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The characteristics of sea bass *(Dicentrarchus labrax)* eggs : description, biochemical composition and hatching performances

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Abstract

This paper concerns eggs of captive fish and gonads of wild fish, from the Brittany coast obtained in the 7-18 °C thermal range. The diameters of eggs, 1.07-1.32 mm are compared to those observed in other areas, especially along the Mediterranean coast. The duration of embryogenesis as a function of temperature and the efficiency of different incubators are discussed. The quality of eggs in relation with larval survival is discussed through the biochemical composition of the eggs collected in captivity compared to that of wild fish gonads. The absolute values of lipids, proteins, fatty acids, lipid classes, phosphatides and minerals measured, also the positive relations obtained between egg dry weight, diameters, mortality during incubation are presented as criteria of egg quality.

Keywords : Sea Bass, *Dicentrarchus labrax*, eggs, embryos, aquaculture.

Caractéristiques des œufs de bar (Dicentrarchus labrax) *description, composition et taux d'éclosion.*

Résumé

Ce papier rend compte d'une étude effectuée sur les œufs de bars captifs (*Dicentrarchus labrax*) et des gonades de bars sauvages, collectés sur le littoral breton où la température de l'eau de mer varie de 7 à 18 °C. Les diamètres des œufs (1,07-1,32 mm) sont comparés à ceux des œufs récoltés dans d'autres sites, en particulier des sites du pourtour méditerranéen. Une relation entre durée de l'embryogenèse et température est proposée. L'efficacité de plusieurs principes d'incubation est discutée. L'étude de la qualité des œufs, en relation avec la survie larvaire est abordée à travers l'étude de la composition biochimique des œufs pondus en bassins par comparaison à celle des gonades de poissons sauvages. Les teneurs en lipides, protéines, différentes classes de lipides, phosphatides, acides gras et minéraux, ainsi que les relations obtenues entre poids secs, diamètres et survie des œufs en incubation sont analysées et discutées comme pouvant être des critères de qualité des œufs.

Mots-clés : Bar, *Dicentrarchus labrax*, œufs, embryons, aquaculture.

INTRODUCTION

In captivity, the period of incubation of marine fish eggs carries various different risks. For any particular species, it is necessary to know the optimal conditions for normal embryogenesis, for the geographical area in question. These factors are already well defined for sea bass, *Dicentrarchus labrax*, in the Mediterranean coast. Here we describe a study carried out on this species on the Atlantic coast of France. In aquaculture, there is a widespread need to define the quality of eggs and to predict larval rearing performances.

We looked at two parameters: (1) biometric data of eggs in relation to the water temperature and biochemical composition of eggs and ovules; (2) since rearing conditions of broodstock (social relations, food, confinement...) are unnatural, we compared wild fish gonads with early eggs from captive fish. The gonads of captive fish are less easily obtained and were not analyzed.

MATERIAL AND METHODS

Eggs naturally fertilized in tanks were spawned by sea bass [caught from the wild and adapted to captivity for 4 to 6 years in experimental facilities situated in Brittany (Devauchelle, 1986)]. They were spawned under natural or artificial temperature and light conditions *i. e.* during normal or out of normal spawning periods. Each batch of eggs collected over the period 1976-1983 was submitted to microscopic observations. The data obtained from aliquots (minimum 20 eggs) of around 300 different spawns (=egg batches from 1 female) *i. e.* 78×10^6 eggs provide information on the shape, size, viability and hatching percentages of sea bass eggs.

Effect of temperature on period of embryogenesis

The experiments were conducted in an automatic incubation device described by Devauchelle *et al.* (1986) and designed for small numbers of eggs (from 100 to 2000) in a total volume of 1 dm^3 sea water at 35×10^{-3}. The duration of embryogenesis was precisely measured at 15 ± 0.2 °C. Its duration was also measured at 13, 15, 17 and 22 °C on three different batches of eggs from the two cell-stage to hatching. Each batch was divided into three replicates each containing 200 to 600 eggs.

Biochemical composition of eggs spawned in captivity compared to that of "wild" gonads

Samples of eggs and gonads were biochemically analyzed: five gonads from five mature wild females caught during the natural spawning period along the Brittany coast and 12 groups of morula-stage eggs of various quality obtained from normal and two out of normal spawning seasons obtained from wild sea bass adapted to captivity for 5 to 6 years. The proximal composition of eggs, lipid classes, fatty acids, phosphatides, and minerals were estimated by techniques already described (Devauchelle *et al.*, 1987).

Relationship between egg characteristics

Relationships between seven egg characteristics (diameter, wet weight, dry weight, water content, viability, hatching and deformity percentages were evaluated on 17 different batches of eggs issued from natural and shifted spawning seasons. The temperature of the sea water was recorded at the spawning time. The dry weight was obtained by store drying at 110 °C for 24 hours. The viability was expressed as the percentage of eggs normally developed at morula stage to the total number of eggs collected. Hatching was the percentage of hatching eggs to viable eggs. Deformity was the percentage of deformed larvae to total hatched larvae.

RESULTS

General description of eggs

The fecund egg is pelagic, spherical and translucent. It becomes deformed by half-way through the incubation period. At maturation, the eggs contain one to five globules (*fig.* 1) representing an average 2 to 3% of the total volume. Their water content is increased by an average of 25% at fertilization. A perivitelline space appears, 15 to 60 minutes post oviposition, whether or not the eggs are fertilized. The mean diameter of eggs collected at 9.5-16.5 °C, mostly at 13-15 °C, was 1.2 mm with 1.07 mm and 1.32 mm as minimum and maximum values. The mean variation of diameters among eggs collected over 12 years was ±6%. The viability percentage was estimated to be 89% and the hatching percentage from viable eggs 74%. Less than 5% of the one day old larvae has skeletal deformities.

Duration of embryogenesis

Stages of embryonic development at 15 °C are in *table* 1. At temperatures ranging from 13 to 22 °C, the relation between temperature (T °C) and the incubation duration (D in hours) was: $D = 414.455 - 119.728 \text{ Ln } T$ ($r = 0.928$). Between 13 and 17 °C, the development from the start of neurulation to hatching represents an average 22.4% of the total incubation duration. If temperatures changed the total duration of incubation, they did not modify the relative proportions of each stage.

Biochemical composition of eggs compared to those of gonads

The data were subjected to one way analysis of variance test. The water content was much higher in eggs than in gonads. The ash content of eggs was higher ($p < 0.01$) than that of gonads while total protein was lower in eggs than in gonads ($p < 0.01$). Total lipid was not significantly different. However, comparison of gonads with eggs spawned out of the normal spawning season (batches 5 to 12) gave significant difference in the total lipid ($p < 0.5$) (*table* 2).

Lipids were mainly represented by triglyceride and cholesterol ester and waxes (*table* 3). Phospholipids only constituted 22.4 to 27.4% of the total lipid. There were significant differences between eggs and gonads, for total phospholipid ($p < 0.05$) and for cholesterol ester+waxes ($p < 0.05$). Considering fatty acids (*table* 4), the relative proportions of Saturated Fatty

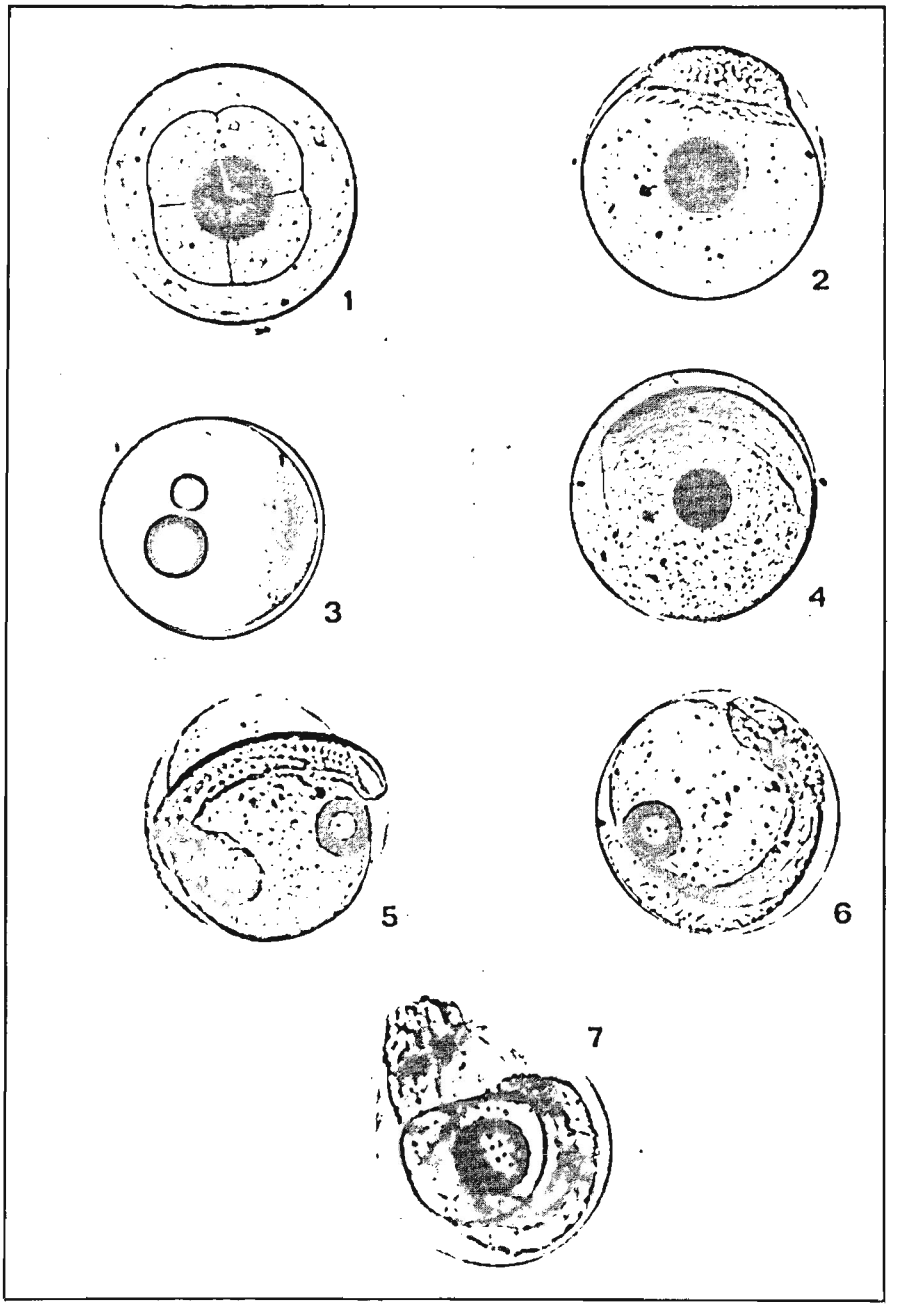

Figure 1. — Sea bass embryogenesis at 15±0.2°C. 1: Four cell-stage (30 min); 2: morula (8.20 hrs); 3: beginning of gastrulation (24 hrs.); 4: beginning of neurulation (34 hrs.); 5: embryo (45 hrs.); 6: the heart beats (74 hrs.); 7: hatching (115 hrs.).

Table 1. — Stages of embryo development of sea bass at 13, 15 and 17°C (hour).

Egg stage	Temperature (°C)		
	13	15	17
Morula	8.20	7.20	5.30
Beginning of gastrulation	24	19	16.40
Beginning of neurulation	34	24	21
Embryo in the half circumference	45	31	24
Heart beats	74	48	51
50% hatching	115±5 h	81±3 h	72±2 h

Acids (SFA) and Unsaturated Fatty Acids (UNFA) were similar in both groups. The lower total lipid (% DW) of gonads was the reason for lower SFA and UNFA levels when expressed as % DW ($p<0.05$). Among UNFA, the groups analysed were the $n-7$, $n-9$, $n-6$ and $n-3$ groups. The $n-7$ and $n-6$ groups were slightly but not significantly lower in eggs while the $n-9$ ($p<0.05$) and especially $n-3$ groups ($p<0.01$) were significantly higher in eggs.

The analysis of classes of phosphoaminolipids (*table 5*) indicated a strong predominance of the phosphatidylcholine and phosphatidyl ethanolamine. The

Table 2. – Proximal composition of sea bass eggs at morula stage and fonads of wild fish caught during the spawning season. Viability is a measure of the quality of the egg batches used.

	Sample	Viability (%)	Water content (%)	Total protein	Total lipid (% dry weight)	Ash
Eggs	1	100	89.6	49.2	22.5	7.2
	2	100	88.6	52.2	19.5	6.8
	3	100	89.5	54.2	30.8	6.4
	4	89	88.5	55.0	28.5	6.4
	5	92	87.9	51.5	34.6	5.1
	6	99	87.5	49.8	31.2	6.0
	7	85	88.9	52	33.6	6.4
	8	93	88.7	54.3	33.8	6.6
	9	96	88.7	51.1	35.1	6.7
	10	82	88.5	57.5	30.7	6.7
	11	88	87.1	41.7	32.6	6.1
	12	–	90.0	–	33.0	6.5
	Mean values ± S.E.		88.63 ± 0.22	51.68 ± 1.23	30.74 ± 1.29	6.41 ± 0.15
Gonads	13		68.8	63.4	26.5	4.3
	14		–	62.8	25.6	4.0
	15		65.9	62.5	27.2	4.6
	16		64.1	68.8	26.6	3.9
	17		65.4	62.8	26.0	4.1
	18		64.0	63.0	25.5	4.3
	Mean values ± S.E.		65.64 ± 0.94	63.88 ± 1.04	26.23 ± 0.28	4.20 ± 0.11

Table 3. – Lipid composition of sea bass eggs and gonads.

	Sample	Phospholipid	Triglyceride	Cholesterol	Cholesterol ester + waxes
			Total lipids (%)		
Eggs	3	23.9	31.6	4.4	40.6
	5	23.2	34.1	4.6	39.4
	6	23	35.2	4.6	38.8
	7	25.3	32.3	4.6	35.6
	8	22.8	34.4	5.3	36.5
	9	22.5	36.1	4.6	35.9
	10	22.5	34.1	6.1	38.6
	11	24.9	29.6	4.5	41.6
	12	22.4	28.4	3.8	41.6
	Mean values ± S.E.	23.4 ± 0.36	32.87 ± 0.86	4.72 ± 0.21	38.73 ± 0.77
Gonads	13	24.1	29.8	4.9	37.2
	14	27.4	30.4	4.8	35.4
	15	24.2	32.5	5.2	34.8
	16	24.3	28.4	5.3	38.7
	17	23.8	32.6	4.7	35.1
	18	25.9	33.1	4.8	33.3
	Mean values ± S.E.	24.94 ± 0.58	31.13 ± 0.77	4.95 ± 0.10	35.75 ± 0.78

levels were slightly higher in eggs for all the classes considered, except LPC, but only the PS levels were significantly lower in eggs ($p<0.01$); the LPC ratio was significantly higher in eggs ($p<0.01$). Some mineral levels differed: *table* 6. Calcium and magnesium were more abundant in eggs ($p<0.01$). Phosphorus was lower in eggs ($p<0.01$).

Relationship between several egg characteristics (*table* 7)

The data were submitted to statistical Bravais Pearson test (Sokal and Rohlf, 1969) which revealed the main correlation at $p<0.05$. Positive correlations were found between egg diameter and egg dry weight ($r=<0.597$) and between egg dry weight and percentage of deformities of newly hatched larvae ($r=0.558$) and a negative correlation was found between egg dry weight and hatching percentage of viable eggs ($r=-0.551$). No correlation was evident between temperature and egg diameter.

DISCUSSION-CONCLUSION

Most other studies of sea bass eggs give descriptions and information concerning incubation period similar

Table 4. — Fatty acid composition (F.A.) of sea bass eggs and gonads.

	Sample	Total F.A./ Total lipids (%)	Dry weight (%)	Saturated F.A. Dry weight (%)	Unsaturated F.A. (% dry weight)			
					$n-7$	$n-9$	$n-6$	$n-3$
Eggs	1	64.6	16.5	3.09	1.91	4.45	0.57	5.45
	2	63.1	12.3	2.08	1.39	3.30	0.41	4.13
	5	68.9	21.7	4.59	2.1	5.52	0.74	7.73
	6	70	19.0	4.02	1.82	4.76	0.66	7.03
	7	68.3	20.6	4.81	1.98	5.03	0.70	7.63
	8	70.3	22.8	4.81	2.57	5.85	0.71	7.97
	9	70.2	23.0	4.88	2.41	5.94	0.74	8.06
	10	69.4	19.0	4.56	1.69	4.57	0.57	6.76
	11	65.6	20.4	3.73	2.63	5.22	0.63	7.18
	12	65.3	19.6	3.73	2.90	4.30	0.84	6.83
	Mean value ±S.E.	67.6±0.71	19.5±1.0	4.03±0.29	2.14±0.15	4.90±0.25	0.66±0.04	6.28±0.20
Gonads	13	66.9	16.9	3.23	2.06	3.92	0.82	6.10
	14	66.5	17.0	3.49	3.28	3.90	1.02	4.53
	15	67.5	16.5	3.07	2.11	4.30	0.73	5.5
	16	66.6	16.1	3.0	2.73	3.90	0.53	5.11
	17	68.4	15.8	3.16	2.39	4.27	0.97	4.60
	18	68.4	15.4	3.1	2.49	3.84	0.55	4.68
	Mean value ±S.E.	67.38±0.35	16.28±0.26	3.18±0.07	2.51±0.18	4.02±0.09	0.77±0.09	5.4 ±1.37

Table 5. — Lipid phosphorus content of sea bass eggs and gonads. The phosphorus contents of the various phosphatides are expressed as percentage of total lipid phosphorus. LPC, lysophosphatidylcholine; SPH, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; DPG, diphosphatidylglycerol; AP, phosphatidic acid.

	Sample	Total lipid phosphorus (% dry weight)	LPC	SPH	PC	PS	PI	PE	DPG	AP
			(% lipid phosphorus)							
Eggs	1	0.20	1.35	2.89	71.51	0.79	3.58	16.55	1.51	0.75
	2	0.17	3.58	3.51	68.40	0.80	6.40	13.97	2.23	0.95
	3	0.27	1.60	2.22	75.26	0.79	4.58	13.98	0.54	0.69
	4	0.25	1.11	2.20	75.30	0.79	3.36	13.62	1.75	0.82
	5	0.29	3.78	3.31	85.02	0.4	1.69	8.91	1.75	0.67
	6	0.27	1.88	2.51	76.19	0.5	3.13	11.59	2.01	0.56
	7	0.29	1.99	2.12	80	0.38	1.54	11.35	2.05	0.10
	8	0.31	2.42	2.85	73.4	0.68	1.98	14.38	1.76	0.5
	9	0.33	3.27	2.94	74.25	0.78	3.46	11.37	2.10	0.59
	10	0.31	4.28	4.54	74.59	1.14	3.41	8.38	1.43	0.35
	11	0.32	2.30	3.12	77.89	0.61	2.51	11.70	1.11	0.75
	12	0.25	3.13	5.04	72.32	0.70	2.22	12.04	3.52	1.04
	Mean value±S.E.	0.27±0.02								
Gonads	13	0.29	8.75	3.72	73.90	1.55	1.47	10	–	0.62
	15	0.25	9.24	2.25	69.91	1.58	1.03	15.56	–	0.43
	16	0.25	6.57	2.36	79.81	1.04	0.76	8.42	0.52	0.43
	17	0.24	11.03	2.68	73.68	1.36	0.89	9.37	0.43	0.55
	18	0.26	8.47	2.62	77.83	1.12	1.17	8.14	–	0.65
	Mean value±S.E.	0.26±0.09								

to ours for eggs incubated in large tanks. Small static incubators, both the incubation period (Marangos *et al.*, 1986), and the hatching process are longer (Huelvan, 1982). The duration of fertilization and hatching processes as well as the number of abnormalities of mitoses can be increased under stressful incubation conditions (Blaxter, 1969; Rosenthal and Alderdice, 1976; Hempel, 1979; Kjorsvisk *et al.*, 1984) (low oxygen levels, bacteria infections, mechanical shocks, etc.) or with eggs of poor quality (Marangos *et al.*, 1986). Thus it is not surprising to find differences in the embryogenesis of eggs developed in very different incubation systems. However in general, egg development is not a major problem in the rearing of sea bass. According to Marangos *et al.* (1986), more than 50% of the viable eggs hatch at temperatures ranging from 8 to 20 °C at salinity 47×10^{-3}, from 8 to 14 °C and salinities from 29 to 47×10^{-3}. Long incubation

Table 6. — Mineral composition of sea bass eggs and gonads.

	Sample	Calcium	Phosphorus	Magnesium 10^{-6} dry weight	Iron	Zinc	Copper	Manganese
Eggs	1	1500	9400	1060	90	110	19	*
	2	2520	9400	709	68	145	34	*
	3	708	9400	1050	38	123	19	*
	4	1590	9700	1071	53	159	27	*
	5	815	7845	6616	57	137	18	*
	6	1260	7885	907	81	134	16	*
	7	559	8450	1190	27	95	18	*
	8	808	3390	591	17	304	17	*
	9	784	2500	914	52	103	17	*
	10	2144	9730	658	135	99	18	*
	11	876	8180	785	74	140	17	*
	12	1670	6630	979	63	95	21	*
	Mean values	1270	7709	878	63	137	20	
Gonads	13	250	11500	410	59	132	42	*
	15	386	10900	357	65	136	12	*
	16	219	11100	338	47	136	19	*
	17	155	12100	457	63	149	29	*
	18	218	11300	380	58	127	50	*
	Mean values	246	11380	388	58.4	136	30.4	

* not detectable.

Table 7. — Characteristics of sea bass eggs. Temperature = rearing temperature of broodstock at spawning. Diameter (±0.01 mm) S.E.

Batch n°	Temperature (°C)	Diameter (mm)	Wet weight (10^{-3} g)	Dry weight (10^{-3} g)	Water content (%)	Viability (%)	Hatching from viable eggs (%)	Deformities (%)
1	9.5	1.22	0.930	0.124	86.6	91	98	32.8
2	9	1.19	0.883	0.105	88.1	100	82	18.8
3	9.5	1.25	0.560	0.122	78.2	84	–	–
4	10	1.27	1.016	0.113	88.8	99	44	4.4
5	11	1.17	0.807	0.099	87.5	95	78	1.9
6	10.5	1.23	0.981	0.122	87.6	100	–	–
7	9.1	1.33	1.233	0.147	88.1	87	21	25.0
8	10.5	1.22	0.899	0.129	85.7	100	24	62.6
9	10.2	1.19	0.884	0.132	85.1	100	15	38.7
10	10.3	1.25	1.029	0.135	86.9	96	81	28.7
11	11.1	1.30	1.136	0.140	87.7	95	47	12.0
12	11.7	1.15	0.836	0.107	87.2	96	56	23.4
13	12	1.29	1.219	0.142	88.3	97	51	40.1
14	11.8	1.24	0.951	0.120	87.4	90	91	–
15	11.9	1.16	0.899	0.107	88.1	97	–	–
16	11.7	1.20	1.048	0.094	91.0	97	88	3.4
17	11.9	1.14	0.826	0.109	86.8	100	–	–

periods are inadvisable as bacterial contamination can become a problem, especially when high densities of incubation are practised. In such a situation, it is necessary to change the incubator water regularly as in the case of halibut (Kjorsvisk *et al.*, 1984). Incubation at 13-17 °C and salinity $35-38 \times 10^{-3}$ gave satisfactory results, with hatching percentages always close to 80-90% and low percentages of deformity.

Eggs diameters vary from place to place. In Brittany, values from 1.07 to 1.32 were obtained (Devauchelle, 1986). Along the Mediterranean coast, the eggs are smaller (Villani, 1974; Barnabé, 1976): 1.02–1.296, while the largest eggs are obtained from the North Sea, up to 1.386 (Kennedy and Fitzmaurice, 1972). It was suggested by Ware (1975) that egg diameter may be related to water temperature and/or food availability. This has not been demonstrated experimentally but observations (Devauchelle, 1980) suggest that eggs spawned from normal spawning season broodstock maintained at constant temperature are smaller than those from broodstock submitted to the natural thermal cycles especially low winter temperatures.

Until now, no clear relationship has been found between the egg diameter and the larval performance, although, such a relationship has often been discussed

for marine fish, salmonids and freshwater fish (Blaxter, 1969; Hempel, 1979). Thus egg diameter still cannot be considered as an indicator of larval quality. Rana (1987) considered that the egg size had little influence on the onset of feeding but could modify the resistance of fish to the stressful environment of the rearing tanks; the egg dry weight which is correlated with both diameter and hatching percentage might be a better indicator of quality. Similar observations could be made for total proteins and total lipids. Craik and Harvey (1984) demonstrated that the wet weight, dry weight, lipid or protein contents were positively linked to the rainbow trout survival before first feeding. The aim of this study was not to prove a such relationship, but to examine how the egg composition might be used in further research in sea bass broodstock management and larval rearing. Such data may be useful in the definition of quality, for commercial hatcheries and in the improvement of larval nutrition. Compared to turbot (*Scophthalmus maximus*) and sole (*Solea solea*) which are also involved in European aquaculture projects sea bass, has two peculiarities: egg protein levels are higher and egg lipid levels lower (Devauchelle *et al.*, 1987, 1988). Moreover phospholipids, which represent around 40% of the total lipid in flatfish, only reach 20 to 24% in sea bass eggs. In addition, the level of total lipid seems to be affected by the rearing conditions. The composition of eggs spawned during the normal season is closer to that of gonads, while eggs spawned outside the normal spawning season contain more lipid. Since, during gametogenesis, the oocytes first accumulate lipid, then protein (Caporiccio, 1976), the stage of maturity of the gonads cannot explain such differences. Rather the relative ratio of lipid to protein seems to be inadequate in the spawners food. Since in captivity physical activity is low, the fish do not metabolize their reserves and probably accumulate more lipids than in the wild. Previous work has shown a harmful effect on the larval rearing of too high lipid reserves in eggs of molluscs (Gallager and Mann, 1986). For fish, Luquet and Watanabe (1986) pointed out controversial results of the literature concerning the effects of food energy and egg quality. As long as these relations will remain vague, we think that the biochemical composition might be a good quality criterion. On the other hand, such criteria can also be obtained by physical shocks as already tested by Devauchelle (1980) and Divanach (1985).

The analysis of inorganic and amino-phospholipid composition showed large differences between eggs and gonads. The stage of maturity of the gonads and eggs have to be taken in account here. It is well known that changes in levels of water and ions occur during maturation, ovulation and fertilization (Holliday, 1969; Potts and Rudy, 1969; Davenport *et al.*, 1981; Craik and Harvey, 1987). Embryogenesis is also a period of high variation in phospholipid content. Since the ratio of different aminophospholipids might change very easily depending on the precise stage of eggs considered, the differences in *table* 5 have to be treated with caution.

The problems of ability of eggs to develop in larvae with high growth or survival rates are now important for the aquaculture development. As emphasized by Billard (1987), many practical problems raised in these areas (biology of gametes, fertilization, embryogenesis, hatching, chorion fragility...) have been neglected in studies of fish reproductive physiology. We hope that these field work will soon be more attractive to scientists.

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