Aquaculture, 66 (1987) 125–147
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

Spawning of Sole (*Solea solea*) in Captivity

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(Accepted 9 February 1987)

ABSTRACT

Devauchelle, N., Alexandre, J.C., Le Corre, N. and Letty, Y., 1987. Spawning of sole (*Solea solea*) in captivity. *Aquaculture*, 66: 125–147.

Sole eggs have been naturally spawned and fertilized in captivity for the last 12 years. Spawners, submitted to natural temperature and photoperiod, produced 17 300 000 eggs in 229 different batches. Those for which the spawning season was shifted by artificial combinations of temperature and light produced 1 900 000 eggs in 55 different spawnings.

Each batch of eggs was closely observed in order to study the optimal conditions required for gametogenesis and spawning. This article gives data on egg weight, diameter, biochemical composition and viability rate. The embryonic salinity and temperature tolerance is also discussed on the basis of experiments run in very efficient automatic open water circulation incubators.

INTRODUCTION

Since it is not feasible to base the rearing of sole on viable embryos or fry collected from the wild (Fabre-Domergue and Bietrix, 1905; Girin, 1979; Divanach, 1985) controlled reproduction of captive spawners was developed in the period 1965–1970, parallel with the setting up of larval rearing programs in Europe. The efforts were mostly focused on larval and juvenile stages. Thus, maturation and spawning were successfully completed in different areas, either naturally (Shelbourne, 1968; Flüchter, 1970; Fluchter and Trommsdorff, 1974; Irvin, 1974; Fonds, 1979) or after hormonal induction of maturation (Brasola, 1974; Ramos, 1977; Villani, 1977), but always on a small scale. These authors provided very interesting but, for aquaculture purposes, insufficient information. Today, rearing activities require the production of large numbers of eggs all year round.

As a consequence it is necessary to get a better knowledge of maturation, spawning and incubation processes of sole in order to develop reliable production of viable embryos. For this purpose, the performance of spawners maintained in different situations was monitored. The first target was to obtain newly hatched larvae under natural conditions of temperature and light. Later

0044-8486/87/$03.50

on, since there was much interest in shifting the spawning seasons, the effect was tested of artificial combinations of temperature and light on the spawning season. Embryos, whose characteristics often change according to many external or internal factors (Blaxter, 1969; Hempel, 1979) were also submitted to experiments in order to determine factors affecting quality and thus improve incubation success. Major results which were collected in 12 successive years for *Solea solea* are presented.

MATERIAL AND METHODS

Part 1

Two groups of spawners were studied. They were trawled or seined in the wild and then placed at low stocking densities (0.65 to 3 kg/m^3) in 7-m^3 to 17-m^3 circular tanks. The sex ratio could be determined after 1982, when sexing methods were improved (Devauchelle, 1984). The fish were maintained on a drained sand bottom (Girin, 1979; Devauchelle, 1980). Seawater was pumped directly from the entrance of the Bay of Brest, Brittany, and filtered across Lacron sand filter when seawater supplying the broodstock tanks had to be heated or cooled through exchangers, and when the incubator facilities were used, whatever the water temperature. The flow rate was adjusted to one-tenth of the total volume of the tanks per hour. No recirculating system was used. One group of spawners (I), kept in the 17-m^3 tank was submitted to natural variations of temperature and light except during June 1983 when the seawater was cooled to 11–12°C. The other group (II) was maintained under artificial cycles for 7 years.

The experiment began in October 1976 by abruptly advancing the daylength and temperature conditions by 4 months. In 1977, normal cycles were regularly contracted to 10 instead of 12 months, in order to reach, at the end of 1977, cycles advanced by 6 months.

Thereafter, the spawners were maintained under artificial photoperiodic cycles of 12 to 13 months duration. From the results of previous experiments, the temperature cycle was considered to be less important for the initiation of gametogenesis. Therefore in the experiments carried out between 1980 and 1983 the temperature levels were decreased and variations were not cyclical. These fish, supposed to spawn out of the normal spawning season, were kept in a 13-m^3 tank between 1976 and 1979 and in a 7-m^3 tank after this period.

Artificial light was provided by six fluorescent tubes of 40 watt each. The average light intensity was 1500 lux at the surface. Artificial temperature ranging from 8 to 18°C was obtained through heat exchangers.

Sole spawners were fed ad libitum with fresh molluscs (*Callysta chione, Glycimeris glycimeris, Laevicardium crassum*) and polychetes (*Nereis diversicolor,*

Nephtys hombergii) from 1972 to 1982. Thereafter, frozen molluscs (*Glycimeris glycimeris*) were the exclusive feed.

Since sole spawners are very sensitive to the development of *Endobtella solea*, an ectoparasite, they were systematically treated once or twice a year during summer and autumn, in a formalin bath. The fish were placed for 3 h in small volumes (100 to 200 liters) of aerated seawater containing 300 ppm of pure formalin and 1 ppm of Malachite green. Sometimes the treatment had to be repeated three times, every other day.

The fish matured and spawned without human or hormonal help. The pelagic eggs were concentrated near the outflow in a plankton net device designed by Girin (1979). They were collected at the morula stage once or twice a day. Aliquots of each new batch were systematically sampled in order to estimate their characteristics: the number of eggs spawned was estimated by counting 3 to 6 aliquots of an average of 50 eggs each; the mean diameter was estimated on an aliquot of at least 20 eggs, and the viability rate on an aliquot of at least 50 eggs. Since the eggs become deformed at the beginning of neurulation (Devauchelle, 1976), their diameter was always estimated under the microscope before the end of gastrulation. Hatching and skeletal deformity rates of newly hatched larvae were systematically followed during seven spawning cycles: five normal and two shifted cycles. Thus, aliquots of each batch of eggs were sampled and placed in 0.1-liter incubators. These units, made of PVC tubes and plankton net (250 μm), were maintained floating with small polystyrene floats in rectangular tanks containing 120 liters and directly supplied with running filtered water at the rate of 0.4 l/min. Each incubator received 100 to 600 eggs. Incubation temperature was adapted to the temperature of the spawning tank, i.e. 9 to 12°C. Artificial light provided an average intensity of 2000 lux at the surface of the incubators. Daylength was adapted to that of the spawning tanks. No antibiotic or antifungal treatment was given during the incubation period.

Part 2

Eggs and gonad analyses were conducted in order to provide basic data on their proximal composition and to determine the relative composition of the different lipid classes and oligoelements or minerals which could be involved in embryogenesis or energy transfer processes.

The egg composition was estimated on 40-g samples of viable embryos obtained from five different groups of eggs spawned during the 1980 spawning season. Their composition was compared to those of gonads sampled from females which were trawled near the hatchery site during the natural spawning season. Before analysis, egg and gonad samples were rinsed with distilled water and carefully dried on filter paper.

The moisture content was estimated after desiccation at 90 to 105°C to a constant weight. The protein content was determined by analyzing the total

nitrogen of fresh samples, using the Kjeldahl method. Ash was estimated by ignition for 12 h in a muffle-furnace (550°C). The total lipid content was determined by weighing after extraction in chloroform–methanol in the presence of ethylgallate and washing twice to eliminate non-lipid contamination, followed by vacuum evaporation of the solvents. Different groups of lipids were estimated: the phospholipids, by calorimeter, in comparison to total lipids and after separation by two-dimensional thin-layer chromatography; the cholesterol was determined in the non-saponifiable part of the total lipid; the triglycerides as well as the hydrocarbon fraction + the esters of cholesterol + waxes were estimated after separation on a column of acetyl salicyclic acid Kieselgel (Ref. Merck 7734). The fatty acids were evaluated by saponification with KOH and methylation with methanol. They were separated by chromatography.

The minerals and oligoelements Ca, P, Mg, Fe, Zn, Cu and Mn were determined by mass spectrophotometry.

Since phosphoaminolipids play an important role in the molecular architecture of the membrane configuration of the cells, the relative proportions of eight components were estimated (Table 7). Their values were expressed in percentage of the total deposited lipidic phosphorus, after separation by two-dimensional gel chromatography after elimination of the neutral lipids in the presence of diethyloxide.

Part 3

Relations between seawater temperature at spawning time and diameter, wet and dry weight, water content, viability and hatching rate of eggs were examined on 13 different groups of eggs samples over the natural 1981 spawning season. Five hundred to eight hundred embryos were carefully counted, rinsed with distilled water, dried on filter paper and weighed. The dry weight was estimated after 48 h in the oven (90°C). The other parameters were estimated following the above-mentioned indications.

Part 4

Accurate estimations were made of incubation duration and salinity/temperature tolerance of the embryos. The experiments were run in an automatic device (Devauchelle et al., 1986) which allows accurate controls in the range of 0–35‰ and 8–26°C. Embryonic development was first described at 15°C. Then the incubation duration was estimated for four developmental stages (morula, half blastula, end of gastrulation and time to 50% hatching) on three different groups of eggs at 13, 15 and 17°C. Three samples of 200 to 250 viable embryos per batch of eggs were placed at the 2-cell stage in small incubators containing 1 liter of water.

The combined effects of salinity and temperature were studied in a second

step, for the following values: 0, 5, 10, 15, 20, 25, 30 and 35‰ and 12, 14 and 16°C. Experiments were run on nine different batches of eggs divided into two to four samples of 200 to 250 viable embryos which were distributed in the 1 litre incubators. For each condition, tests were run on three stages: morula, gastrula and neurula. Before testing, the embryos were incubated at 35‰. Seawater temperature ranged from 12.5 to 16°C.

RESULTS

Part 1

A high mortality of wild spawners, following fishing, may occur during the first month of captivity. However, it depends directly on the fishing techniques: trawling usually induces 50% of fish deaths while seining causes only 20% mortality. After this period, mortality of spawners reached 10% a year. No difference between shifted and normal spawning season was noted.

During the first 3 years of captivity, egg production remained at a very low level. Spawners seemed to enter a refractory period. Females became swollen before the presumed spawning season but did not spawn efficiently. After this period, most females clearly became swollen 1 to 2 months before spawning and the number of collected eggs suddenly increased. Most often spawning occurred early in the morning.

The first spawnings of group I (Table 1) took place by the beginning of March when the temperature rose to 8–9°C. The last spawnings were recorded when the temperature reached 12–12.5°C, most often in mid-May. However, oviposition stopped before this data in the case of hot springs (1984) or went on longer when temperatures were naturally or artificially (1983) maintained at levels lower than 12°C. The effect of daylength on spawning seemed to be less evident: the photoperiod ranged from 11.00 to 16.00 h of light per day.

The mean annual number of eggs ranged from 1500 to 138 000 per batch and reached 140 000 eggs/kg female in 1983. The number of eggs collected varied widely between individual spawns with 1500 and 421 000 as extreme values. The peak of production corresponded to temperatures ranging from 10 to 10.5°C and to daylight lengths ranging from 11.50 to 12 h (Fig. 1).

The variations in production must be prudently interpreted from year to year, since the broodstock composition changed. However, it is clear that a winter seawater temperature above 10°C resulted in low production even when the temperature level allowed normal oviposition.

Annual mean diameters were rather similar and their small variations could not be correlated to the age of the female or to the number of eggs collected. However, the mean diameter of eggs tended to decrease with increasing temperatures and daylengths, over each spawning season. The recorded extreme

TABLE 1

Composition of group I: spawning conditions and egg characteristics

Year	Fish (no.)	Fish (kg)	Spawning period			Eggs (no.×10³)	Spawns (no.)	Diameter (mm)	Viability (%)
			Dates	Daylength (hours min)	Temperature (°C)				
1973	20	17	3/3- 4/5	11.00-14.40		78	9	—	—
1974	40		10/4- 3/5	13.20-14.40		3	2	—	—
1975	40	34	16/4-28/4	13.35-14.25		18	4	1.40±0.121	—
1976	40		7/3-27/4	11.15-14.00	8 -12	770	18	1.39±0.11	—
1977	40		5/3-27/4	11.15-14.50		840	23	1.33±0.114	91
1978	69		9/3-15/5	11.30-15.10		1990	24	1.38±0.09	91
1979	82		24/3-12/5	12.25-15.00		2390	23	1.41±0.11	73
1980	47	33	4/3-12/5	12.30-15.10	9.2-12	2600	29	1.39±0.117	95
1981	47	34	15/3-14/5	11.00-15.00	10 -11.8	3600	24	1.37±0.123	61
1982	43♂+24♀	51	11/3- 6/5	11.40-14.45	9.6-11.9	1400	22	1.39±0.116	73
1983	43♂+23♀	51	4/3-23/6	12.10-16.00	8.4-12	3160	51	1.36±0.096	56
1984	44♂+15♀	42	27/3-23/4	12.30-15.30	8.4-12	440	10	1.40±0.085	53

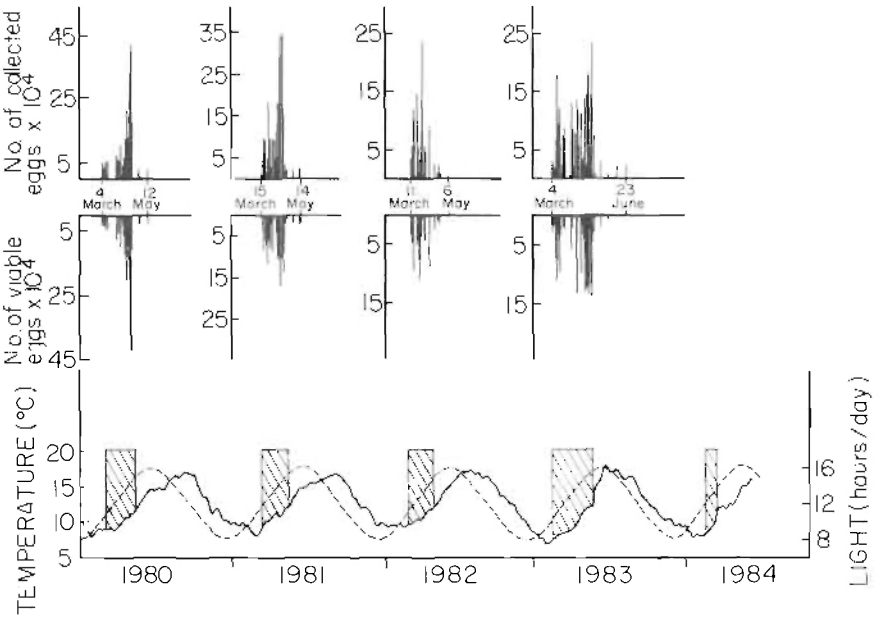

Fig. 1. Number and viability of each group of eggs of group I collected under natural light (--) and temperature (—) cycles.

diameters were 1.20 and 1.57 mm. The dispersion for a specific batch was weak: an average $\pm 1.8\%$ of the mean value.

Annual egg viability was always higher than 60% except during two seasons, 1983 and 1984, when polychetes were absent from the meals. During the spawning period, viability varied strongly from batch to batch, ranging from 0 to 100%. No correlation between the number of eggs collected, their diameter or the age of the spawner could be noted. However, a rapid decrease in egg quality was alway observed with temperatures higher than 11.5°C.

Finally, in group I, the weekly food consumption, estimated over a 2-year period, reached an average of 10% of mollusc flesh and 2% of polychete of the fish weight. The extreme values were 4 and 24 kg of mollusc flesh, corresponding to April and September, respectively. (Fig. 2).

Group II, for which light and temperature cycles were controlled, produced fewer eggs, with lower and, especially, more variable viability rates (Table 2). Spawning occurred at temperatures ranging from 8 to 15°C between 11 and 16.10 h of light per day (Fig. 3). The expected seasons were respected. Therefore the annual average number of eggs per batch was low: 14 000 to 33 000 when considering only the females which had been captive for more than 3 years. The number of eggs collected did not exceed 114 000 per individual spawn. The maximum fertility recorded was 90 000 eggs/kg female (1983).

Annual diameters were close to those calculated for group I: 1.36–1.43 mm, with less marked extreme individual values: 1.31–1.44 mm. It was interesting

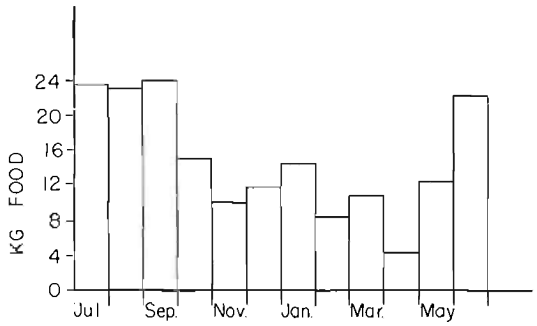

Fig. 2. Variations in mollusc flesh intake (kg) in group I.

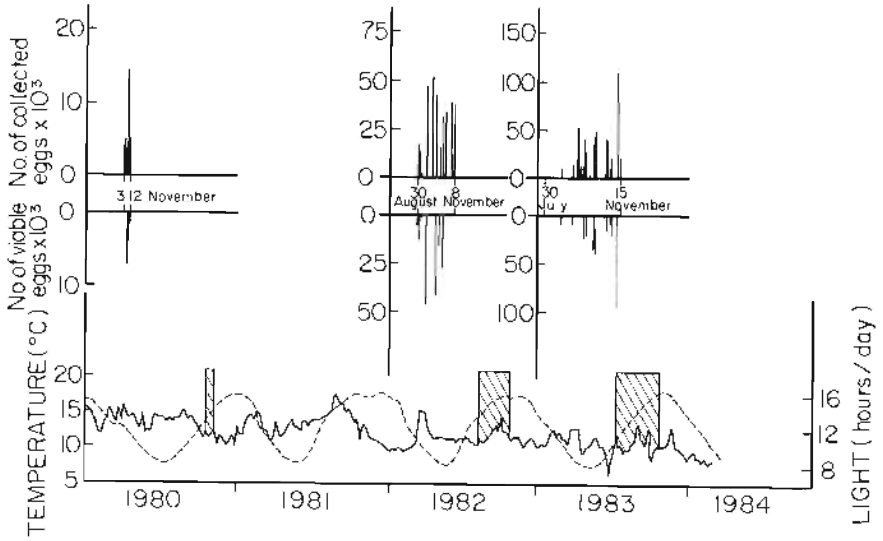

Fig. 3. Spawning frequency, number and viability of each batch of eggs of group II collected under artificial light (---) and temperature (—) conditions.

to notice that since controlled temperature did not increase during the spawning season, the egg diameters did not clearly decrease, as was the case with group I. When temperature and light cycles simulated the natural variations of Brittany (1978–1979), egg viability was acceptable. As soon as photoperiodic cycles were maintained without strong seawater cooling, the egg viability decreased. In 1979, temperatures as high as 14–15 °C, recorded during the maturation period, induced a short spawning period and poor egg quality, in spite of normal temperature levels during oviposition. Similar conditions recorded during gametogenesis, with maximum temperatures at the time when spawning should have occurred, resulted in no spawning. In 1982, 2.5 months before the expected spawning period, temperature was lowered to an average 11 °C. Technical incidents did not permit this level to be maintained throughout the spawning period. As a consequence, the quantity of eggs collected was in-

TABLE 2

Composition of group II: spawning conditions and egg characteristics

Year	Fish (no.)	Fish (kg)	Spawning period			Eggs (no.×10^3)	Spawns (no.)	Diameter (mm)	Viability (%)
			Dates	Daylength (hours. min)	Temperatures (°C)				
1978	39	18.5	11/9 - 3/11	12.00-16.00	10-12.5	312	12	1.36±0.105	62
1979	33	16.0	29/10-18/11	11.00-15.30	9.5-12	271	14	1.38±0.09	76
1980	26	16.5	3/11-15/11	13.20-13.40	11-12	185	13	1.37±0.11	14
1981	26	18	–						
1982	25	19	30/8 - 8/11	13.55-16.00	10.6-15	383	14	1.39±0.085	27
1983	8♂+7♀	12	30/7 -15/11	11.00-16.10	8-10.5	717	22	1.43±0.121	41

creased, but egg quality was rather poor. In 1983, since the temperature was lowered with more success during gametogenesis and half of the spawning period, the spawning results were immediately improved: good viability rates once more seemed to depend on temperatures lower than 11.5–12°C. Meanwhile the number of collected eggs could be related to the cold thermal regime which covered the maturation period, as previously observed for group I.

TABLE 3

Proximal composition of sole eggs at the morula stage, and sole gonads sampled from wild fish during the spawning season. The viability rate gives an indication of the quality of the batch from which the eggs samples were obtained

Sample	Viability (%)	Water content (%)	(% dry weight)		
			Crude proteins	Total lipids	Ash
Eggs					
a	100	91.9	67.4	11.3	16.1
b	100	92.2	67.4	13.8	9.8
c	100	92.4	63.1	14.8	9.7
d	56	92.2	69.5	12.1	7.2
e	86	93.8	71.5	11.5	5.7
Gonads					
f		64.1	75.0	18.5	5.6
g		67.8	72.5	20.1	6.3
h		63.5	74.6	19.1	5.2
i		66.6	73.3	19.1	5.3
j		64.3	73.3	20.1	5.3
k		69.7	74	19.2	5.7

TABLE 4

Different lipid classes in sole eggs and gonads

Sample	(% total lipids)			
	Phospholipids	Triglycerides	Cholesterol	Cholesterol ester + waxes
Eggs				
a	41.8	–	11.6	–
b	41.3	–	10.4	–
c	41.8	–	10.4	–
d	47.7	37.60	10.2	6.9
e	39.8	–	10.3	
Gonads				
f	40.7	39.3	11.7	11.4
g	34.3	44.4	12.3	14.1
h	41.1	39.2	11.7	12.0
i	41.1	37.3	12.8	14.2
j	41.2	39.8	11.4	10.9
k	41.7	36.3	12.0	10.9

Part 2

The proximal composition of eggs and gonads is indicated in Table 3.

Water content was higher in eggs than in gonads. The content of lipids and proteins was significantly higher in the gonads. The average ash percentage was higher in eggs than in gonads. However, the considerable variations in individual values were not found to be significant.

Lipids (Table 4) were represented mainly by phospholipids and triglycerides. The proportion of different lipid groups was similar in eggs and gonads, except for cholesterol ester and waxes.

Eggs contained lower fatty acids levels than gonads (Table 5). This observation has to be directly linked to the lower total lipid content. The proportion of saturated and unsaturated fatty acids, expressed as percentage of total lipid content was very similar in eggs and gonads, with small differences: the *n*-6 unsaturated fatty acid levels tended to be lower in eggs while other unsaturated fatty acid levels could be considered as slightly increased. However, no significant difference was noted. On the other hand, when these results were expressed as percentage of dry weight, the differences in egg and gonad composition were significantly different ($P<0.01$) for the *n*-3, *n*-6 and *n*-9 groups.

The analyses of minerals, oligoelements and phosphoaminolipids were made on six samples of gonads and only two samples of eggs. Consequently, the results could not be statistically tested.

Table 6 clearly shows the predominance of phosphorus which represents an average 1.13% of the dry weight. The variations in calcium and magnesium

TABLE 5

Different fatty acids in sole eggs and gonads

Sample	Total fatty acids		Saturated fatty acids (% dry weight)	Unsaturated fatty acids (% dry weight)			
	(% total lipids)	(% dry weight)		*n*-7	*n*-9	*n*-6	*n*-3
Eggs							
a	65.4	7.38	3.5	1.48	1.78	0.62	3.27
b	67.3	9.28	4.29	1.88	2.17	0.74	3.96
c	69.1	10.22	4.33	2.23	2.23	0.79	4.38
d	70.1	8.50	3.17	2.91	1.76	0.68	3.81
e	64.6	7.42	3.04	1.91	1.60	0.72	3.29
Gonads							
f	71.3	13.18	4.87	2.86	2.03	1.22	5.04
g	71.1	14.27	5.1	3.69	2.09	1.50	4.84
h	69.8	13.34	5.35	3.08	2.35	1.16	5.20
i	69.6	13.26	5.01	3.37	2.18	1.30	5.01
j	70.3	14.10	4.91	3.09	2.5	1.65	5.50
k	69.4	13.33	5.09	3.74	1.82	1.08	5.30

TABLE 6

Some oligoelements and minerals in sole eggs and gonads

Sample	(10^{-6} dry weight)						
	Calcium	Phosphorus	Magnesium	Iron	Zinc	Copper	Manganese
Eggs							
d	615	10800	948	769	115	*	*
e	1419	14840	1355	145	$\leqslant 81$	*	*
Gonads							
f	242	10270	446	36	89	*	*
g	311	11025	758	43	102	*	*
h	167	10000	230	30	77	*	*
i	269	12126	913	48	105	*	*
j	370	10364	807	36	90	*	*
k	403	10890	901	53	79	*	*

*Not detectable.

TABLE 7

Total phosphorus content of sole eggs and gonads and relative importance of various phosphoaminolipids, expressed as percentage of total lipidic phosphorus

Sample	Lipidic phosphorus (% dry weight)	(% lipidic phosphorus)							
		LPC	SPH	PC	PS	PI	PE	DPG	AP
Eggs									
d	0.27	1.63	3.99	76.21	1	3.10	12.03	1.6	0.43
e	0.28	1.67	3.25	73.98	0.73	2.52	15.74	1.84	0.29
Gonads									
f	0.26	1.84	4.62	78.27	0.85	1.89	9.24	2.92	0.38
g	0.21	1.36	4.98	70.09	1.06	1.66	17.80	2.31	0.60
h	0.28	3.15	4.84	69.43	0.76	2.57	16.80	2.04	0.41
i	0.28	2.58	5.87	74.18	1.20	1.92	10.96	2.64	0.66
j	0.29	2.99	6.79	72.83	1.15	1.90	11.21	2.58	0.54
k	0.28	2.75	3.98	70.70	0.60	1.85	17.78	1.89	0.47

LPC, lysophosphatidylcholine; SPH, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; DPG, diphosphatidylglycerol; AP, phosphatidic acid.

TABLE 8

Characteristics of 13 different egg batches spawned in captivity under natural conditions of light and temperature

Batch no.	Temperature (°C)	Diameter (mm)	Wet weight (10^{-3}g)	Dry weight (10^{-3}g)	Humidity (%)	Viability (%)	Hatching from viable eggs (%)	Deformities (%)
1	9.1	1.411 ± 0.019	1.4923	0.1237	91.7	82.6	–	–
2	9	1.412 ± 0.015	1.4528	0.1251	91.4	100	4	50
3	9.5	1.444 ± 0.026	1.4935	0.1299	91.3	66.3	21	8.2
4	9.5	1.404 ± 0.018	1.4170	0.1212	91.45	100	81	8.2
5	10.5	1.386 ± 0.020	1.4557	0.1209	91.70	81.25	13	13.3
6	10	1.446 ± 0.015	1.4945	0.1279	31.44	60.44	88	7.8
7	10.2	1.385 ± 0.015	1.4221	0.1365	90.40	97.06	74	16.8
8	10.1	1.408 ± 0.015	1.4468	0.1159	92	94.67	81	2.7
9	11.1	1.384 ± 0.023	1.4528	0.1244	91.44	42.86	24	15.6
10	11.6	1.381 ± 0.017	1.4506	0.1253	91.36	70.91	54	19.9
11	12.7	1.319 ± 0.023	1.2729	0.1115	91.24	75	81	6.6
12	11.7	1.325	1.3860	0.081	94.13	86	95	0
13	11.9	1.368	1.4981	0.1003	93.30	100	100	3

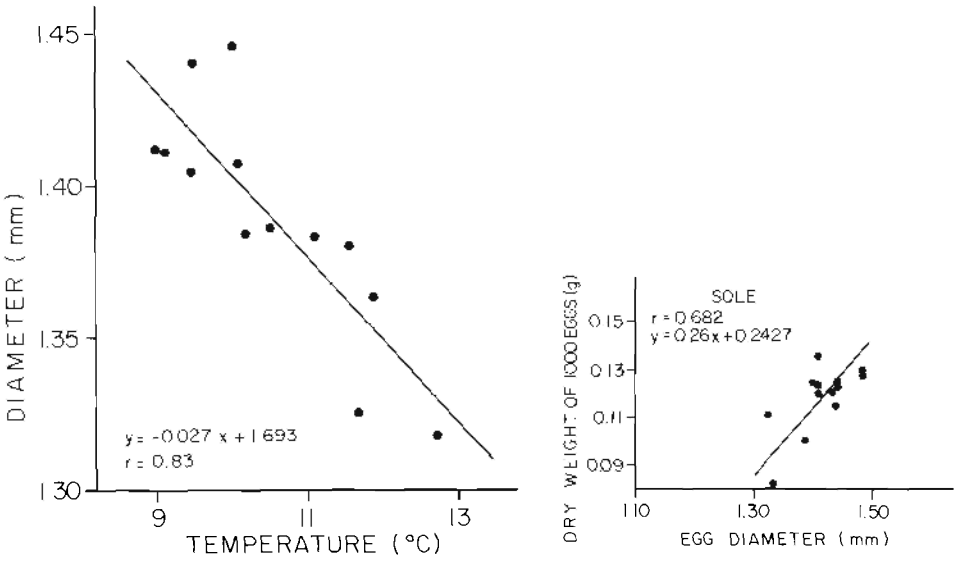

Fig. 4. Relation between the egg diameter and the recorded temperature of the seawater at spawning time.

Fig. 5. Relation between egg diameter and dry weight.

levels tend to reflect those of egg waxes. Magnesium is more abundant than calcium in the gonads. Unlike phosphorus, these levels differed markedly among samples. Iron and zinc were present in the eggs, but no copper and manganese were detected.

The analysis of different phosphoaminolipids (Table 7) indicated similar levels in eggs and gonads, with a strong predominance of phosphatidylcholine (=lecithine) and phosphatidylethanolamine. The variations in results suggest that in such investigations it is important to sample the eggs or gonads according to a very precise developmental stage.

Part 3

The results are presented in Table 8. Temperature and egg diameter were inversely related (Fig. 4). The dry weight tended to increase with the egg size (Fig. 5). The water content was stable and estimated at $91.76 \pm 2.08\%$. Apart from these observations, no other significant relations were discovered.

Part 4

The embryonic development is detailed in Fig. 6. At temperatures ranging from 13 to 17°C, the relation between temperature (T) and the incubation

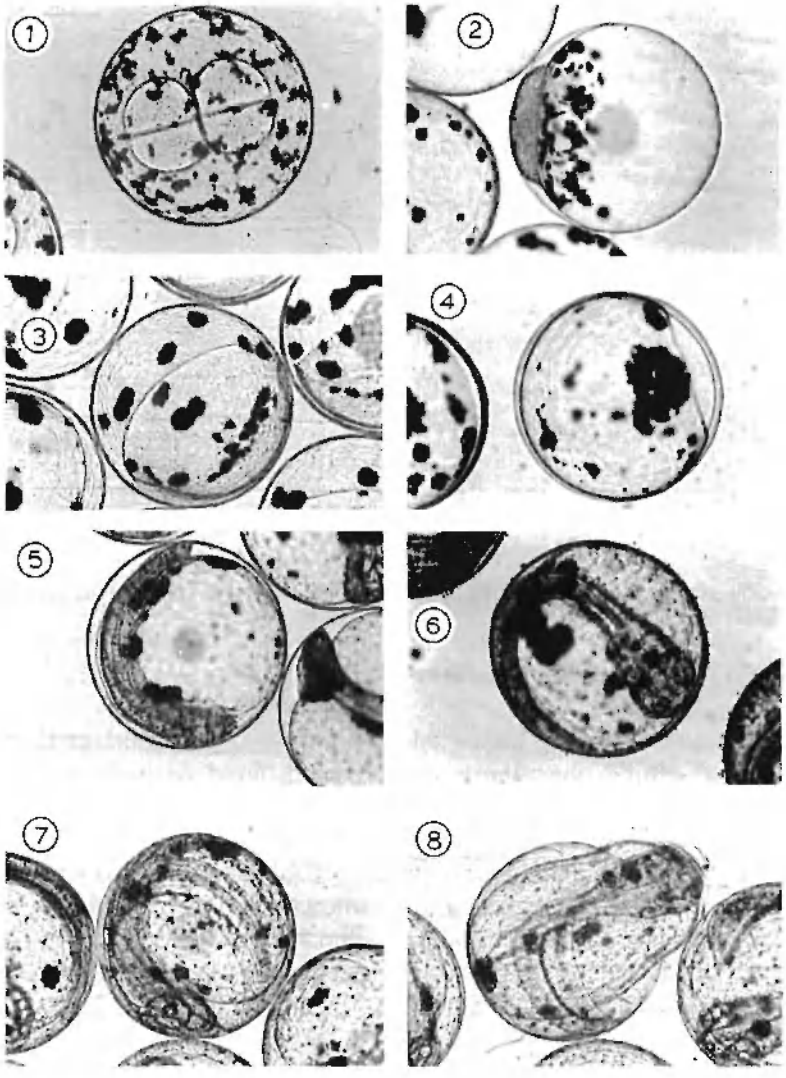

Fig. 6. Duration of embryogenesis of sole eggs incubated at 15°C, from 2-cell stage ($t=0$) to hatching. (1) 30 min: 4 cells. (2) 15 h: morula. (3) 20 h: half gastrula. (4) 30 h: end of gastrulation. (5) 38 h: neurula. (6) 50 h: the heart beats. (7) 80 h: prehatching; embryo often moves. (8) 82 h: hatching.

duration (D) was linear: $D=-9T+220.67$ ($r=-0.9522$). Among the four stages considered, morula duration seemed to be least influenced by temperature levels (Table 9). No effect of salinity on the duration of embryogenesis was detected.

The results of embryonic salinity and temperature tolerance are detailed in Tables 10 and 11 while Fig. 7 presents mean numbers of normal newly hatched

TABLE 9

Duration of four developmental stages of sole: a = beginning of morula; b = half blastula; c = blastopore closed; d = time to 50% hatching.

Temperature (°C)	Egg stage	Duration (hours.min)
13	a	5.20
	b	24.00
	c	29.00
	d	107 ± 7
15	a	5.15
	b	20.00
	c	27.00
	d	79 ± 4
17	a	5
	b	18
	c	21
	d	71 ± 2

TABLE 10

Hatching rates and percentage of deformed newly hatched larvae at various temperature/salinity/egg stage combinations

Temperature (°C)	Stage	Batch	Sample	Hatching (%)				Deformities (%)			
				Salinity (‰)				Salinity (‰)			
				10	15	25	35	10	15	25	35
16	Morula	A	1	63.6	91.4	90.8	94.7	100	73.1	36.2	14.1
			2	73.5	90.6	89.7	94.5	100	72.5	24.7	16.4
	Gastrula	A	1	85.6	93.1	96	–	50.8	24	24	–
			2	90.9	91.1	90.3	–	50.9	26.3	22.1	–
		B	1	16.3	88.5	87.5	87.1	100	18.5	9.8	8.7
			2	18.5	83.8	84.3	92.5	100	19.3	10.8	12.2
	Neurula	A	1	93.4	93	92.4	–	36	18.7	21.4	–
			2	92.4	88.1	95.4	–	45.2	24.2	21.9	–
		B	1	62.4	92.2	88.4	91.2	100	15.3	13.1	7.8
			2	59.1	94	91.2	91.7	100	26.3	15.8	9.6
12.5	Morula	A	1	86.7	91.9	93.8	90.6	73.9	38.5	15.3	17.1
			2	82.9	93.6	91.8	93.5	79.4	25.3	16.2	15.6
	Gastrula	A	1	94.8	94.1	92.9	–	51.1	15.8	14.9	–
			2	90.5	93.5	94.1	–	47.3	16.6	18.7	–
		B	1	84.1	83.2	88.6	88.3	68.9	17	16.7	18.4
			2	75.0	82.5	91.4	88.1	83.3	16.8	15.4	22.5
	Neurula	A	1	93.1	95.6	94.1	–	46.8	12.4	16.8	–
			2	94.5	94.2	97.4	–	39.6	13.6	14	–
		B	1	86	94.3	93.9	89.9	52.8	13.4	8.4	13.8
			2	82.3	90.4	93.6	93.2	57	19.1	8.8	9

TABLE 11

Hatching rates and percentages of deformed newly hatched larvae at various salinities and embryonic stages at 14°C

Stage	Batch	Sample	Hatching (%)							Deformities (%)						
			Salinity (‰)							Salinity (‰)						
			5	10	15	20	25	30	35	5	10	15	20	25	30	35
Morula	A	1	0	75.4	92.4	94	95.5	94	95.1	–	100	67.9	24.9	8.8	8.2	7.8
		2	0	75.6	88.9	93.6	93.5	94.9	81	–	100	70.6	23.6	14.8	9.2	18.2
	B	1	0	49.1	66.5	70.1	73.4	–	67.3	–	100	29.9	27.7	13.1	16.4	14.5
		2	0	61.3	75.2	74.3	78.1	70.4	75.6	–	100	19.9	20	14.8	8.5	11
	C	1	0	58	84.6	88	93.1	88.8	91.4	–	100	30.2	24.1	18.3	14.2	13.6
		2	0	67.1	80.5	86.9	89.1	92.5	87.2	–	100	38.5	34.4	12.7	12.9	15.9
	D	1	0	75.2	87.6	90.8	90.3	91.2	91.7	–	100	84.9	28.3	7.2	7.9	7.4
		2	0	76.7	90.1	91.7	92.4	91.3	91	–	100	72.5	32.1	6.8	7.5	11.8
	E	1	1.2	53.4	75.5	88.3	89	86.6	83.8	100	100	33.5	26	8.2	10.5	14.4
		2	0.9	49.2	75.6	85.2	79.1	86.4	81.1	100	99	36.8	21.7	16	9.4	14.7
Gastrula	E	1	1.5	83	91.3	95.5	92.6	94	94.5	100	93.4	29.2	16.3	9.4	14.4	14.2
		2	1.2	86.8	91.7	93.4	96.9	95.6	92.4	100	88	27.8	16.5	7	12.9	12
	F	1	0	91.2	93.1	95	95.1	91.2	91.5	–	92.5	19.1	6.6	2.3	3.2	10.3
		2	0	88.9	92.5	95.1	93.1	93.1	91.7	–	94	24.7	8.9	8.1	8.8	12.1
	G	1	3.3	73.9	99.3	99.2	98.6	99.3	100	100	100	15.9	15.3	19	14.8	22.5
		2	1.4	68.7	99.3	99.2	96.6	98.6	98.5	100	100	20.6	18.9	15.8	18	17.7
Neurula	E	1	7.7	98.4	100	99.3	99.3	99.4	100	100	50	11.3	7.4	2.8	3.3	5.3
		2	5.8	96.9	99.4	100	99.3	99.3	100	100	57.1	11	12.5	3.3	3.4	5
	G	1	21.8	69.4	98.4	100	100	98.5	100	100	100	15.3	10.5	10.4	12.3	9.6
		2	13.6	72.9	98.6	100	99.1	100	98.5	100	100	14.1	95.7	30.2	22.5	17.7

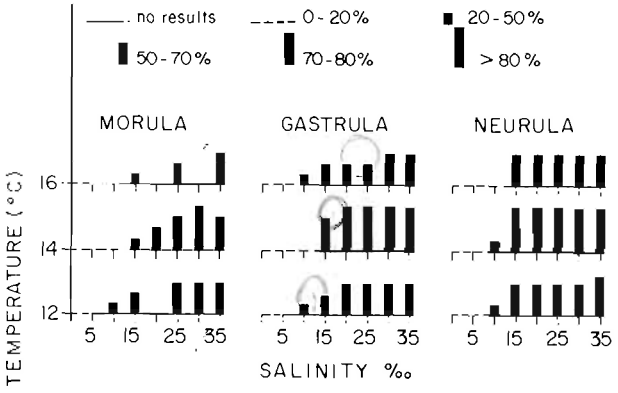

Fig. 7. Production of normal newly hatched larvae from viable eggs at different salinity/temperature combinations.

larvae. The best incubation performances were obtained at 14°C and 30–35‰. The tolerance increased with the age of embryos: at the neurula stage, more than 80% of viable eggs hatched to give normal larvae at 12, 14 and 16°C, for salinities ranging from 15 to 35‰. Similar results were recorded during gastrulation at 14°C and 20–35‰. At the morula stage only the combination 14°C/30‰ could be associated with this performance. In any case, the incubation processes were considerably disrupted at salinities below 10‰, and even at 14°C both hatching rates and deformities of newly hatched larvae were affected. On the other hand, at the morula stage (unlike the gastrula or neurula stages) deformities first increased at 15‰.

Salinity resistance depended on the temperature levels. At 14°C, best results were obtained at 35‰, while the number of normally hatched larvae was higher at 25–30‰ and 14°C, or 15–25‰ and 12.5°C.

When considering the differences between egg batches, it is seen that eggs were more sensitive under conditions which preserved the embryos from drastic mortality but did not allow high hatching values; i.e. 16°C/15–25‰, 14°C/15‰, 12.5°C/10–15‰. Since under these conditions, the highest mortality was recorded at the beginning of the tests, we suggest that they could be used to select egg batches of high quality. The duration of hatching processes could also indicate the quality of eggs; thus, an inverse relation between the two parameters was observed.

DISCUSSION

The duration of acclimation of sole spawners to captivity is long compared to that of sea-bass (*Dicentrarchus labrax*) and sea-bream (*Sparus auratus*) reared in similar conditions, but is close to that of turbot (*Scophthalmus maximus*) (Devauchelle, 1986). The origin of such different behavior might be found in the completely different habitats of these fish. Unlike the two roundfish, sole live and spawn on the bottom (Lagardere, 1982) at depths ranging from 20 to 100 m or more, with high pressure and low light. Hence, the brutal transfer from the wild to small tanks only 1 m deep might explain the long delay between the catch and the observation of regular spawns, as well as the low viability of flatfish eggs compared to eggs of some pelagic fish.

However, the successful repetition of natural and shifted spawning seasons which follows this period is encouraging, and the variability in both quality and quantity of viable eggs warrants discussion. It is probably caused by a combination of factors, but among them, temperature appeared to be the determinant one. Its effects on both spawn characteristics and timing were observed.

The lowest fecundity rates of sole succeeded the warmest winters. This phenomenon, which was also observed by Downing (1980) leads us to conclude that high temperature levels, with increasing food intake, did not contribute

to the production of successful spawning seasons. Since it is generally admitted that half or more of the ovarian growth occurs during starvation (Hoar, 1969), we suggest that more attention be paid to the summer and autumnal feeding of sole in order to satisfy the energy demand for the winter growth of the ovaries. During this period, the reserves have to be supplied from elsewhere in the body.

The second role of temperature appeared to be the control of the spawning dates. They differ from place to place, in the wild or in tanks. Along the coast of Brittany the first eggs are collected in the wild as early as December/January (Deniel, 1981), while the season begins in the early spring along the North-Sea coast (Fonds, 1976; Riley, 1974). In the tanks, the seasons seem to be less constant. But in both cases, viable eggs were collected mostly in the temperature range of 8–9°C to 12°C (Irvin, 1974; Riley, 1974; Fonds, 1976; Downing, 1980; present data). Consequently, the variation in timing of spawning might be interpreted as a direct effect of the thermal regimes. In our area, tank temperatures are more variable, depending on windy cold winters or sunny weather, than those of the sea bottom. For this reason, it is evident that sole spawners kept in tanks cannot spawn as early as in the wild. In the same way, the production of viable eggs generally stops in mid-May when temperatures rise above 12–12.5°C, except when the water temperature is kept low artificially. These tendencies were also noted for other marine fish species maintained in similar conditions of captivity (Devauchelle, 1986); however, sole was the only one which required such low levels and narrow temperature ranges. Moreover, our results provide data on the association of photoperiod and temperature in the control of reproductive processes: maturation and spawning can be obtained by cyclic daylength variations only; cyclic annual variations are not necessary for temperature, but its level has to be carefully adjusted during spawning (8–12°C), while maturation is not suppressed at temperatures lower than 8°C. Sole spawners respond to photoperiodic and thermal stimulation in similar ways to those of most fish species living in temperate areas. As mentioned by Downing (1980) for sole, Htun Han (1975) for plaice and turbot, and Bye (1984) for most freshwater fish spawning in early spring, the photoperiodic autumnal drop to short daylengths may trigger gonad maturation. During this period, temperature would tend to control the speed of vitellogenesis. On the other hand, the spawning processes could be considered to be mostly under thermal control. It is evident that the roles of both factors have to be further investigated, and such knowledge would contribute to improved spawning performances, and aid in detecting the effect of other parameters such as sex ratio, age, food, which certainly also influence the quantity and quality of the eggs collected (Wooton, 1982). In the same way, the volume of the tanks should be retained among such factors. While the egg quality is not significantly affected by the volume of tanks containing 5 m^3 to 17 m^3 of seawater (Devauchelle, unpublished, 1985), the effect of tank volume on fertility has still to be deter-

mined, in spite of good performances of group II in 1983. Additional experiments would establish the relative effect of tank size, compared to effects of various thermal conditions, on the number of eggs collected.

Sole eggs are rather large, compared to those of most pelagic fish species (Russel, 1976). Moreover, their average diameter changes from batch to batch and their volume can double. Since several authors consider that the size and survival of newly hatched larvae are related to the egg size (Hoar, 1969; Hempel, 1979), it would be useful to get more information concerning the origin of such variations. Over several spawning periods, the variations could be related to different parameters: the age of the fish (Gall, 1974; Schoenberr, 1977), the number of ovipositions (Kuo et al., 1973), the number of eggs spawned (Nikolsky, 1963) or genetic factors (Scott, 1962). But Bagenal (1971) and Ware (1975) suggested that the seasonal decline of fish egg diameters appeared to be linked to food availability. Since the spawners strongly reduced their food intake during maturation, it seems that the variations in seasonal egg diameters should rather be a consequence of events closer to oviposition, for instance, the relationships between temperature and metabolism. This would change the protein and carbohydrate contents of eggs, and thus the egg dry weight as observed in the present work. In this event, consequences for larval rearing success might be important. In 1975, Nash and Kuo suggested that more effort should be focused on the biochemical aspects of eggs in relation to finfish larval rearing. It is still true today and, in this respect, our preliminary data on the biochemical composition of eggs provide interesting information.

In fact, the sole egg dry weight is close to that of another flatfish, *Scophthalmus maximus* (Devauchelle et al., 1982), but is less than most other marine and freshwater fish species presented by Hempel (1979). As in most species, the egg dry matter depends mainly on its protein content. Thus an analysis of the major amino acids would be worthwhile since it has been suggested by Statova et al. (1982) that yolk proteins are transformed into new tissues and are not only lost in combustion during the first days of development.

On the other hand, the results show similar proportions of the different lipid classes, main fatty acids and polar lipids of the total fat, in ova both from the wild and from eggs spawned in captivity. In the hatchery context this information is very encouraging.

Many specific points still remain to be determined. Thus, a low value for the total fat in eggs may be a consequence of the mollusc diets used in our hatchery: the lipids represent only 8–9% of the dry weight. Moreover, while phosphatidylcholine and phosphatidylethanolamine were found to be the dominant constituents of the polar lipids in sole, as well as in the marine fish eggs studied by Torcher and Sargent (1984), the relative proportions of other constituents (PI/DPG) differ in eggs and gonads.

Little is known as yet about the content of minerals and oligoelements in the eggs of fish. In spite of the capacity of fish to absorb certain minerals in

their environment through their gills, the effects of dietary deficiencies have been observed. Luquet and Watanabe (1986) showed that a phosphorus deficiency was associated with a fall in fecundity of ayu. Similarly, low-phosphorus diets in red sea-bream (Watanabe et al., 1984a,b) reduced egg production and quality. As phosphorus clearly appears as the main constitutive mineral, these results are not surprising. However, results concerning the effect of low magnesium, zinc, iron or copper diets were less evident on Atlantic or coho salmon reproduction (Takeuchi et al., 1981; Hardy et al., 1984). On the other hand, the negative effect of manganese deficiency on reproductive performance was recently proved by Lall and Hines (1985) in brook trout. Since manganese, as well as copper, could not be detected in sole eggs, unlike sea-bass for instance (Devauchelle et al., 1982), it should be interesting to determine the relation between these oligoelements and egg production and hatchability, which are rather poor for these captive spawners. Should such a relation exists more attention should be given to the balance between these oligoelements and minerals such as calcium. Much work still remains to be done in this field. However, these preliminary basic data will help to formulate successful broodstock diets.

Much is known concerning the salinity and temperature tolerance of embryos. Thus relations between temperature and incubation duration were expressed by Irvin (1974), Riley (1974) and Fonds (1979). Our results, dealing with a narrow temperature range, indicate a linear relation, although these authors described an exponential relation $D=aT^b$ for a wider temperature range. In this instance our observations agree with Riley's (1974).

The best temperature for incubation (13–15 °C) was higher than that for spawning (10.5 °C). The optimal salinity range was 20–35‰ according to Fonds (1979). The tolerance of the embryos also depended on the stage of development (after neurulation, sole embryos are more resistant, like most fish embryos – Hempel, 1979) and on the temperature level. The interaction of salinity and temperature was apparent, development at low salinities being more successful at low temperatures. These tendencies agree with the findings of Alderdice and Forrester (1968, 1971) and Alderdice et al. (1979) for *Parophrys vetulus, Eopsetta jordani* and *Clupea pallasi.* Under our conditions, the best hatching rates were obtained at 14 °C and 30‰. With this combination the eggs died prior to gastrulation and among the egg batches the differences in hatching performances were more important. These observations suggest that testing the quality of spawnings by application of short periods of stress to the eggs at the morula stage may be worthwhile. However, other factors are involved in the egg tolerance. As shown by May (1975) on *Bairdiella iciestia*, the parental acclimation might also be determinant and could explain the differences in optimal values recorded from place to place.

In conclusion, this study shows that sole spawning and incubation require low temperatures for the best results. In most Mediterranean hatchery sites,

the production of a large quantity of sole eggs might depend on water cooling. Moreover, unlike other marine fish involved in European aquaculture projects, sole spawns over a narrow temperature range, although embryos may tolerate a wider salinity range.

This paper provides new technical information which might help brookstock maintenance. Studies on egg quality still remain to be done. For this purpose, the egg biochemical composition might be considered as a useful support in determining the relation between egg characteristics and larval rearing success.

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