Vol. 163: 53–61, 1998 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published March 12

Effect of biotic and abiotic factors on the biochemical composition of wild eggs and larvae of several fish species

C. Guisande*, I. Riveiro, A. Solá, L. Valdés*****

Facultad de Ciencias del Mar, Universidad de Vigo, Aptdo. 874, E-36200 Vigo, Spain

ABSTRACT: The aim of this study is to establish possible associations between temperature, salinity and egg and larval abundance, and the biochemical composition of wild fertilised eggs and larvae of marine fish species. Eggs and larvae of the most abundant species at each station at the time of sampling were collected during 2 surveys carried out from 25 March to 14 April 1995 (MPH-95) for collecting eggs and from 30 May to 16 June (SEFOS-95) for collecting larvae. Both surveys were carried out on the N-NW coast of the Iberian Peninsula. Egg size, egg abundance, egg stage, temperature and salinity explained a small variance in the high variation observed within and between stations in protein, carbohydrate and lipid content of the eggs in both *Trachurus trachurus* and *Scomber scombrus*. Conversely, protein, carbohydrate and lipid content of larvae in *Sardina pilchardus*, *Engraulis encrasicolus* and *T trachurus* seemed to vary according to either larval body length, temperature, salinity and/or larval abundance. Protein, carbohydrate and lipid content increased as larval body length increased for the 3 species. However, a percentage of lipid and protein in the larvae of the 3 species varied according to prevailing buoyancy conditions. An increment of larval lipid percentage and a reduction of larval protein percentage was observed as temperature increased and salinity decreased, the opposite case causing an increase in protein percentage and a decrease in lipid percentage of the larvae. This trade-off between protein and lipid production is interpreted as a mechanism to achieve optimal larval buoyancy.

KEY WORDS: Protein · Carbohydrate · Lipid · Egg · Larva · Temperature · Salinity · *Sardina pilchardus* · *Trachurus trachurus* · *Scomber scombrus* · *Engraulis encrasicolus*

INTRODUCTION

Most of the knowledge on energy reserves and energy metabolism in developing fish eggs and larvae has been primarily based on eggs that have been naturally or artificially fertilised in culture tanks (Watanabe 1982, Vetter et al. 1983, Fyhn & Serigstad 1987, Fyhn 1989, Miranda et al. 1990, Finn et al. 1991, Srivastava & Brown 1991, Tamaru et al. 1992). Little information exists in the literature on bioenergetic studies on fertilised eggs and larvae directly collected from the natural environment where the quality of eggs produced by females can be affected by biotic factors such as egg and larval abundance and abiotic factors such as temperature and salinity.

Egg abundance could affect egg biochemical composition from the viewpoint of an optimum balance between offspring number and size (Buckley et al. 1991b). Larval abundance could also affect larval biochemical composition due to a possible food depletion caused by a high intraspecific competition.

Temperature and salinity affect egg and larval buoyancy. Egg buoyancy has been shown as an important factor affecting success of fish spawning, mainly for 2 reasons. Firstly, eggs must stay buoyant in the upper water to avoid low oxygen conditions of deep layers (Nissling & Westin 1991) and, secondly, eggs must

*E-mail: castor@uvigo.es
Present addresses:
**Instituto Español de Oceanografía, Avda. de Brasil, 31, E-28020 Madrid, Spain
***Centro Oceanográfico de Santander, Instituto Español de Oceanografía, Aptdo. 240, E-39080 Santander, Spain

© Inter-Research 1998
Resale of full article not permitted

reach an appropriate water depth for larval feeding by the time of hatching (Page et al. 1989). As there is a spatial and temporal variability in both temperature and salinity, hence in egg buoyancy (Coombs et al. 1985), any change in egg density directed toward achieving optimal buoyancy could benefit the recruitment processes. Egg buoyancy can vary according to egg water content (Craik & Harvey 1987) and chorion thickness (Kjesbu et al. 1992). Differences in egg density can also occur as a result of changes in egg quality, with eggs' low specific weight relative to sea water when egg quality is poorer (Kjesbu et al. 1992). On the other hand, many studies have also pointed out the importance of water density for larval spatial distribution. Lee et al. (1995) showed that surface water temperature and salinity were important factors associated with the formation of larval anchovy fishing grounds. Anderson & DeYoung (1995) demonstrated that vertical distribution of cod eggs and larvae was sensitive to changes in water temperature and salinity, showing a progression from deeper to shallower depths as cod eggs develop through to larvae. Page et al. (1989) pointed out the importance for larval survival of staying in the upper more productive layers. Therefore, changes in either protein, carbohydrate or lipid percentages of the eggs and larvae could be observed as a mechanism to achieve optimal egg and larval buoyancy.

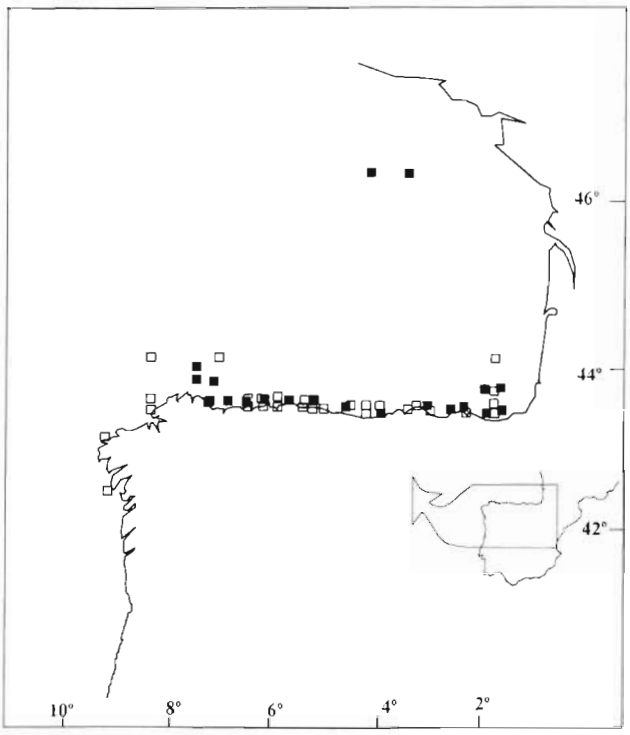

Fig. 1. Map of study area, showing location of sampling stations during MPH-95 (□) and SEFOS-95 (■) cruises

The aims of this study were to determine changes in protein, carbohydrate and lipid composition during the development of eggs and larvae collected from natural fish populations and to discover whether protein, carbohydrate and lipid content of eggs and larvae could be associated to changes in temperature, salinity and/or egg and larval abundance.

METHODS

Wild fertilised eggs and larvae of the most abundant species at each station at the time of sampling were collected during 2 surveys carried out from 25 March to 14 April 1995 (MPH-95) for collecting eggs and from 30 May to 16 June (SEFOS-95) for collecting larvae on the RV 'Cornide de Saavedra'. Fig. 1 shows the location of sampling stations for both surveys. These sampling stations and periods were chosen because previous studies have shown that the main spawning of the most abundant species in the Cantabrian Sea occurs in these areas: from April to May for *Scomber scombrus*, from May to June for *Trachurus trachurus* and, although the spawning period of *Sardina pilchardus* extends almost throughout the whole year, the main and consistent peak occurs from April to May (Solá et al. 1990).

As this survey formed a part of an EEC project, we used integrated tows following the ICES basis of the Mackerel/Horse mackerel egg production workshop (Anonymous 1994) and, hence, information on the depth distribution of eggs and larvae was not obtained. Oblique tows were performed using a 50 cm diameter bongo net fitted with 200 µm mesh. Eggs and live larvae were immediately removed from the plankton samples using pipettes and, after examination under a binocular microscope equipped with an ocular micrometer, egg diameter or larval body length was measured and embryonic developmental stages were determined according to the morphological criteria of Lockwood & Nichols (1977) for *Scomber scombrus* and Pipe & Walker (1987) for *Trachurus trachurus*. Eggs or larvae were then transferred to individual 1.5 ml ultracentrifuge plastic tubes where each egg was homogenised in either 300 µl (to analyse protein and lipid) or 500 µl (to analyse carbohydrate and lipid) and each larva in 675 µl (to analyse protein, carbohydrate and lipid) of distilled water using a pipette tip adapted to fit the shape of the vial. Therefore, for each larva protein, carbohydrate and lipid content were analysed, whereas for each egg either protein and lipid or carbohydrate and lipid were analysed. These samples were immediately frozen on board for further laboratory analysis of egg and larval biochemical composition.

Protein, carbohydrate and lipid percentage were calculated considering total organic content as the combined total protein, carbohydrate and lipid content of eggs and larvae.

Egg and larval biochemical composition analysis. For each individual egg or larva homogenate, 200 µl was used for protein analysis, 400 µl for carbohydrate analysis and 75 µl for lipid analysis. The method described by Lowry et al. (1951) and modified by Markwell et al. (1978) was used to analyse the protein content of the eggs. To sample volumes of 200 µl, 50 µl NaOH (0.5 N) (prepared just before the test) and 750 µl of solution C were added. Solution C was prepared shortly before the beginning of the analysis and was composed of solutions A and B in the proportions of 100A:1B. These solutions were as follows: solution A, Na_2CO_3 (2%), NaOH (0.4%), $NaK(COO)_2(CHOH)_24H_2O$ (0.16%), SDS (1%); solution B, $CuSO_45H_2O$ (4%). The samples were shaken and maintained at 30°C for 15 min. Then 75 µl of diluted 1:1 Folin-Cioccalteus was added to each sample. Subsequently, the samples were incubated for 30 min at the same temperature. Absorbance was read at 660 nm and compared with bovine serum albumin (BSA) standard.

Egg and larval carbohydrate content was measured by the phenol-sulphuric acid method of Dubois et al. (1956). To sample volumes of 400 µl, 10 µl of 81% phenol was added and, after gently shaking, 1 ml of concentrated sulphuric acid was added. The samples were shaken again and maintained at room temperature for 30 min. Finally, the absorbance was read at 485 nm. A standard curve was established by using reagent-grade glucose.

Egg and larval lipid content was determined using the sulfophosphovanillin method of Zöllner & Kirsch (1962). 50 µl of absolute ethanol was added to sample volumes of 75 µl immediately after the sample was shaken and maintained at 4°C for at least 2 h. Then 375 µl of concentrated sulphuric acid was added and later, after samples had been homogenised by shaking, the tubes were maintained at 100°C for 15 min. After hydrolysis, when the tubes had cooled down, 2 ml of vanillin reagent was added. Absorbance was read at 530 nm, with cholesterol as standard, after tubes had been shaken and incubated at 30°C for 30 min. Vanillin reagent was prepared by mixing 1.976 g of vanillin with 800 ml of 85% H_3PO_4 and distilled water to 1000 ml.

Hydrographic sampling. Hydrographic sampling was carried out by CTD cast. The CTD used was a SeaBird 25. Water samples were taken from the surface, 500 and 1000 m depth for CTD calibrations. Salinity analysis of the water samples was made on board using a 2100 MINISAL salinometer calibrated with standard water. Since we used integrated tows, there was no information on egg and larval depth distribution and, hence, temperature and salinity at each station were calculated as the mean of the values taken every 5 m as far as the bongo tow depth. However, it is important to mention that temperature and salinity at the depth at which eggs or larvae are distributed could be different from these mean values.

RESULTS

Table 1 shows tow characteristics and mean temperature and salinity of the whole water column for all the stations sampled in both surveys.

A high variation between and within stations in protein, carbohydrate and lipid content of eggs was observed for *Scomber scombrus* and *Trachurus trachurus* (Table 2). Stepwise regressions show that neither egg size, egg stage, temperature, salinity nor egg abundance explained a high proportion of this variation found in protein, carbohydrate and lipid content of the eggs for both species (Table 3).

In contrast, a high proportion of the variation observed in larval protein, carbohydrate and lipid contents for *Sardina pilchardus*, *Trachurus trachurus* and *Engraulis encrasicolus* between and within stations (Table 4) was explained either by larval size, temperature, salinity and/or larval abundance (Table 5). An increment in total protein, carbohydrate and lipid content of larvae was obtained as larval size increased for the 3 species (Table 5). However, protein and lipid contents of larvae were also affected by the temperature and salinity of the water. At higher temperature and lower salinity, hence lower buoyancy for the larvae, a reduction in larval protein percentage and an increment in larval lipid percent were observed, the opposite being the case as temperature decreased and salinity increased (Table 5, Fig. 2). In the case of *T. trachurus* and *E. encrasicolus* the relationships between protein and lipid percentage with temperature were not as significant as for *S. pilchardus* (Fig. 2). This is probably due to the low number of data and the small temperature range.

These changes in the biochemical composition of the larvae as temperature varied were not associated to changes in larval organic content. Fig. 3 shows that, considering the 4 species, there was a significant relationship between total organic content (combined total protein, carbohydrate and lipid content of eggs and larvae) and either egg or larval size. Therefore, for the same larval size, there is a trade-off between protein and lipid production according to a larva's specific weight relative to sea water, so that the organic content remains the same.

Table 1. Summary of tow characteristics and mean ± SD temperature and salinity of the whole water column at sampling stations located on the N-NW coast of the Iberian Peninsula from 25 March to 14 April 1995 (MPH-95) and from 30 May to 16 June 1995 (SEFOS-95)

Stn	Lat. N	Long. W	Depth	Bongo tow depth (m)	Temperature (°C)	Salinity (‰)
MPH-95						
31	42.4509°	9.2007°	125	121	12.51 ± 0.46	35.29 ± 2.36
38	43.1506°	9.1465°	127	103	12.50 ± 0.68	35.32 ± 2.27
45	43.3736°	8.3038°	162	133	12.53 ± 0.37	35.22 ± 3.06
46	43.5239°	8.3008°	266	179	12.62 ± 0.09	35.46 ± 0.83
47	44.0752°	8.3017°	360	173	12.30 ± 0.41	35.45 ± 1.38
54	44.0774°	7.0983°	995	197	12.40 ± 0.29	34.81 ± 0.73
55	43.5255°	7.2973°	145	113	12.51 ± 0.32	34.91 ± 0.35
56	43.3757°	6.4993°	92	81	12.42 ± 0.27	34.99 ± 3.77
57	43.5298°	6.5027°	125	84	12.09 ± 0.22	34.39 ± 3.34
63	43.5269°	6.3032°	262	194	12.30 ± 0.21	35.27 ± 0.66
64	43.3751°	6.2988°	100	79	12.18 ± 0.12	35.22 ± 2.14
65	43.3760°	6.0999°	87	71	12.38 ± 0.22	35.37 ± 1.85
66	43.5289°	6.1001°	674	192	12.38 ± 0.26	35.37 ± 2.20
69	43.5261°	5.5011°	218	178	12.39 ± 0.39	35.35 ± 1.87
70	43.4269°	5.5015°	116	105	12.34 ± 0.35	35.01 ± 1.27
71	43.3748°	5.2988°	92	87	12.26 ± 0.30	35.29 ± 1.79
72	43.5275°	5.3012°	245	215	12.33 ± 0.37	35.12 ± 0.60
79	43.3746°	5.1001°	138	118	12.31 ± 0.26	35.30 ± 3.46
80	43.3757°	4.4989°	153	120	12.62 ± 0.36	35.28 ± 1.64
86	43.3789°	4.3001°	198	180	12.47 ± 0.37	35.22 ± 2.51
87	43.2750°	4.3003°	125	105	12.31 ± 0.40	34.99 ± 0.95
88	43.3781°	4.1004°	957	231	12.42 ± 0.42	35.24 ± 3.48
94	43.3770°	3.4937°	176	176	12.52 ± 0.43	35.32 ± 1.57
95	43.3768°	3.2995°	1089	221	12.37 ± 0.56	35.34 ± 1.41
97	43.2754°	3.0999°	77	68	12.64 ± 0.61	34.94 ± 2.64
101	43.2749°	2.2990°	96	92	12.72 ± 0.24	34.58 ± 3.15
104	43.2738°	1.4518°	145	74	12.66 ± 0.34	35.59 ± 2.91
105	43.3735°	1.4515°	416	251	12.31 ± 0.50	35.15 ± 1.91
106	43.5234°	1.4500°	116	86	12.59 ± 0.36	34.68 ± 2.96
107	44.1490°	1.4493°	116	103	12.45 ± 0.51	34.05 ± 4.06
SEFOS-95						
2	43.5250°	7.4596°	133	125	13.33 ± 1.09	35.52 ± 0.11
3	44.0736°	7.4532°	988	250	12.73 ± 0.99	35.56 ± 0.06
9	43.5234°	7.1518°	154	120	13.34 ± 1.27	35.26 ± 1.54
10	43.3743°	7.1512°	46	38	15.07 ± 0.33	35.55 ± 5.54
11	43.3760°	6.4511°	90	72	14.41 ± 0.79	35.48 ± 0.05
12	43.5219°	6.4532°	120	100	13.24 ± 1.53	35.50 ± 0.11
20	43.3757°	6.1501°	77	57	13.66 ± 1.60	35.53 ± 0.10
21	43.3766°	5.4360°	44	32	15.24 ± 1.29	35.35 ± 0.13
30	43.3737°	5.1505°	135	115	13.10 ± 1.48	35.26 ± 1.20
31	43.3892°	4.4490°	89	85	13.74 ± 1.79	35.38 ± 0.22
40	43.2725°	4.1519°	89	79	13.81 ± 1.71	35.30 ± 0.28
46	43.3770°	3.1492°	530	190	12.73 ± 1.20	35.56 ± 0.70
55	43.3004°	2.4522°	110	88	13.46 ± 1.91	35.08 ± 0.63
56	43.5251°	2.1508°	670	217	13.06 ± 1.77	35.31 ± 0.47
58	43.2261°	2.1499°	101	95	13.19 ± 1.52	35.11 ± 0.67
60	43.3849°	1.4410°	130	116	12.66 ± 1.39	35.35 ± 0.46
61	43.5246°	1.4508°	120	106	13.73 ± 1.71	35.07 ± 0.50
108	46.3731°	3.4517°	127	89	13.20 ± 1.99	35.17 ± 0.33
109	46.3747°	4.1508°	144	136	13.04 ± 1.54	35.39 ± 0.16

Table 2. *Scomber scombrus* and *Trachurus trachurus*. Means ± SD of egg size (mm), egg stage, protein, carbohydrate and lipid content (in µg egg^{-1}) and egg abundance (eggs m^{-2}) at each station. Number of eggs analysed at each station for protein, carbohydrate and lipid was between 3 and 28

Stn	*Scomber scombrus*						*Trachurus trachurus*					
	Size	Stage	Protein	Carbohydrate	Lipid	Abundance	Size	Stage	Protein	Carbohydrate	Lipid	Abundance
31	1.000 ± 0.00	1.0 ± 0.0	7.0 ± 0.8	3.8 ± 1.6	1.1 ± 0.0	9.4						
38	1.113 ± 0.09	2.0 ± 1.0	12.3 ± 8.5	5.7 ± 0.6	6.5 ± 0.0	7.6						
45	1.153 ± 0.07	3.3 ± 1.1	16.0 ± 3.1	6.5 ± 3.3	0.3 ± 0.0	30.7						
46	1.115 ± 0.08	2.5 ± 0.9	17.7 ± 6.0	5.1 ± 1.7	7.9 ± 5.5	996.4	0.950 ± 0.000	2.5 ± 0.5	22.7 ± 0.0	2.9 ± 0.0	2.8 ± 0.0	43.9
47	1.156 ± 0.04	3.0 ± 0.9	19.5 ± 7.8	5.1 ± 1.1	8.4 ± 4.7	42.0						
54	1.152 ± 0.03	2.8 ± 1.2	17.2 ± 2.3	6.7 ± 1.7	6.1 ± 3.4	118.7						
55	1.128 ± 0.05	1.8 ± 1.2	15.0 ± 3.4	5.4 ± 2.4	5.0 ± 3.0	61.8	0.900 ± 0.000	1.0 ± 0.0		3.0 ± 0.0	0.8 ± 0.0	29.5
56	1.150 ± 0.09	1.8 ± 0.8	16.5 ± 0.0	4.3 ± 1.4	5.0 ± 3.2	25.8	0.900 ± 0.000	1.0 ± 0.0	6.6 ± 0.0		1.7 ± 0.0	16.1
57	1.157 ± 0.04	1.4 ± 0.6	23.3 ± 3.3	4.5 ± 1.1	1.5 ± 0.9	51.0						
63	1.163 ± 0.05	1.8 ± 0.9	24.4 ± 4.9	7.2 ± 1.9	5.0 ± 6.6	73.3						
64	1.140 ± 0.03	2.0 ± 0.6	24.0 ± 6.1	5.8 ± 1.5	1.9 ± 1.5	40.4	0.875 ± 0.025	2.0 ± 0.0	13.9 ± 0.0	3.5 ± 0.0	1.3 ± 0.6	8.1
65	1.137 ± 0.03	1.4 ± 0.5	19.3 ± 6.1	6.1 ± 1.6	1.2 ± 0.9	31.4						
66						21.9	0.967 ± 0.047	4.0 ± 0.0	21.1 ± 0.7	5.3 ± 0.0	0.8 ± 0.4	13.1
69	1.160 ± 0.04	1.9 ± 1.1	31.4 ± 5.0	6.5 ± 2.3	1.7 ± 1.2	162.9						
70	1.163 ± 0.03	1.5 ± 0.5	22.2 ± 3.3	5.8 ± 1.1	1.1 ± 0.7	95.8						
71	1.150 ± 0.04	1.5 ± 0.6	17.7 ± 2.0	2.5 ± 1.7	5.8 ± 5.7	66.8	0.950 ± 0.000	2.0 ± 0.0				13.1
72	1.160 ± 0.04	1.9 ± 0.7	21.0 ± 3.3	4.6 ± 1.3	2.6 ± 2.3	82.1						
79	1.165 ± 0.03	2.3 ± 1.3	22.3 ± 4.5	5.2 ± 1.3	3.7 ± 1.3	48.9	0.833 ± 0.024	2.0 ± 0.8	7.6 ± 1.8	4.2 ± 0.0	3.5 ± 3.4	31.5
80	1.130 ± 0.02	2.3 ± 1.2	22.0 ± 1.6	3.7 ± 1.2	1.1 ± 0.6	52.6						
86	1.143 ± 0.03	1.4 ± 0.5	23.1 ± 4.3	8.0 ± 3.1	2.5 ± 1.2	34.9						
87	1.160 ± 0.04	2.2 ± 1.2	17.7 ± 4.4	6.4 ± 1.7		25.7	0.917 ± 0.037	1.3 ± 0.7	6.2 ± 0.9	2.0 ± 0.6	1.0 ± 0.0	35.2
88	1.141 ± 0.05	2.6 ± 1.0	19.3 ± 6.7	3.4 ± 1.3	1.5 ± 1.1	48.8	0.963 ± 0.013	2.5 ± 0.5	12.4 ± 0.0	3.1 ± 0.0	0.9 ± 0.0	24.4
94	1.158 ± 0.04	1.3 ± 0.6	22.8 ± 5.4	4.0 ± 0.5	1.5 ± 0.5	36.6	0.900 ± 0.000	1.0 ± 0.0	12.9 ± 0.0			13.3
95	1.170 ± 0.02	2.6 ± 0.8	34.8 ± 2.5	5.1 ± 3.6	6.1 ± 0.3	26.7						
97	1.157 ± 0.04	1.4 ± 0.5	26.3 ± 6.2	3.4 ± 0.6	12.1 ± 5.0	24.0	0.930 ± 0.031	1.5 ± 0.8	11.5 ± 2.8	4.1 ± 1.5	2.4 ± 0.0	75.5
101	1.158 ± 0.04	1.6 ± 0.7	7.9 ± 2.0			37.0	0.875 ± 0.000	1.0 ± 0.0				8.2
104	1.750 ± 0.03	1.9 ± 0.9	16.8 ± 10.8			26.1	0.925 ± 0.000	1.0 ± 0.0				2.9
105	1.625 ± 0.04	2.0 ± 1.3	16.3 ± 5.7	8.4 ± 2.5	11.6 ± 6.8	79.1	0.850 ± 0.000	1.0 ± 0.0	5.1 ± 0.0		4.5 ± 0.0	5.3
106	1.153 ± 0.04	1.3 ± 0.5	24.6 ± 5.2	6.2 ± 1.4	3.2 ± 3.8	27.9						
107	1.153 ± 0.04	1.5 ± 0.9	17.8 ± 3.9	5.3 ± 0.8	8.3 ± 3.5	38.7						
108	1.150 ± 0.00	2.0 ± 0.0	17.0 ± 0.0		4.1 ± 0.0							

Table 3. *Scomber scombrus* and *Trachurus trachurus*. Stepwise linear regressions between total protein, carbohydrate and lipid content of the eggs (in µg egg^{-1}) with egg size (in mm), egg stage, temperature (°C), salinity (‰) and egg abundance (eggs m^{-2}). Partial correlations are shown in parentheses

	Constant	Egg size	Egg stage	Temperature	Salinity	Egg abundance	r^2	df	F	p
Scomber scombrus										
Total protein	−23.832	0.999 (0.32)		−9.327 (−0.22)	3.247 (0.12)		0.17	3,150	10.0	0.000
Total carbohydrate	4.626		0.505 (0.21)				0.04	1,152	6.7	0.011
Total lipid	9.599	0.260 (0.06)	0.567 (0.15)	5.046 (0.22)	−2.302 (0.06)	0.003 (0.20)	0.10	5,200	4.6	0.001
Trachurus trachurus										
Total protein	−1267.081				36.261 (0.63)	0.151 (0.10)	0.73	2,23	31.6	0.000
Total lipid	1.264					0.032 (0.42)	0.18	1,35	7.7	0.009

Table 4. *Sardina pilchardus*, *Trachurus trachurus* and *Engraulis encrasicolus*. Means ± SD of larval size (mm), protein, carbohydrate and lipid content (in µg larva^{-1}) and larval abundance (larvae m^{-2}). Number of larvae analysed at each station for protein, carbohydrate and lipid was between 3 and 16

Stn	Size	Protein	Carbohydrate	Lipid	Abundance
Sardina pilchardus					
2	9.73 ± 2.83	145.6 ± 120.2	24.3 ± 14.8	57.6 ± 35.7	124.5
3	6.70 ± 0.00	39.2 ± 0.0	8.6 ± 0.0	14.6 ± 0.0	6.5
9	12.36 ± 2.70	216.8 ± 119.2	25.0 ± 8.9	138.5 ± 52.0	31.7
10	5.43 ± 2.45	37.5 ± 40.1	7.6 ± 8.2	66.6 ± 67.5	416.9
11	9.67 ± 4.52	144.7 ± 142.1	26.2 ± 10.1	108.4 ± 100.8	153.8
12	7.72 ± 2.54	105.2 ± 140.7	12.3 ± 5.6	95.3 ± 39.8	118.5
20	8.17 ± 5.70	222.6 ± 264.2	7.1 ± 0.6	104.9 ± 69.6	91.6
21	7.00 ± 1.81	19.2 ± 13.6	12.0 ± 6.4	137.6 ± 49.0	70.8
30	7.79 ± 2.22	113.4 ± 140.4	20.1 ± 8.9	83.5 ± 47.7	238.7
31	6.99 ± 1.58	76.9 ± 41.8	10.5 ± 3.3	102.1 ± 27.6	109.2
40	7.95 ± 1.78	74.1 ± 32.0	16.8 ± 8.8	98.9 ± 27.5	68.1
Trachurus trachurus					
30	3.93 ± 0.51	52.6 ± 25.7	9.4 ± 5.5	30.4 ± 5.8	153.3
31	2.64 ± 0.55	13.3 ± 8.6	10.4 ± 3.0	24.3 ± 0.0	77.1
40	3.82 ± 1.13	33.2 ± 17.5	13.9 ± 5.4	44.1 ± 23.2	38.9
108	3.96 ± 0.64	83.3 ± 41.4	12.7 ± 5.5	26.9 ± 18.9	76.6
109	4.75 ± 0.72	62.4 ± 27.8	10.6 ± 6.0	26.1 ± 17.0	394.5
Engraulis encrasicolus					
46	8.72 ± 2.25	172.0 ± 139.7	20.1 ± 11.2	22.2 ± 18.4	85.4
55	5.70 ± 2.05	57.5 ± 82.4	12.3 ± 8.9	18.7 ± 8.1	95.1
56	4.94 ± 1.85	31.9 ± 36.1	8.8 ± 5.0	25.5 ± 15.0	144.1
58	7.03 ± 0.52	27.6 ± 20.3	19.1 ± 11.6	13.0 ± 14.2	155.9
60	6.6 ± 32.05	49.3 ± 38.5	11.9 ± 10.4	12.1 ± 9.7	115.9
61	9.01 ± 2.52	96.4 ± 67.3	23.6 ± 15.9	45.7 ± 33.6	254.5

DISCUSSION AND CONCLUSIONS

It has been shown that either protein (*Sparus aurata*; Fyhn 1989, Rønnestad et al. 1994) or lipid (*Sciaenops ocellata*; Vetter et al. 1983) is the most important source of energy during egg development in fish. In other fish species, e.g. Atlantic halibut *Hippoglossus hippoglossus*, egg biochemical composition was found to be more size-dependent than egg stage-dependent (Finn et al. 1991), although in further species, e.g. *Pseudopleuronectes americanus*, protein and lipid concentration of the eggs were independent of egg size (Buckley et al. 1991b). In this study, however, the biochemical composition of wild eggs of *Scomber scombrus* and *Trachurus trachurus* was not highly correlated to either egg stage, egg size or egg abundance (Table 3). The lack of correlation between egg biochemical composition and egg abundance is hardly surprising because it has been shown that egg abundance in the field cannot be equated with offspring numbers collected from a single female under controlled conditions (Buckley et al. 1991a).

Egg biochemical composition of *Scomber scombrus* and *Trachurus trachurus* did not vary according to

Table 5. *Engraulis encrasicolus*, *Trachurus trachurus* and *Sardina pilchardus*. Stepwise linear regressions between total protein, carbohydrate and lipid content of the larvae (in μg larva^{-1}) and percent of larval protein, carbohydrate and lipid with larval size (in mm), temperature (°C), salinity (‰) and larval abundance (larvae m^{-2}). Partial correlations are shown in parentheses

	Constant	Larval size	Temperature	Salinity	Larval abundance	r^2	df	F	p
Engraulis encrasicolus									
Total protein	−9802.599	28.200 (0.76)	97.331(−0.15)	240.009 (0.36)	−0.421(−0.12)	0.70	4,47	27.7	0.000
Total carbohydrate	−8.145	3.508 (0.84)				0.70	1,44	104.6	0.000
Total lipid	−2929.387	2.592 (0.42)	42.311(0.10)	66.954 (0.11)	0.130 (0.07)	0.40	4,33	5.4	0.002
Percent protein	−15.855	0.043 (0.58)		0.457 (0.54)		0.50	2,33	16.1	0.000
Percent carbohydrate	22.842		−0.224 (0.257)	−0.559 (−0.49)		0.36	2,33	9.2	0.001
Percent lipid	−1.573	−0.045 (−0.62)	0.163 (0.401)			0.44	2,33	13.3	0.000
Trachurus trachurus									
Total protein	4505.034	22.941 (0.68)		−128.663 (−0.27)		0.60	2,32	23.7	0.000
Total lipid	−23.310	14.570 (0.51)			−0.050 (−0.15)	0.41	2,25	8.6	0.001
Percent protein	676.697		−46.398 (−0.54)		−0.026 (0.18)	0.33	2,23	5.5	0.011
Percent carbohydrate	−17.812	−5.490 (−0.38)	14.464 (0.52)	45.132 (0.26)		0.67	3,25	16.9	0.000
Percent lipid	−298.632		24.831 (0.41)			0.17	1,24	4.9	0.037
Sardina pilchardus									
Total protein	−3774.232	33.748 (0.84)	−24.936 (−0.24)	111.051 (−0.06)	0.128 (−0.16)	0.73	4,88	60.1	0.000
Total carbohydrate	−3.809	2.541 (0.76)				0.58	1,90	124.8	0.000
Total lipid	−165.648	5.297 (0.50)	11.770 (0.19)			0.33	2,85	20.5	0.000
Percent protein	−7.189	0.029 (0.47)	−0.131 (−0.46)	0.263 (−0.04)		0.41	3,75	17.4	0.000
Percent carbohydrate	0.184	−0.006 (−0.23)				0.05	1,77	4.2	0.043
Percent lipid	−1.183	−0.025 (−0.39)	0.128 (0.47)			0.37	3,75	14.8	0.000

changes in either temperature or salinity (Table 3). Therefore, for these 2 species, changes in protein, carbohydrate and lipid content of the eggs were not observed as a mechanism to modify egg buoyancy. This result is in agreement with those that considered the function of lipid in the eggs as nutritive and not as hydrostatic (Craik & Harvey 1987). As vertical distribution of pelagic eggs is more influenced by wind-induced mixing than by egg buoyancy (Coombs et al. 1985, Sundby 1991), to assure optimal buoyancy, it is probably enough to produce eggs of a density which allows the eggs to stay buoyant in the upper layers according to the prevailing range of salinity in the spawning areas, additional strategies such as changes in the biochemical composition of the eggs not being necessary.

The variation in the egg biochemical composition observed for these 2 species could be due to the influence of other factors, such as parental effect. Other studies have shown that female size and spawning time affect egg size, fecundity and egg viability (Buckley et al. 1991b, Kjesbu et al. 1992).

Conversely, larval size, temperature and salinity were found to be important factors which affected the biochemical composition of larvae in *Sardina pilchardus*, *Trachurus trachurus* and *Engraulis encrasicolus* (Table 5). The trade-off observed between protein and lipid as temperature changed (Fig. 2) could be explained as a response in order to achieve optimal larval buoyancy. From total organic investment either a higher proportion of protein or lipid is synthesised according to prevailing buoyancy conditions.

The differences found between egg and larval stages in protein, carbohydrate and lipid contents, as water density changed, could be explained according to some of these assumptions: in the egg stage it is not possible to modify the biochemical composition; to achieve optimal buoyancy in the larval stage is of higher value in the recruitment process than in the egg stage; and finally, as has been mentioned above, vertical distribution of pelagic eggs is mainly influenced by wind-induced mixing, possible changes in egg buoyancy due to possible variations in egg biochemical composition being of minor importance.

Larval abundance could affect the biochemical composition of larvae due to a possible food depletion at high larval densities. Although some of the larval densities found in this study for the 3 species were similar to the maximum values observed in this area (Dicenta 1984), larval abundance was not as important a factor as larval size and temperature and, moreover, the significant relationships observed showed no clear pattern of the possible influence of intraspecific competition on larval protein, carbohydrate and lipid contents in *Sardina pilchardus*, *Trachurus trachurus* and *Engraulis encrasicolus* (Table 5).

Larval carbohydrate content was not affected by changes in water temperature and salinity. This component was found to be size-dependent in the larval stage. The proportion of carbohydrate was very low in both eggs (Table 2) and larvae (Table 4) when compared with the amounts of protein and lipid. It seems this component is not an important source of energy

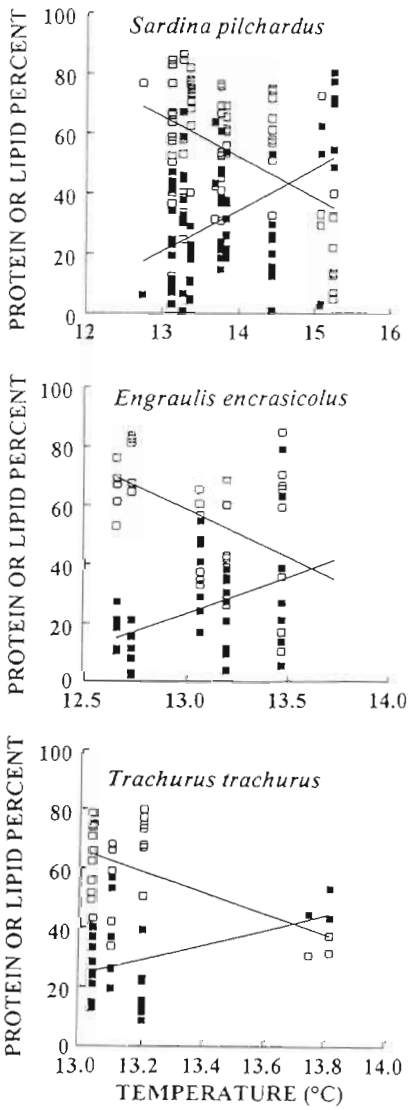

Fig. 2. Relationships between percentage of protein (■) and lipid (□) and mean temperature of the whole water column for *Sardina pilchardus* (for protein $r^2 = 0.21$, $F_{1,77} = 20.3$, $p < 0.001$ and for lipid $r^2 = 0.22$, $F_{1,77} = 22.1$, $p < 0.001$), *Trachurus trachurus* (for protein $r^2 = 0.28$, $F_{1,24} = 9.6$, $p = 0.005$ and for lipid $r^2 = 0.17$, $F_{1,24} = 4.8$, $p = 0.037$) and *Engraulis encrasicolus* (for protein $r^2 = 0.22$, $F_{1,34} = 9.9$, $p = 0.003$ and for lipid $r^2 = 0.16$, $F_{1,34} = 6.5$, $p = 0.015$)

during embryonic and larval development, the main proportion of total carbohydrate probably being structural (Heming & Buddington 1988).

In summary, the variation found in egg protein, carbohydrate and lipid content of *Scomber scombrus* and *Trachurus trachurus* was not related with changes in either egg size, egg stage or egg abundance, nor was it a mechanism to modify egg buoyancy. However, a high proportion of the variation found in larval biochemical composition was explained by changes in larval size, temperature and/or salinity. An increment in larval organic content of *Sardina pilchardus*, *T. trachurus* and *Engraulis encrasicolus* was observed as larval size increased. However, a higher proportion of either protein or lipid was synthesised according to water density, probably as a strategy directed toward achieving optimal larval buoyancy.

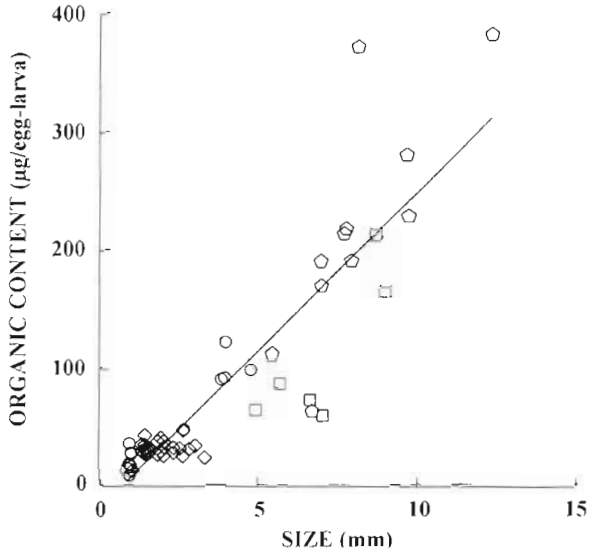

Fig. 3. Relationship between egg and larval organic content (*O*, combined total protein, carbohydrate and lipid content in µg) and size (*S*, in mm) for *Sardina pilchardus* (○), *Trachurus trachurus* (○), *Scomber scombrus* (◇) and *Engraulis encrasicolus* (□). $O = -18.827 + 26.673S$, $r^2 = 0.8$, $F_{1,55} = 219.7$, $p < 0.001$

Acknowledgements. We thank J. M. Cabanas for supplying temperature and salinity data for the field stations, M. Varela and I. Soler for useful help in the laboratory, the ichthyoplankton group of I.E.O. of Madrid for supplying egg and larval abundance data and for their help during both cruises, and 3 anonymous referees for their useful comments.

LITERATURE CITED

Anderson JT, DeYoung B (1995) Application of a one-dimensional model to vertical distributions of cod eggs on the northeastern Newfoundland Shelf. Can J Fish Aquat Sci 52:1978–1989

Anonymous (1994) Report of the Mackerel/Horse mackerel egg production workshop. ICES CM 1994/H:4

Buckley LJ, Smigielski AS, Halavik TA, Caldarone EM, Burns BR, Laurence GC (1991a) Winter flounder *Pseudopleuronectes americanus* reproductive success. I. Among-location variability in size and survival of larvae reared in the laboratory. Mar Ecol Prog Ser 74:117–124

Buckley LJ, Smigielski AS, Halavik TA, Caldarone EM, Burns BR, Laurence GC (1991b) Winter flounder *Pseudopleuronectes americanus* reproductive success. II. Effects of spawning time and female size on size, composition

and viability of eggs and larvae. Mar Ecol Prog Ser 74: 125–135

Coombs SH, Fosh CA, Keen MA (1985) The buoyancy and vertical distribution of eggs of sprat (*Sprattus sprattus*) and pilchard (*Sardina pilchardus*). J Mar Biol Assoc UK 65: 461–474

Craik JCA, Harvey SM (1987) The causes of buoyancy in eggs of marine teleosts. J Mar Biol Assoc UK 67:169–182

Dicenta A (1984) Aportación al conocimiento del ictioplancton de la costa vasca. Bol Inst Esp Oceanogr 1:94–105

Dubois M, Gilles KA, Hamilton JK, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

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

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

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

Heming TA, Buddington RK (1988) Yolk absorption in embryonic and larval fishes. In: Hoar WS, Randall DJ (eds) Fish physiology, Vol 11A. Academic Press Inc, Boston, p 407–446

Kjesbu OS, 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. J Fish Biol 41:581–599

Lee MA, Lee KT, Shiah GY (1995) Environmental factors associated with the formation of larval anchovy fishing grounds in the coastal waters of southwest Taiwan. Mar Biol 121:621–625

Lockwood SJ, Nichols JH (1977) The development rates of mackerel (*Scomber scombrus* L.) eggs over a range of temperature. ICES CM/J:13

Lowry OH, Rosenbraugh NJ, Farr AL, Randall RJ (1951) Protein measurements with the Folin phenol reagent. J Biol Chem 193:256–275

Markwell MAK, Haas SM, Bieber LL, Tolbert NE (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem 87:206–210

Miranda A, Cal RM, Iglesias J (1990) Effect of temperature on the development of eggs and larvae of sardine *Sardina pilchardus* Walbaum in captivity. J Exp Mar Biol Ecol 140: 69–77

Nissling A, Westin L (1991) Egg buoyancy of Baltic cod (*Gadus morhua*) and its implications for cod stock fluctuations in the Baltic. Mar Biol 111:33–35

Page F, Frank KT, Thompson KR (1989) Stage dependent vertical distribution of haddock (*Melanogrammus aeglefinus*) eggs in a stratified water column: observations and model. Can J Fish Aquat Sci 46:55–67

Pipe RK, Walker P (1987) The effect of temperature on development and hatching of scad *Trachurus trachurus* L., eggs. J Fish Biol 31:675–682

Rønnestad I, Koven WM, Tandler A, Harel M, Fyhn HJ (1994) Energy metabolism during development of eggs and larvae of gilthead sea bream (*Sparus aurata*). Mar Biol 120: 187–196

Solá A, Motos L, Franco C, Lago de Lazós A (1990) Seasonal occurrence of pelagic fish eggs and larvae in the Cantabrian Sea (IIXc) and Galician (IXa) from 1987 to 1989. ICES CM 1990/H:26

Srivastava RK, Brown JA (1991) The biochemical characteristics and hatching performance of cultured and wild Atlantic salmon (*Salmo salar*) eggs. Can J Zool 69:2436–2441

Sundby S (1991) Factors affecting the vertical distribution of eggs. ICES Mar Sci Symp 192:33–38

Tamaru CS, Ako H, Lee CS (1992) Fatty acid and amino acid profiles of spawned eggs of striped mullet, *Mugil cephalus* L. Aquaculture 105:83–94

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 Sci 40:627–634

Watanabe T (1982) Lipid nutrition in fish. Comp Biochem Physiol 73(B):3–15

Zöllner N, Kirsch K (1962) Über die quantitative Bestimmung von Lipoiden (Mikromethode) mittels der vielen natürlichen Lipoiden (allen bekannten Plasmalipoiden) gemeinsamen Sulfophosphovanillin-Reaktion. Z Ges Exp Med 135:545–561

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: July 21, 1997; Accepted: January 2, 1998
Proofs received from author(s): February 19, 1998