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Changes in egg size of plaice (*Pleuronectes platessa* L.) during incubation, and the effect of fixation

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Changes in the size of plaice eggs during incubation were studied in tank experiments with 11 batches stripped from 6 individual females and fertilized artificially. Live egg size increased by 1% from fertilization to hatching. Fixation in 4% formaldehyde in sea water resulted in a decrease in egg size. This shrinkage increased with developmental stage from 0% in eggs that had just been fertilized to 1.4% in eggs prior to hatching. Mean size of fixed eggs decreased slightly but significantly by 0.7% between fertilization and hatching. The implications of these changes for studies of size-selective mortality in pelagic fish eggs are discussed.

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Introduction

The problem of changes in egg size during development has been raised in a study of the mortality of pelagic eggs in the sea. Rijnsdorp and Jaworski (1990) showed that egg size in plaice and cod increased during incubation by 3% and 2.5%, respectively, and concluded that egg mortality in plaice and cod was size-dependent. The estimated mortality rate of smaller eggs was higher than that of larger eggs, both within and between species. Their conclusion, however, was based on the assumption that the size of an individual egg would not change during incubation. Independent evidence for this assumption was not available (Hoar and Randall, 1988). The present paper presents the results of an experimental study on the changes in egg size of plaice between fertilization and hatching in live eggs and on the effect of fixation in 4% formaldehyde in sea water. Besides the relevance of changes in fixed egg size for studies of egg mortality, changes in live egg size may have important implications for the study of egg mortality in relation to the physical processes affecting the vertical distribution of pelagic fish eggs (Westgård, 1989).

Material and methods

Ripe male and female plaice were caught in the southern North Sea during the first half of January 1990, transported to the laboratory and placed in a circular tank, with a diameter of 2 m and water depth of 0.5 m, at a temperature of 13°C. The ovulating females were stripped every other day and ripe eggs were fertilized artificially with the semen of one to three males. The buoyant fertilized eggs were separated from unfertilized eggs by decanting after raising the salinity from 32‰ to 33‰. Eleven individual batches of fertilized eggs from six females (30–45 cm) were placed in 5-l glass containers in one controlled temperature room at 8°C (nine experiments) and in another at 5°C (two experiments). The water in the tanks was treated with natrium penicillin G (50 000 iu./l) and streptomycin sulphate BP (50 mg/l) and was replaced once, halfway through the experiment.

Samples were taken four to six times during incubation and fixed in 4% formaldehyde in sea water (~32‰) buffered with 0.5% sodium-b – glycerophosphate. The last samples of stage 5 eggs were taken when less than 5% of the eggs had hatched. Egg size was determined before and after fixation of the samples. Size of live eggs was determined in sea water in a subsample of about 35 eggs; the size of fixed eggs was determined in the fixation medium after 10–15 days. Repeated measurements of 10 samples after 5 months of fixation showed that no further change in size had occurred. Developmental stages were determined according to Ryland and Nichols (1975). Egg size was determined under a binocular microscope to the nearest

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Table 1. Results of the ANOVA of egg size according to the model: ES = E + S; with ES = egg size (mm). E = experiment number, and S = developmental stage.

	SS	df	MS	F	p
Live eggs					
Experiment	0.3941	10	0.03941	206.0	≪0.01
Stage	0.00301	4	0.00075	3.94	<0.01
Error	0.00746	39	0.0001		
Total	0.4086	53			
Eggs fixed in 4% formaldehyde in sea water					
Experiment	0.4022	10	0.04022	713.8	≪0.01
Stage	0.00120	4	0.00030	4.549	<0.01
Error	0.00248	44	0.000056		
Total	0.4048	58			

Table 2. Parameter estimates of the change in egg size (mm) relative to that of stage 1 eggs; obtained from the ANOVA according to the model ES = E + S, with ES = egg size (mm). E = experiment number, and S = developmental stage. %GM = the fitted size of stage 1 eggs in experiment 1.

	Live eggs		Fixed eggs	
	Mean	s.e.	Mean	s.e.
%GM	1.828	0.0080	1.848	0.0042
Stage 2	0.0037	0.0073	−0.0075	0.0033
Stage 3	0.0176	0.0062	−0.0038	0.0031
Stage 4	0.0190	0.0067	−0.0069	0.0034
Stage 5	0.0179	0.0058	−0.0127	0.0029

eye-piece unit of 0.033 mm. A pilot study of replicated measurements showed that with a probability of 95% the measured mean size did not differ by more than 0.01 mm from the real value.

Results

The change in egg size during incubation was studied using analysis of variance (ANOVA), with experiment number and developmental stage as independent factors with respectively 11 and 5 levels. Both experiment number and developmental stage were significant, indicating that size differed between individual batches and between developmental stages (Table 1). The parameter estimates of the relative changes in live and fixed eggs for the five developmental stages are given in Table 2.

Figure 1 shows the percentage change in size with age relative to the size of stage 1 eggs. The size of live eggs increased after fertilization up to 1.0%. The main change occurred between days 4 and 10 at the transition between stages 2 and 3. The size of fixed eggs decreased by 0.7% between fertilization and the beginning of hatching. ANOVA of ratios of fixed egg size over live egg size showed that shrinkage after fixation increased significantly ($p < 0.01$) with development stage. The parameter estimates given in Table 3 indicate that shrinkage did not differ significantly from 0% in stage 1 eggs, but increased up to 1.4% in stage 5 eggs. Although ratios will not normally be distributed, the results of the ANOVA are consistent with the analysis of size changes in live and fixed eggs separately.

Discussion

As the changes were studied in groups of eggs and not in individual eggs, the observed increase in live egg size could in theory be due to a higher mortality among the smaller eggs of a batch than among the bigger ones. According to Jones (1958; see also Rijnsdorp and Jaworski, 1990) the size selective mortality coefficient b is given by the equation $b = d/s^2$, where d is the change in size and s is the standard deviation. With the estimated increase in live egg size during incubation of d =

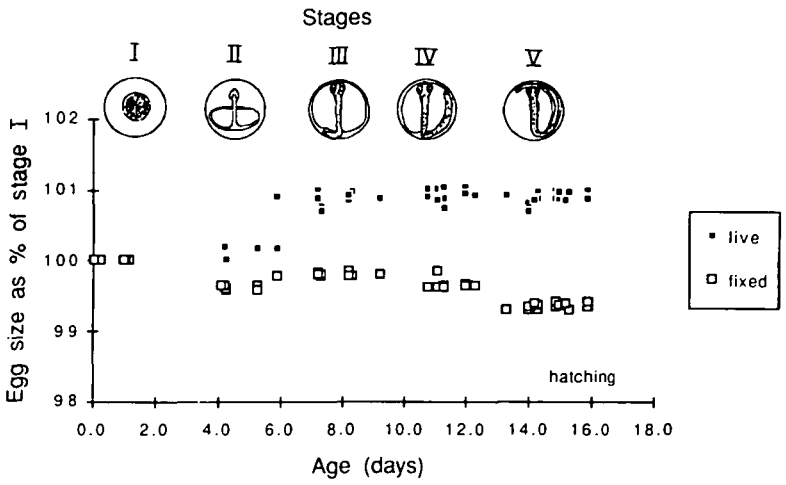

Figure 1. Percentage change in egg size of live- and formaldehyde-fixed eggs during embryonic development. Age in experiments 10 and 11 at T = 5°C was converted to the corresponding value at T = 8°C.

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Table 3. Parameter estimates of the change in ratio of fixed to live egg size as obtained from the ANOVA of egg size ratio against developmental stage. %GM = the fitted ratio of stage 1 eggs.

	Mean	s.e.
%GM	1.001	0.0029
Stage 2	−0.006	0.0045
Stage 3	−0.009	0.0039
Stage 4	−0.011	0.0042
Stage 5	−0.014	0.0037

0.02 mm (Table 2) and the average s = 0.03 mm within a batch, the size-selective mortality coefficient can be calculated as b = 22.22.

The difference in mortality coefficient between eggs differing two standard deviations in size thus amounts to $2 \times s \times b = 1.33$ and the difference in survival of $\exp(-1.33) = 0.26$. In other words, if all large eggs with a size of the mean plus one standard deviation survive, then only 0.26 of the small eggs (mean minus one standard deviation) will survive. This implies that, if the increase in live egg size is due to size-selective mortality, a rather high mortality must have occurred during the incubation experiments, in particular between days 5 and 8 when the major change took place. Although some mortality occurred, it was not sufficiently high to explain the increase in live egg size.

The small decrease of 0.7% in size during embryonic development of formaldehyde fixed eggs implies that size-dependent mortality in eggs in the sea might be studied from changes in egg size. The estimate of size-dependent mortality by Rijnsdorp and Jaworski (1990) would be an underestimate even when fixation causes a decrease in egg size of 0.7% as observed in the present study. Preliminary results for mackerel and cod also suggest that changes in fixed egg size are very small and negligible (pers. comm., J. H. Nichols, Lowestoft, UK and O. Kjesbu, Bergen, Norway).

Fixation of eggs just after fertilization does not lead to a significant shrinkage (Table 3), which is in agreement with the observations of Heincke and Ehrenbaum (1900). However, fixation of unfertilized eggs results in a significant shrinkage of 2.9% (Hislop and Bell, 1987). This difference between unfertilized and fertilized eggs is not surprising given the structural changes in the chorion at fertilization (Davenport *et al.*, 1981).

This study showed that the size of live eggs increased slightly during development (by 1.0%). Changes in size, and therefore probably in buoyancy, during development are of significance for studies of how physical processes determine the distribution of pelagic eggs and may also affect the contact rate with predators (Rothschild and Osborn, 1988; Westgård, 1989). Developmental changes in buoyancy were not determined, but evidence exists for other species that the specific gravity increases during incubation (Franz, 1910; Sundnes *et al.*, 1965).

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