Scophthalmus maximus (L.)[15]	0·74	—		90·7	c
Scophthalmus maximus (L.)[15]	0·69	—	—	90·4	c
Scophthalmus maximus (L.)[3]	—	16	—	92·8	a
Solea solea (L.)[3]	?†	14·1	—	90·8	c
Solea senegalensis Kaup[9]	?†	11·7	—	91·5	b
Solea senegalensis Kaup[16,17]	?†	16·3	21·1	90·8	b
Sparus auratus L.[18]	1·04	14·6	22·4	—	c
Leiostomus xanthurus Lacepède[19]	2·14¶	—	20·4	—	?
Molva molva (L.)[3]	2·2§	26·6	—	88·6	c
Dicentrarchus labrax (L.)[20]	3·74	28‖	21·6	—	c
Coryphaenoides rupestris Gunner[21,3]	6·1	34·9	—	81·4	a

*Sampling method: a unactivated eggs; b activated washed eggs; c activated unwashed eggs; ? unknown. †Multiple small oil globules. ¶% of yolk volume. §calculated from data in Russell (1976). ‖Estimated from size of oil globule. References: [1]Finn *et al.* (1995*b*); [2]Fyhn & Serigstad (1987); [3]Craik & Harvey (1987); [4]Thorsen *et al.* (1996); [5]Lønning *et al.* (1988); [6]Thorsen & Fyhn (1996); [7]Rønnestad *et al.* (1992*a*); [8]Cetta & Capuzzo (1982); [9]Vazquez *et al.* (1994); [10]Whyte *et al.* (1993); [11]Finn *et al.* (1991); [12]Riis-Vestergaard (1982); [13]Falk-Petersen *et al.* (1986); [14]Rønnestad *et al.* (1992*b*); [15]Finn *et al.* (1995*c*); [16]Mourente & Vázquez (1996); [17]Yúfera *et al.* (1999); [18]Rønnestad *et al.* (1994); [19]Fyhn & Govoni (1995); [20]Rønnestad *et al.* (1998); [21]Grigor'ev & Serebryakov (1981).

calling for high concentrations of FAA and NH_4^+ to yield the observed osmolarity of 400 mOsm. Contrary to this, FAA and NH_4^+ in the Atlantic halibut eggs studied by Finn *et al.* (1991) contributed only 175 mmol l^{-1}. It may

be noted that this FAA concentration was almost identical to the result by Østby *et al.* (2000) and (as % dry mass) identical to the result by Evans *et al.* (1996). Taken together, these pieces of evidence indicate that the specific gravity of Atlantic halibut eggs is higher than in most pelagic eggs, and that unknown osmolytes contribute substantially to hydration of Atlantic halibut eggs. The egg batch in Finn *et al.* (1991), which originated from a domesticated female maintained in 30‰ salinity, was neutrally buoyant in 33‰ salinity. The likely explanation arises from the fact that the combined mass of chorion, FAA and ovoplasmic PAA makes up only 78% of the washed dry mass estimated for this batch of eggs. Carbohydrate and ash are expected to contribute 4% of washed dry mass (Table II). The balance of 18% dry mass is assumed to be lipid in eggs from the domesticated female, which will explain both the lower specific gravity, the relatively low water content, and the observed high energy density of 2·4–2·5 J μl^{-1} of egg volume. The assumed lipid content is not contradicted by the rather differing data on the lipid content of Atlantic halibut eggs (Table III), which indicate that yolk of Atlantic halibut eggs may tend to have a higher lipid concentration and a lower relative water content than most other pelagic eggs.

It was assumed for the present purpose that the FAA : TAA ratios calculated for cod, lemon sole and Pacific halibut represent typical values for pelagic eggs without oil globules, indicating also the typical relation of 'light' H_2O to 'heavy' TAA as well as the typical energy density of OML.

The lipid contents varied from 10 to 15% dry mass in eggs without visible oil globules, except one of the results for Atlantic halibut eggs. Unactivated eggs gave rise to the majority of the results in Table III, for which reason the lipid content calculated as % dry mass of washed cod eggs (Table II) is the better choice for comparison. This shows that the lipid content of the batch of eggs in Table II is close to the upper end of the range.

Assuming that the energy density of OML is independent of the lipid content within the range of 10–15% dry mass, the energy density of the egg batch in Table II will vary from 1·25 J μl^{-1} of total egg volume at 10% lipid to 1·36 at 15%. The expected variation in energy density due to variation in lipid content of pelagic eggs without visible oil globules is therefore <10%. The value calculated for the midpoint of the interval, 12·5% lipid, is 1·31 J μl^{-1}. This is assumed to represent 97·9% of the true energy density by analogy with the analysis in Table II. It is, therefore, recommended to apply an energy density of 1·34 J μl^{-1} of total egg volume for calculation of the energy content of pelagic eggs without visible oil globules.

PELAGIC EGGS WITH VISIBLE OIL GLOBULES

The majority of pelagic egg species described by Russell (1976) contain oil globules. The data enabled estimation of fractional volumes of oil globules for 28 species, using the mean of the volumes calculated from the minimum and maximum diameters specified. Frequency analysis (Fig. 2) showed that fractional volumes between 0·25 and 1% of the egg volume are the more common ones.

Eight species with visible oil globules are included in Table III. The data available for five of the species enabled calculation of the FAA : TAA ratio including the contribution from the chorion. The ratios are lower than for

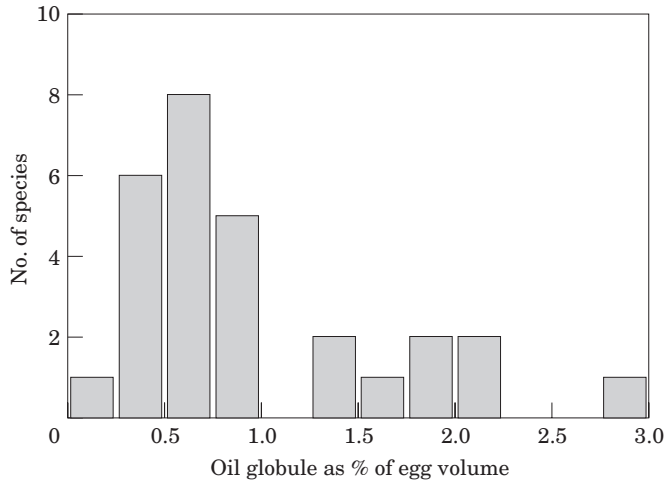

FIG. 2. Frequency analysis of the relative size of oil globules in pelagic fish eggs. For each species, the volumes of oil globule and egg were approximated as the mean of volumes calculated from minimum and maximum diameters in Russell (1976).

species without oil globules, indicating that hydration due to protein hydrolysis contributes less to buoyancy. The assumption that the contribution to buoyancy from the oil globule is fully counteracted by less protein hydrolysis lead to modelling energy density as a function of the relative size of the oil globule. The model (Appendix) was derived in a situation where both volume and mass, and thus specific gravity, were kept constant while varying the FAA : TAA ratio according to the size of the oil globule. The main result is that the average energy density σ of the whole egg is a linear function of x, the fractional volume of the oil globule: $\sigma = 1{\cdot}34 + 40{\cdot}6\ x$ (J μl^{-1}). The intercept is the recommended energy density for eggs without oil globules, and the slope coefficient is a composite result of fractional volumes, specific gravities, and energy densities (Tables I and II).

Essential features of the model will be exemplified by applying cod egg data (Tables I and II) to eggs of ling *Molva molva* (L.), a gadoid species spawning pelagic eggs of diameter 0·97–1·13 mm with oil globules of diameter 0·28–0·31 mm (Russell, 1976). Calculated from the mean volumes, the oil globule of ling eggs makes up 2·2% of the egg volume, the second highest value calculated from data in Russell (1976) (Fig. 2). Imagine an oil globule replacing this amount of yolk in an otherwise unmodified egg with properties as in Tables I and II. The replacement decreases the wet mass and the specific gravity by 0·3% [Appendix (1)]. This change is very small but it cannot be regarded as unimportant. The imaginary egg will be neutrally buoyant in sea water of 30‰ salinity, as opposed to 33‰ for the unmodified egg. Spawned in sea water of 33–35‰ salinity, the egg will ascend substantially faster towards the surface in stagnant water and stay closer to the surface in turbulent water with an enlarged risk of incurring mechanical damage, to which fish embryos are very sensitive especially during the early stages of development (Holmefjord & Bolla, 1988). Neutral buoyancy in 33‰ salinity will be restored if a fractional volume of 0·98% OML is replaced by protein [Appendix (2)], which is the way of illustrating less

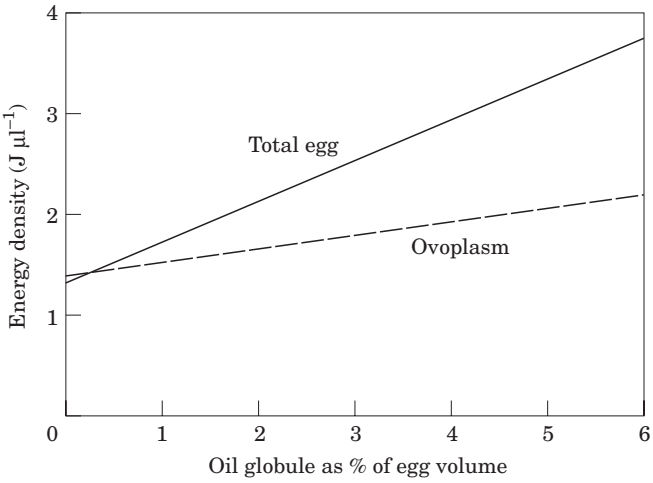

FIG. 3. Average energy density of pelagic fish eggs and ovoplasm as a function of the relative size of oil globule, calculated according to the Appendix.

protein hydrolysis without changing the volume of the egg. The combined effects of the two modifications are that the calculated energy density of the ovoplasm increases from 1·43 to 1·73 J µl^{-1} [Appendix (7)], whereas the average energy density of the whole egg is increased from 1·35 to 2·26 J µl^{-1} [Appendix (9)], primarily due to the high energy content of the oil globule. The FAA : TAA ratio decreases from 0·37 to 0·29, a displacement that matches fairly well to the general difference between eggs with and without oil globules (Table III).

The largest known fractional oil globule volume is, probably, 6% in eggs of grenadier *Coryphaenoides rupestris* Gunner (Grigor'ev & Serebryakov, 1981, 1983; Table III). The ovoplasmic energy density of eggs with oil globules up to this size, predicted according to the Appendix, is given in Fig. 3 together with the energy density of the whole egg. The ovoplasmic energy density is increased by 60% solely due to differences in the FAA : TAA ratio, while the overall energy density is increased by 186%, caused by the combined effect of the oil globule and changes in the ovoplasm.

DISCUSSION

The expressions for energy density rely heavily on the single batch of cod eggs analysed by Finn *et al.* (1995*a*, *b*). Comparisons, where possible, of the properties of this batch to those of other eggs indicated that this particular batch of eggs was actually representative for eggs without oil globules. Direct validation of the expressions requires that accurate data on energy content and volume of the egg, and volume of the oil globule if present, are available or can be calculated for individual batches of eggs. More data that those presented in the paper seem hard to find.

The assumption of low interspecific variation in the energy density of pelagic eggs without oil globules is supported by the evidence (Table III), with the possible exception of Atlantic halibut eggs. Further evidence is found in Hislop

& Bell (1987) who observed little variation in the energy density (J g^{-1} of ash free dry mass) of eggs of six gadoid species, indicating little variation in the proportion of lipid to TAA.

The close relationship between the FAA : TAA ratio, relative water content and buoyancy is demonstrated by eggs of Baltic cod, a stock genetically adapted to produce eggs having neutral buoyancy in salinities of 11–16‰ (Thorsen *et al.*, 1996). FAA made up 65% of the mass of TAA in these eggs as contrasted to 37–39% in marine cod eggs, and the relative water content was higher, showing that hydrolysis of protein and concomitant uptake of water is more extensive in eggs of the brackish water stock. Buoyancy in low salinities, however, is not obtained merely by dilution as judged from the fact that the absolute content of TAA was lower in eggs of Baltic cod than in those of marine cod. The lipid content was not measured. Similarly to cod eggs, eggs of Baltic flounder *Platichthys flesus* (L.) are expected to be less dense than their marine counterparts are (Solemdal, 1967, 1973). Because changes in proximate composition are part of the adaptation to buoyancy in low salinity but are poorly described, the principles for estimating egg energy outlined in this paper cannot be adapted to eggs having aberrant specific gravities. Neither can the principles be applied to the few species of pelagic eggs that develop large perivitelline volumes during activation, e.g. members of the genus *Hippoglossoides* (Howell & Caldwell, 1984). Doing so will ascribe the average energy density, mainly originating from the yolk, to the extra perivitelline volume which in reality contributes no energy. The obstacle may be circumvented by adding *c.* 20% (Table I) to the volume of the unactivated eggs, if known, because a large PVS is never present before activation (Ahlstrom & Moser, 1980).

The hypothesis that the influence of oil globules on buoyancy is neutralized by a lower relative water content and thus a higher energy density of the ovoplasm is supported by the data in Table III showing that the FAA : TAA ratio in eggs with an oil globule is lower than in eggs without one. It may also be noted that the average salinity of neutral buoyancy of mackerel *Scomber scombrus* L. eggs, in which the fractional oil globule volume (*c.* 2%) belongs to the top five calculated from data in Russell (1976), is 32·5–33‰ salinity (Coombs, 1981), similar to marine cod eggs (Thorsen *et al.*, 1996), and that eggs of grenadier, with a very high relative oil content, are bathypelagic like Atlantic halibut eggs (Bergstad & Gordon, 1994; Ekelseyev, 1995), and therefore buoyant only in high salinities. A consequence of the hypothesis is a substantial increase in the calculated energy density in ovoplasm of eggs containing oil globules. The true energy density may be lower than calculated in case that some of the lipid attributed to ovoplasm is actually deposited in the oil globule. Based on a review of the literature, it has been suggested that the presence of an oil globule reflects a lipid content of >15% dry mass (Finn, 1994). The volume of oil globules seems to remain constant until a late stage of embryonic development (Rønnestad *et al.*, 1992*b*, 1994, 1998; Fyhn & Govoni, 1995) indicating that the lipid necessary for formation of the increasing amount of membrane around cells and organelles is located in the ovoplasm also in this type of eggs. The content of oil globules of the size reported for eggs of turbot *Scophthalmus maximus* (L.) by Rønnestad *et al.* (1992*b*) corresponds to 8% of the estimated dry mass of turbot eggs, only half of the amount measured by Craik & Harvey (1987). These pieces

of evidence support the assumption of no relocation of lipid but do not exclude the alternative. Therefore, the energy densities predicted by the suggested formula may be interpreted as maximum values.

Less hydration in eggs with oil globules may be caused partly by a lower content of osmolytes other than FAA in the ovoplasm. K^+ and Cl^- are the major inorganic osmolytes, but data about their concentrations in fish eggs are too scarce to enable generalizations.

The expression for the energy density of eggs with oil globules was based on the assumption that the optimum specific gravity of pelagic eggs will be as high as possible without causing the eggs to sink to suboptimal depths. The imaginary cod egg equipped with an oil globule [Appendix (1)] would be neutrally buoyant in sea water of 30‰ salinity, similar to ' the extremely light fraction ' of cod eggs (Kjesbu *et al.*, 1992). These authors simulated the vertical distribution of eggs as a function of their specific gravity, wind-induced water turbulence and the salinity profile at the spawning area at Lofoten (northern Norway). The salinity increased from 33·4‰ salinity at the surface to 34·7‰ at 125 m. The ' extremely light ' eggs were distributed much closer to the surface than average eggs with neutral buoyancy in 33‰ salinity, and the very fact that their specific gravity was considered ' extreme ' indicates that natural selection has favoured eggs of higher specific gravity.

This work was supported by the EU FAIR project CT-95-0604.

References

Ahlstrom, E. H. & Moser, H. G. (1980). Characters useful in identification of pelagic marine fish eggs. *Report of California Cooperative Oceanic Fisheries Investigations* **21,** 121–131.

Beamish, F. W. H., Niimi, A. J. & Lett, P. F. K. P. (1975). Bioenergetics of teleost fishes: Environmental influences. In *Comparative Physiology—Functional Aspects of Structural Materials* (Bolis, L., Maddrell, H. P. & Schmidt-Nielsen, K., eds), pp. 187–209. Amsterdam: North-Holland Publishing Company.

Bergstad, O. A. & Gordon, J. D. M. (1994). Deep-water ichthyoplankton of the Skagerrak with special reference to *Coryphaenoides rupestris* Gunnerus, 1765 (Pisces, Macrouridae) and *Argentina silus* (Ascanius, 1775) (Pisces, Argentinidae). *Sarsia* **79,** 33–43.

Cetta, C. M. & Capuzzo, J. M. (1982). Physiological and biochemical aspects of embryonic and larval development of the winter flounder *Pseudopleuronectes americanus. Marine Biology* **71,** 327–337.

Coombs, S. H. (1981). A density-gradient column for determining the specific gravity of fish eggs, with particular reference to eggs of the mackerel *Scomber scombrus. Marine Biology* **63,** 101–106.

Craik, J. C. A. & Harvey, S. M. (1984). Biochemical changes occurring during final maturation of eggs of some marine and freshwater teleosts. *Journal of Fish Biology* **24,** 599–610.

Craik, J. C. A. & Harvey, S. M. (1987). The causes of buoyancy in eggs of marine teleosts. *Journal of the Marine Biological Association of the United Kingdom* **67,** 169–182.

Elekseyev, F. Y. (1995). Reproductive cycle of the roundnose grenadier, *Coryphaenoides rupestris* (Macrouridae), from the North Atlantic. *Journal of Ichthyology* **35,** 123–134.

Evans, R. P., Parrish, C. C., Brown, J. A. & Davis, P. J. (1996). Biochemical composition of eggs from repeat and first-time spawning captive Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture* **139,** 139–149.

Falk-Petersen, S., Falk-Petersen, I., Sargent, J. R. & Haug, T. (1986). Lipid classes and fatty acid composition of eggs from the Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture* **52,** 207–211.

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

Finn, R. N., Fyhn, H. J. & Evjen, M. S. (1991). Respiration and nitrogen metabolism of Atlantic halibut eggs (*Hippoglossus hippoglossus*). *Marine Biology* **108,** 11–19.

Finn, R. N., Henderson, J. R. & Fyhn, H. J. (1995*a*). Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). II. Lipid metabolism and enthalpy balance. *Marine Biology* **124,** 371–379.

Finn, R. N., Fyhn, H. J. & Evjen, M. S. (1995*b*). Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). I. Respiration and nitrogen metabolism. *Marine Biology* **124,** 355–369.

Finn, R. N., Widdows, J. & Fyhn, H. J. (1995*c*). Calorespirometry of developing embryos and yolk-sac larvae of turbot (*Scophthalmus maximus*). *Marine Biology* **122,** 157–163.

Flüchter, J. & Pandian, T. J. (1968). Rate and efficiency of yolk utilization in developing eggs of the sole *Solea solea. Helgoländer wissenschaftlichen Meeresuntersuchungen* **18,** 53–60.

Fyhn, H. J. & Govoni, J. J. (1995). Endogenous nutrient mobilization during egg and larval development in two marine fishes—Atlantic menhaden and spot. *ICES Marine Science Symposia* **201,** 64–69.

Fyhn, H. J. & Serigstad, B. (1987). Free amino acids as energy substrate in developing eggs and larvae of the cod *Gadus morhua. Marine Biology* **96,** 335–341.

Fyhn, H. J., Finn, R. N., Reith, M. & Norberg, B. (1999). Yolk protein hydrolysis and oocyte free amino acids as key features in the adaptive evolution of teleost fishes to seawater. *Sarsia* **84,** 451–456.

Grigor' ev, G. V. & Serebryakov, V. P. (1981). Eggs of roundnose grenadier, *Coryphaenoides rupestris* Gunnerus 1765. *Journal of Northwest Atlantic Fishery Science* **2,** 73–74.

Grigor' ev, G. V. & Serebryakov, V. P. (1983). Eggs of rock grenadier, *Coryphaenoides rupestris* (Macrouridae). *Journal of Ichthyology* **23,** 161–163.

Haug, T., Kjørsvik, E. & Solemdal, P. (1984). Vertical distribution of Atlantic halibut (*Hippoglossus hippoglossus*) eggs. *Canadian Journal of Fisheries and Aquatic Sciences* **41,** 798–804.

Haug, T., Kjørsvik, E. & Solemdal, P. (1986). Influence of some physical and biological factors on the density and vertical distribution of Atlantic halibut *Hippoglossus hippoglossus* eggs. *Marine Ecology Progress Series* **33,** 207–216.

Hislop, J. R. G. & Bell, M. A. (1987). Observations on the size, dry weight and energy content of the eggs of some demersal fish species from British marine waters. *Journal of Fish Biology* **31,** 1–20.

Holmefjord, I. & Bolla, S. (1988). Effect of mechanical stress on Atlantic halibut eggs at different times after fertilization. *Aquaculture* **68,** 369–371.

Howell, W. H. & Caldwell, M. A. (1984). Influence of temperature on energy utilization and growth of embryonic and prolarval american plaice, *Hippoglossoides platessoides* (Fabricius). *Journal of Experimental Marine Biology and Ecology* **79,** 173–189.

Hølleland, T. & Fyhn, H. J. (1986). Osmotic properties of eggs of the herring *Clupea harengus. Marine Biology* **91,** 377–383.

Kjesbu, O. S., Kryvi, H., Sundby, S. & Solemdal, P. (1992). Buoyancy variations in eggs of Atlantic cod (*Gadus morhua* L.) in relation to chorion thickness and egg size: theory and observations. *Journal of Fish Biology* **41,** 581–599.

Lowry, O. H., Rosebrough, N. J., Fall, A. L. & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* **193,** 265–275.

Lønning, S., Kjørsvik, E. & Falk-Petersen, I. (1988). A comparative study of pelagic and demersal eggs from common marine fishes in Northern Norway. *Sarsia* **73,** 49–60.

Mahler, H. R. & Cordes, E. H. (1966). *Biological Chemistry*. New York: Harper & Row.

Mangor-Jensen, A. (1986). Osmoregulation in eggs and larvae of the cod (*Gadus morhua* L.). Basic studies and effects of oil exposure. In *Fish Larval Physiology and Anatomy. Basic Studies and Effects of Oil* (Fyhn, H. J., ed.), pp. 117–166. Bergen: University of Bergen.

Mangor-Jensen, A. (1987). Water balance in developing eggs of the cod *Gadus morhua* L. *Fish Physiology and Biochemistry* **3,** 17–24.

Mangor-Jensen, A. & Waiwood, K. G. (1995). The effect of light exposure on buoyancy of halibut eggs. *Journal of Fish Biology* **47,** 18–25. doi:10.1006/jfbi.1995.0106.

Mourente, G. & Vázquez, R. (1996). Changes in the content of total lipid, lipid classes and their fatty acids of developing eggs and unfed larvae of the Senegal sole, *Solea senegalensis* Kaup. *Fish Physiology and Biochemistry* **15,** 221–235.

Oppen-Berntsen, D. O., Helvik, J. V. & Walther, B. T. (1990). The major structural proteins of cod (*Gadus morhua*) eggshells and protein crosslinking during teleost egg hardening. *Developmental Biology* **137,** 258–265.

Østby, G. S., Finn, R. N., Norberg, B. & Fyhn, H. J. (2000). Osmotic aspects of final oocyte maturation in Atlantic halibut. In *Proceedings of the 6th International Symposium on the Reproductive Physiology of Fish* (Norberg, B., Kjesbu, O. S., Taranger, G. L., Andersson, E. & Stefansson, S. O., eds), p. 185. Bergen: University of Bergen.

Riis-Vestergaard, J. (1982). Water and salt balance of halibut eggs and larvae (*Hippoglossus hippoglossus*). *Marine Biology* **70,** 135–139.

Riis-Vestergaard, J. (1984). Water balance in cod eggs. In *The Propagation of Cod* Gadus morhua *L.* (Dahl, E., Danielssen, D. S., Moksness, E. & Solemdal, P., eds), pp. 87–103. Arendal: Institute of Marine Research, Flødevigen Biological Station.

Riis-Vestergaard, J. (1987). Physiology of teleost embryos related to environmental challenges. *Sarsia* **72,** 351–358.

Rønnestad, I., Finn, R. N., Groot, E. P. & Fyhn, H. J. (1992*a*). Utilization of free amino acids related to energy metabolism of developing eggs and larvae of lemon sole *Microstomus kitt* reared in the laboratory. *Marine Ecology Progress Series* **88,** 195–202.

Rønnestad, I., Fyhn, H. J. & Gravningen, K. (1992*b*). The importance of free amino acids to the energy metabolism of eggs and larvae of turbot (*Scophthalmus maximus*). *Marine Biology* **114,** 517–525.

Rønnestad, I., Koven, W. M., Tandler, A., Harel, M. & Fyhn, H. J. (1994). Energy metabolism during development of eggs and larvae of gilthead sea bream (*Sparus aurata*). *Marine Biology* **120,** 187–196.

Rønnestad, I., Koven, W., Tandler, A., Harel, M. & Fyhn, H. J. (1998). Utilisation of yolk fuels in developing eggs and larvae of European sea bass (*Dicentrarchus labrax*). *Aquaculture* **162,** 157–170.

Russell, F. S. (1976). *The Eggs and Planktonic Stages of British Marine Fishes*. London: Academic Press.

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

Solemdal, P. (1967). The effect of salinity on buoyancy, size and development of flounder eggs. *Sarsia* **29,** 431–442.

Solemdal, P. (1973). Transfer of Baltic flatfish to a marine environment and the long term effects on reproduction. *OIKOS Supplementum* **15,** 268–276.

Thorsen, A. & Fyhn, H. J. (1991). Osmotic effectors during preovulatory swelling of marine fish eggs. In *Reproductive Physiology of Fish* (Scott, A. P., Sumpter, J. P., Kime, D. E. & Rolfe, M. S., eds), pp. 312–314. Sheffield: FishSymp. 91, University of Sheffield.

Thorsen, A. & Fyhn, H. J. (1996). Final oocyte maturation *in vivo* and *in vitro* in marine fishes with pelagic eggs; yolk protein hydrolysis and free amino acid content. *Journal of Fish Biology* **48,** 1195–1209.

Thorsen, A., Fyhn, H. J. & Wallace, R. A. (1993). Free amino acids as osmotic effectors for oocyte hydration in marine fishes. In *Physiological and Biochemical Aspects of Fish Development* (Walther, B. T. & Fyhn, H. J., eds), pp. 94–98. Bergen: University of Bergen.

Thorsen, A., Kjesbu, O. S., Fyhn, H. J. & Solemdal, P. (1996). Physiological mechanisms of buoyancy in eggs from brackish water cod. *Journal of Fish Biology* **48,** 457–477.

UNESCO. (1981). The practical salinity scale 1978 and the international equation of state of seawater 1980. Tenth Report of the Joint Panel on Oceanographic Tables and Standards (JPOTS), Sidney, B.C., Canada, 1–5 September, 1980. *Unesco Technical Papers in Marine Science* No. **36.**

Vázquez, R., González, S., Rodríguez, A. & Mourente, G. (1994). Biochemical composition and fatty acid content of fertilized eggs, yolk sac stage larvae and firstfeeding larvae of the Senegal sole (*Solea senegalensis* Kaup). *Aquaculture* **119,** 273–286.

Whyte, J. N. C., Clarke, W. C., Ginther, N. G. & Jensen, J. O. T. (1993). Biochemical changes during embryogenesis of the Pacific halibut, *Hippoglossus stenolepis* (Schmidt). *Aquaculture and Fisheries Management* **24,** 193–201.

Young, E. G. & Inman, W. R. (1938). The protein of the casing of salmon eggs. *Journal of Biological Chemistry* **124,** 189–193.

Yúfera, M., Parra, G., Santiago, R. & Carrascosa, M. (1999). Growth, carbon, nitrogen and caloric content of *Solea senegalensis* (Pisces: Soleidae) from egg fertilization to metamorphosis. *Marine Biology* **134,** 43–49.

APPENDIX: ENERGY DENSITY OF PELAGIC FISH EGGS RELATED TO SIZE OF OIL GLOBULE

(1) If ovoplasm (O) corresponding to the fraction x of total egg volume V_{egg} is replaced by an oil globule of equal volume, the change in wet mass is

$$\Delta M_1 = x V_{egg}(\rho_{oil} - \rho_O)$$

PVS is the perivitelline space, ρ denotes specific gravity, and

$$\rho_O = M_O V_O^{-1} = (M_{egg} - M_{chorion} - M_{PVS})\ (V_{egg} - V_{chorion} - V_{PVS})^{-1}$$

(2) If a fraction y of (ovoplasm minus lipid) (OML) is replaced by an equal volume of protein amino acids (PAA), the change in wet mass is

$$\Delta M_2 = y V_{egg}(\rho_{PAA} - \rho_{OML})$$

where

$$\rho_{OML} = M_{OML} V_{OML}^{-1} = (M_{egg} - M_{chorion} - M_{PVS} - M_{lipid})\ (V_{egg} - V_{chorion} - V_{PVS} - V_{lipid})^{-1}$$

Maintaining a constant egg mass demands that

$$\Delta M_1 = -\Delta M_2 \rightarrow y = x(\rho_{oil} - \rho_O)\ (\rho_{OML} - \rho_{PAA})^{-1}$$

(3) Energy in free amino acids (FAA) and protein amino acids (PAA) remaining after withdrawal of material in sections (1) and (2) is

$$E_1=[M_{\mathrm{FAA}}\sigma_{\mathrm{FAA}}+(M_{\mathrm{PAA}}-M_{\mathrm{chorion}})\sigma_{\mathrm{PAA}}] \\ [1-V_{\mathrm{egg}}(xV_{\mathrm{O}}^{-1}+y(V_{\mathrm{O}}-V_{\mathrm{lipid}})^{-1})]+M_{\mathrm{chorion}}\sigma_{\mathrm{PAA}}$$

M represents masses before withdrawal is initiated, σ denotes energy density and

$$V_{\mathrm{O}}=V_{\mathrm{egg}}-V_{\mathrm{chorion}}-V_{\mathrm{PVS}}$$

$$V_{\mathrm{lipid}}=M_{\mathrm{lipid}}\rho_{\mathrm{lipid}}^{-1}$$

The amount of E_1 located in the ovoplasm is

$$E_{1,\mathrm{O}}=E_1-M_{\mathrm{chorion}}\sigma_{\mathrm{PAA}}$$

(4) Energy in ovoplasmic lipid remaining after withdrawal of material in section (1) is

$$E_2=M_{\mathrm{lipid}}\sigma_{\mathrm{lipid}}(1-xV_{\mathrm{egg}}V_{\mathrm{O}}^{-1})$$

(5) Energy added with the oil globule is

$$E_3=xV_{\mathrm{egg}}\rho_{\mathrm{oil}}\sigma_{\mathrm{oil}}$$

(6) Energy added as ‘ new ’ protein in section (2) is

$$E_4=yV_{\mathrm{egg}}\rho_{\mathrm{PAA}}\sigma_{\mathrm{PAA}}$$

(7) The energy density of the ovoplasm (not including the oil globule) becomes

$$\sigma_{\mathrm{O}}=(E_{1,\mathrm{O}}+E_2+E_4)\ (V_{\mathrm{O}}-xV_{\mathrm{egg}})^{-1}$$

which is a nonlinear function of x.
(8) The average energy density in the egg excluding the oil globule but including perivitelline space (PVS) becomes

$$\sigma_{\mathrm{ex\ oil}}=(E_1+E_2+E_4)\ [V_{\mathrm{egg}}(1-x)]^{-1}$$

which is a nonlinear function of x.
(9) The average energy density of the whole egg becomes

$$\sigma=(E_1+E_2+E_3+E_4)V_{\mathrm{egg}}^{-1}$$

which is a linear function of x, i.e.

$$\sigma=a+bx$$

The intercept a is the energy density of the unmodified egg; the coefficient b is a composite function of masses, specific gravities and energy densities of the egg constituents. Insertion of values from Tables I and II and the recommended energy density for eggs without oil globules results in

$$\sigma = 1{\cdot}34 + 40{\cdot}6x$$