Helgoländer wiss. Meeresunters. 18, 53–60 (1968)

Rate and efficiency of yolk utilization in developing eggs of the sole *Solea solea*

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KURZFASSUNG: **Rate und Quote der Dotterausnutzung bei sich entwickelnden Eiern der Seezunge *Solea solea*.** Wie bei anderen planktisch-marinen Fischen sind die Eier der Seezunge durch einen relativ hohen Wassergehalt (91 %) und einen niedrigen Kaloriengehalt (5800 cal/g trockene organische Substanz) charakterisiert; demgegenüber haben die Eier der Substratlaicher einen niedrigeren Wassergehalt (etwa 75 %) und einen höheren Kaloriengehalt (6500 cal/g trockene organische Substanz). Seezungenlarven scheiden kurz vor und nach dem Schlüpfen Schleim aus, wenn sie in destilliertes Wasser überführt werden. Diese Schleimabsonderung stört die Bestimmung des Trockengewichts und des Kaloriengehalts. Sie kann reduziert werden, wenn man die Larven vor Überführung in destilliertes Wasser mit Formalin abtötet. Die Ausnutzungsquote des Dotters frischgeschlüpfter Larven beträgt im Mittel 85,2 % bei 10° C, 79,0 % bei 15° C und 75,5 % bei 20° C. Die Inkubationsdauer beläuft sich auf etwa 176 Stunden bei 10° C, 80 Stunden bei 15° C und 53 Stunden bei 20° C.

INTRODUCTION

Our knowledge on rate and efficiency of yolk utilization in developing fish eggs has been summarized by Smith (1957, 1958). The information so far available is, however, based mostly on freshwater fishes. Of the four papers available on marine fishes, the one by Blaxter & Hempel (1966) deals with *Clupea harengus*, while two others by Dakin & Dakin (1925 quoted in Needham 1931, p. 1114) and Ryland & Nichols (1967) concern *Pleuronectes platessa*. The authors studied the utilization of yolk by measuring the length and dry weight of the embryo or the concentration of nitrogen in the embryo. Lasker (1962), using developing eggs of *Sardinops caerulea*, analyzed yolk and larvae for water, protein and calorific contents, measured oxygen consumption of developing larvae, and reported on rate and efficiency of yolk utilization. The present paper deals with the calorific content of the egg, and rate and efficiency of yolk utilization in relation to temperature in developing eggs of *Solea*

MATERIAL AND METHODS

The eggs used in the present study were collected from spawning North Sea soles *Solea solea* (about 6 year old fishes) maintained in the laboratory for 3 years. The swimming eggs were collected immediately after spawning and allowed to develop in small aquarium cylinders kept in temperature-constant rooms. At desired intervals, the developing eggs were removed for further analyses.

A definite number (about 300) of developing eggs was counted, washed free from adhering sea water by exposing them to distilled water for a period of 3 minutes, and weighed and dried at 80° C for 4 hours. Ash content was estimated by incinerating the sample at 500° C for 5 hours. Calorific measurements were made with a semi-micro bomb calorimeter (Parr Instrument Co., Model No. 1412). All weighings were made using a Sartorius balance sensitive to ± 0.01 mg.

RESULTS

Composition of fresh eggs

Fresh eggs were collected on March 16 and March 29, 1967. On both dates water temperature at spawning was 8° C. A portion of the eggs from March 16 was incubated at 10° ± 0.1° C; the rest were analyzed immediately. The eggs from March 29 were incubated at 15° and 20° ± 0.1° C.

The values obtained for dry weight, ash and calorific contents are presented in Table 1. Dry weight amounted to 9.2 % of the living matter, ash to 1.16 % of the dry matter, and calorific content to 5836 cal/g dry organic substance.

Table 1

Composition of fresh eggs of the sole *Solea solea*. The value on dry weight content of the eggs is based on 4322 eggs (14 groups of about 300 eggs each); standard deviation is related to the mean value of the 14 mean values

Determinations made Number	Kind	Mean values	Standard deviation	Coefficient of variation	Average values for a single egg
14	Dry weight	9.2 %	± 0.31	3.4 %	84.2 μg
6	Ash content	1.16 %	± 0.1	0.9 %	1.0 μg
6	Energy (cal/g organic substance)	5836	± 61	1.0 %	0.486 cal

Of 100 randomly chosen eggs, the diameter of an egg measured to 1.3 mm in 95 individuals, while 4 of them measured to 1.2 mm and the other 1.4 mm. A total number of 4322 eggs collected on two different dates was used to estimate the dry weight of a single egg. The mean dry weight value of a single egg was 83.0 μg for the March 16 material and 88.8 μg for the March 29 material. Values obtained on both series (14 determinations) averaged 84.2 μg (± 1.3 μg) with a coefficient of variation of 1.5 % from the mean value. Using the value of 84.2 μg/egg together with the data presented in Table 1, live (915.2 μg) and dry weights and ash and calorific contents of a single egg were calculated.

Initially, composition of eggs was studied without washing them in distilled water prior to weighing. After 4 hours drying, sea water adhering to the surface of the eggs evaporated, leaving considerable quantities of salt particles. Analyses of such material gave 20.14 % ash and 4513 cal/g dry weight, against 1.16 % ash and 5768 cal/g dry weight in washed eggs (Table 1). Thus, later on all eggs were washed for 3 minutes in distilled water prior to drying.

Fresh eggs or developing eggs (up to the primitive streak formation stage) showed no other side effect upon distilled water treatment. As soon as more advanced developmental stages were brought in contact with distilled water at temperatures between 10° and 20° C, the embryos secreted a jelly-like slime substance in large quantities even if exposure time was only 1 minute. This slime substance increased the weight of the test material considerably; at the same time it reduced the calorific value to less than 2000 cal/g dry weight (5 determinations). Four determinations of ash content of such substances (600° C) gave values of more than 40 %; the ash was lead black in color. In the bomb calorimeter the substance remained charred and uncombusted. Upon drying, the slime secreted by the embryos became so inert that it could neither be reduced to ash after a period of 6 hours incineration at 600° C, nor be combusted in the bomb calorimeter. Due to these unexpected difficulties, much of our precious material was lost and it was subsequently found that slime secretion could be reduced to a considerable extent by killing the larvae or embryos in 5 % sea water formalin prior to distilled water exposure. However, we had only limited material and the results on efficiency of yolk utilization based on a few values only.

Efficiency of yolk utilization

Average changes in dry weight and calorific content of a single egg are given in Table 2. At 10° C, a freshly hatched larva (67.4 μg dry weight) contains 0.414 cal, i. e.

Table 2

Average changes in dry weight and calorific content of developing eggs and freshly hatched larvae of the sole *Solea solea*. The values presented are based on two determinations each

Developmental stage	cal/g dry weight	cal/g dry organic substance	Number of eggs or larvae counted	Average dry weight of one egg or larva (μg)	Average cal/egg or cal/larva
Fresh egg (8° C)	5768	5836	4322	84.2	0.486
"Primitive streak" (10° C)	5748	—	800	78.5	0.451
Just before hatching (10° C)	5772	—	660	72.7	0.420
Just hatched (10° C)	6145	6496	800	67.4	0.414
Just hatched (15° C)	6058	—	450	63.1	0.384
Just hatched (20° C)	6022	6169	600	60.4	0.367

about 85.2 % of the total energy contained in the fresh egg. The corresponding values for larvae hatched at 15° and 20° C were 79.0 % and 75.5 %, respectively.

Freshly hatched larvae contained considerable quantities of yolk; therefore, the values stated above require correction for the yolk content. In other species such as herring it has been possible to dissect out the yolk mass from the embryo (Blaxter & Hempel 1966, Paffenhöfer & Rosenthal 1968). However, the larva of the sole is small (2.6 mm) and delicate, and removal of yolk by dissection could introduce considerable errors. In general, the yolk mass was elliptical in shape. A number of larvae were measured for the lengths and breadths of their yolk masses; and the average volume of the yolk mass was estimated from 41 values. Assuming the specific gravity value of the marine planktonic eggs of the plaice *Pleuronectes platessa* (Smith 1957, p. 330) to be similar to that of the eggs of the sole *S. solea*, the volume of the yolk mass contained in the freshly hatched larva was converted into wet weight (495 μg). There is much evidence to show that water (Gray 1926) and calorific content per unit weight (Lasker 1962) of the yolk do not change during embryonic development. Thus, using the calorific content and water values obtained for fresh eggs, the calorific content of unused yolk mass in a single fresh hatched larva has been estimated to be 0.262 cal.

Since the yolk volume varied only a little in larvae hatched at different temperatures, the calorific value obtained for the yolk mass of a freshly hatched larva at 10° C was used for the larvae hatched at 15° and 20° C.

On the basis of these considerations the following values were estimated: at hatching, a single larva contains (without yolk mass) 0.152 cal at 10° C, 0.122 cal at 15° C and 0.105 cal at 20° C; i. e., of the 0.224 cal used during the embryonic development (till hatching), substances containing only 0.152 cal, 0.122 cal or 0.105 cal were utilized by the embryos at these different temperaturs. Thus, the net utilization efficiency values are 67.9 %, 54.5 % or 46.9 % at the 3 temperatures.

Table 3

Effects of different temperature levels on the embryonic development of *Solea solea*

Temperature (° C)	Observations
4	Cell division leads to beginning embryonic disc stage; no further development
5	Embryonic disc is formed and covers 2/3 of the egg surface; no further development
6	Embryo develops up to "primitive streak" stage; no further development
7	Full normal development; 50 % of the eggs hatched on the 12th day following fertilization
10	Full embryonic disc is formed at 57th hour after fertilization; after 75 hours: formation of primitive streak; after 176: 50 % of the tested eggs hatched
15	After 25 hours: embryonic disc covers 1/2 of the egg surface; after 40 hours: formation of embryonic disc is completed; after 80 hours: 50 % of the tested eggs hatched
20	Development is very fast; after 53 hours 50 % of eggs hatched

Rate of yolk utilization

The effects of temperature on the rate of embryonic development have been summarized in Table 3. These observations relate to material collected in 1966 and 1967; they have, in part, been presented by FLÜCHTER (1966). At the low temperature levels 4°, 5° and 6° C, egg development became arrested at different early stages. At the higher temperature levels full normal development took place. At 7° C, 50 % of the larvae hatched after 12 days, at 20° C after 53 hours. All the other data can be seen from Table 3.

As has already been shown, as much as 0.152 cal have been utilized by a larva hatching at 10° C. This amount of energy was converted into embryonic substance during a period of about 176 hours. The respective values are 0.122 cal/80 hours at 15° C and 0.105 cal/53 hours at 20° C.

DISCUSSION

The data on composition of fresh fish eggs available in the literature have been summarized in Table 4. Data on calorific content of fish eggs are scanty; therefore, we have calculated the calorific values from the data reported by DAKIN & DAKIN (1925, quoted in NEEDHAM 1931, p. 1114), HOLLET & HAYES (1946), HARTMANN et al. (1947) and MENGI (1965) on chemical composition of eggs. The values 9400 cal/g fat, 5650 cal/g protein, 4150 cal/g carbohydrate (MAYNARD & LOOSLI 1962, p. 322) and 4187 cal/g dry egg membrane (FAUSTOV & ZOTIN 1964) were used to convert the values on chemical composition into calorific content. Having a high percentage of water content (91 %) and low calorific content (5836 cal/g dry organic substance), the eggs of *Solea solea* from the North Sea closely resemble those of the Atlantic plaice *Pleuronectes platessa* (DAKIN & DAKIN 1925), the Pacific sardine *Sardinops caerulea* (LASKER 1962), and the Baltic cod *Gadus morhua* (MENGI 1965). High water content and small size may be considered a special adaptation to pelagic life (SVERDRUP et al. 1964, p. 821). While the planktonic eggs of these four marine species have a calorific value of about 5800 cal/g dry organic substance, demersal eggs of either marine fishes – e. g., *Clupea harengus* (PAFFENHÖFER & ROSENTHAL 1968) – or anadromus (euryhaline) fresh water fishes – e. g., *Salmo* (HOLLET & HAYES 1946, HARTMANN et al. 1947), *Acipenser* spp. (FAUSTOV & ZOTIN 1964) – have more than 6200 cal/g weight (ash 5 %) or 6500 cal/g dry organic substance. A relatively higher calorific content per unit egg weight indicates a higher fat content. A high fat content in demersal fish eggs should not be misunderstood; the fat content in fish eggs has little to do with floatation, which is brought about by reducing the specific gravity of the planktonic eggs of the demersal fishes (SMITH 1957, p. 330).

The developing eggs of *Solea solea*, which were not affected by exposure to distilled water during early development, secreted large quantities of slime when exposed to distilled water after having reached the primitive streak formation stage. It is possible that, at this stage, the permeability of the egg membrane increases. PANDIAN (1968) observed that exposure of developing eggs of the slipper limpet *Crepidula fornicata* to distilled water also induced slime secretion. However, reducing

Table 4

Composition of fresh eggs of fishes

Species	Water (%)	cal/g dry weight	cal/g dry organic substance	Remarks	References
Sardinops caerulea	91.2	5386	5791	Marine, planktonic	LASKER (1962)
Solea solea	90.8	5768	5836	Marine, planktonic	FLÜCHTER & PANDIAN (1968)
Gadus morhua	88.0	5423	5757	Marine, planktonic	MENGI (1965)
Pleuronectes platessa	92.6	5699	—	Marine, planktonic	DAKIN & DAKIN (1925)
Clupea harengus	76.0	—	—	Marine, demersal	BLAXTER & HEMPEL (1966)
Clupea harengus	—	6093	6580	Marine, demersal	PAFFENHÖFER & ROSENTHAL (1968)
Misgurnus fosillis	—	5605	—	Fresh water, demersal (?)	FAUSTOV & ZOTIN (1964)
Acipenser stellatus	—	6238	—	Fresh water, anadromus, demersal	FAUSTOV & ZOTIN (1964)
A. güldenstaedti	—	6221	—	Fresh water, anadromus, demersal	FAUSTOV & ZOTIN (1964)
Salmo fario	41.0	—	—	Fresh water, anadromus, demersal	GRAY (1926)
Salmo irrideus	66.0	6156	6395	Fresh water, anadromus, demersal	HARTMANN et al. (1947)
Salmo sp.	—	6207	—	Fresh water, anadromus, demersal	HOLLET & HAYES (1946)

the duration of exposure to less than 15 seconds, the slime produced could be minimized to a negligible amount. Secretion of slime by developing eggs of marine animals may be considered a mechanism for temporarily escaping unfavorable conditions.

A single freshly hatched sole larva contained as much as 85.2 %, 79.0 % and 75.5 % of the total energy contained in the fresh egg, at 10°, 15° and 20° C, respectively. Thus, at hatching, net losses varied from 14.8 % to 24.5 % at these temperatures. Corresponding values reported by FAUSTOV & ZOTIN (1964) are 2.0 % for the stellate sturgeon *Acipenser stellatus* and 3.5 % for the loach *Misgurnus fossilis*. A recalculation of LASKER's (1962) data gives a value of 13.3 % for the Pacific sardine *Sardinops caerulea*. Cumulative net efficiency of yolk utilization (after correcting for the remaining yolk) varied from about 68 % to 47 % at the test temperatures mentioned. These values are low in comparison to that (78.7 %) of the Pacific sardine (LASKER 1962) but are close to those reported for the herring *Clupea harengus* by BLAXTER & HEMPEL (1966). One possible reason for the low efficiency values obtained in sole larvae appears to be that, unlike in sardine larvae, the unused yolk mass undergoes considerable changes in its composition during embryonic development and it seems possible that the unused yolk mass of the sole larvae contained more energy than the values arrived at in the present study.

Hatching size and duration of the incubation period decrease with increasing temperature; this has been amply demonstrated also in other fish species by GRAY (1926, 1928), KINNE & KINNE (1962) and SWEET & KINNE (1964). From the point of ecology it is interesting to note that the lower temperatures (< 7° C) arrest the development at different stages of development. This would suggest that the different sequences of embryonic events have different minimum temperature ranges. In the North Sea, the soles spawn during the spring when the water temperature gradually rises. If spawning occurs at a lower temperature (< 7° C), or if the water temperature decreases after spawning, egg development becomes arrested. Such "arrested eggs" do not die so quickly, however, and may continue to develop as soon as the temperature rises again. Since spawning time and surface temperature vary greatly from spawning place to spawning place and from year to year, initial body weights and sizes of freshly hatched larvae may vary accordingly.

SUMMARY

1. The eggs of *Solea solea*, like many other marine planktonic fish eggs, are characterized by a high water content (91 %) and a relatively low calorific content (5800 cal/g dry organic substance). Demersal fish eggs, on the other hand, have, according to the information available from literature, a low water content (about 75 %) and a high calorific content (6500 cal/g dry organic substance).
2. Older embryos and freshly hatched larvae of *Solea solea* secrete large quantities of slime upon brief exposure to distilled water; this makes the estimation of their dry weight and calorific content difficult. Slime secretion could be reduced to a certain extent by killing the larvae in 5 % sea-water formalin prior to distilled water exposure (to remove adhering salt water).

3. At 10° C, incubation lasts 176 hours and freshly hatched larvae contain 85.2 % of the energy contained in a freshly laid egg (0.49 cal/egg); at 15° C and 20° C, the corresponding values are 80 hours, 79.0 % and 53 hours, 75.5 %, respectively.

Acknowledgements: We are grateful to Professor O. Kinne for valuable suggestions and for improving our manuscript. One of us (T. J. P.) received the financial support from the Daad (Bad Godesberg, Germany) during the course of this study.

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