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Fertilization rate in a natural population of the common sole (*Solea solea* L.)

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An assessment of fertilization rate in a natural population of Dover sole, *Solea solea* (L.), is described. A preliminary study of the morphological changes of fertilized and unfertilized eggs from a captive stock was carried out to aid identification of sea-caught eggs. In addition, changes in specific gravity were measured to determine whether, and for how long, unfertilized eggs would be expected to be distributed similarly to their fertilized counterparts. During the field assessment, in which over 16 000 live eggs were examined, only 38 were judged to be unfertilized. This was estimated to represent a fertilization rate of over 99%. The paper also presents results from similar experiments on the eggs of plaice (*Pleuronectes platessa* L.).

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Introduction

The year-class strength of fish populations is determined mainly during the vulnerable early life-history stages. Fertilization of the egg marks the beginning of this phase, and in the large number of species in which it occurs externally its success is dependent on overcoming the tendency of the gametes to disperse after release. Many authors have thus regarded fertilization to be an uncertain process and cite the occurrence of opaque eggs in plankton samples as evidence of fertilization failure. This contention has been refuted by others who claim that such "dead" eggs were merely the result of damage inflicted during capture (see Markle and Waiwood, 1985).

The characteristically high incidence of unfertilized eggs from cultured stocks of fish and other aquatic animals may be further evidence of the uncertainty of the fertilization process. Certainly, this has been the experience with cultured flatfish. In captive sole, *Solea solea* (L.), and turbot, *Scophthalmus maximus* L., for example, about half their annual egg production may be unfertilized, even under conditions which permit natural spawning (Houghton *et al.*, 1985; Bromley *et al.*, 1986). The extent to which this merely reflects some aspect of the artificiality of the captive environment is, however, unclear.

This paper describes an estimate of fertilization rate in a natural population of sole in the Thames estuary, UK. Field observations were preceded by laboratory studies of naturally spawned eggs from a captive stock of sole. In particular, it was necessary to establish the existence of criteria for distinguishing between fertilized and unfertilized eggs and to determine whether, and for how long, unfertilized eggs would be present in the water column. The use of naturally spawned eggs had the disadvantage that eggs collected from the tanks on any one day could have originated from more than one female and consequently been of mixed ages. The reliance on naturally spawned eggs was necessitated by the difficulty of acquiring ripe gametes from mature sole (Irvin, 1973). This is not the case for the plaice (*Pleuronectes platessa* L.) and an opportunity was taken to compare the changes in fertilized and unfertilized eggs from a single batch of hand-stripped plaice eggs with those observed in the sole.

Materials and methods

Laboratory studies of unfertilized eggs

Egg supply

The stock of captive sole spawned naturally, invariably at night. The eggs were netted from the tank in the mornings and any remaining were allowed to wash out of the tank following removal of a retaining mesh fitted to the overflow. This was replaced in the evening. Thus, the vast majority of the eggs collected had been spawned during the previous night. They included the following types: (a) Normally developing fertilized eggs containing a blastula usually of 64–128 cells. (b) Eggs showing no development and in which the peripherally segmented yolk

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characteristic of sole eggs (Russell, 1976) was clearly visible ("globular" eggs). (c) Eggs containing a germinal disc around which a membrane had been formed ("1-cell" eggs).

Frequently, the collections consisted of eggs of distinctly different size groups, a feature considered to be indicative of the occurrence of multiple spawnings. Thus, only batches in which the developing and non-developing eggs had a similar size range were selected for study. This increased the probability that the eggs had been spawned simultaneously from a single female.

For the artificial fertilization of plaice, gametes were manually extruded from captive fish. Eggs from a mature female were stripped into a clean glass bowl and immediately equally divided between two similar containers. A few drops of sperm were added to one of these and clean sea water to both. After about 30 min, each batch was drained on to a mesh and resuspended in clean sea water.

Incubation

The eggs were incubated under static conditions in 10 l containers placed in a constant temperature room held at 9.0 ± 1.0°C for plaice and 13.0 ± 1.0°C for sole. Eggs of both species were negatively buoyant in the laboratory sea water (28–30‰) but floated after the salinity had been increased by 2‰ by the addition of NaCl. A single initial dose of 50 i.u. benzylpenicillin and 0.05 mg streptomycin sulphate per ml was added to inhibit bacterial development.

Development

Samples of fertilized and unfertilized eggs were examined frequently until the latter lost their buoyancy. The changes observed in the unfertilized eggs were described and the corresponding development of fertilized eggs recorded. The development stages referred to are those defined by Riley (1974).

Specific gravity

The specific gravity of fertilized and unfertilized eggs was measured at frequent intervals in salinity gradients prepared in a manner similar to that described by Coombs (1981). Gradients were prepared with a salinity range of 25–42‰ in 1-m long and 39 mm internal diameter perspex tubes graduated at 1-cm intervals. The tubes were housed in a water jacket in which temperature was controlled to within 0.2°C of the incubation temperature of the eggs. The gradients were calibrated using glass beads of known specific gravity at 23°C. These were corrected for the operating temperature using the formula:

$$S_T = S_{23} + 0.000035(23\text{-}T)$$

where S_T is the specific gravity at T°C and S_{23} is the specific gravity at 23°C. The salinity equivalent to any particular specific gravity value and temperature was obtained from standard Sigma-T tables. The linearity of the gradients was confirmed by calculating the regression of the height of each bead in the column (estimated to the nearest mm) against their corrected specific gravity.

Samples of 10 eggs were pipetted into the column just below the surface. After they had sunk to their position of neutral buoyancy their specific gravity was calculated from the regression equation. Measurements were made of fertilized and unfertilized eggs of different ages for two batches of sole and one batch of plaice eggs.

Examination of eggs at sea

Sole eggs were collected in the Thames estuary during May, 1986 from the RV "Clione". A 2-m diameter ring net was lowered from the freely drifting ship until the ring was within 5 m of the sea bed. It was then hauled at a speed not exceeding 0.1 m sec^{-1}. The catch was washed gently into a collecting jar and immediately transferred to a shallow black plastic tank from which the eggs were removed individually using a pipette. Each egg was examined live under low power magnification within 1 h of capture. Developing eggs were staged and those showing no development or of doubtful status were transferred to 20 ml vials of filtered sea water. These were re-examined after at least 5 h incubation at the ambient sea-water temperature. This was sufficient time for any fertilized eggs to develop recognizable features. The number of opaque (dead) eggs in each haul was recorded but their status was not assessed.

Samples of eggs were collected and examined in this way every hour during five 24 h surveys. In addition, the specific gravities of small numbers of fertilized eggs of various developmental stages were measured for comparison with those of eggs from the captive stocks. Their ages were estimated from known developmental rates at the prevailing temperature (Riley, 1974).

Results

Development of fertilized and unfertilized eggs from captive stocks

The morphological changes of plaice eggs are described first because it was possible to follow these from the time of fertilization.

Plaice

Fertilized eggs completed first cleavage within 4 h of fertilization and reached the 128-cell stage within 14 h. Changes in the unfertilized eggs were more gradual, a detectable concentration of cytoplasm at the animal pole being only evident after $5\frac{1}{2}$ h. After 10–11 h a germinal disc of homogeneous cytoplasm with a distinct margin had been formed (1-cell stage). None of the unfertilized eggs cleaved. The margin of the germinal disc gradually

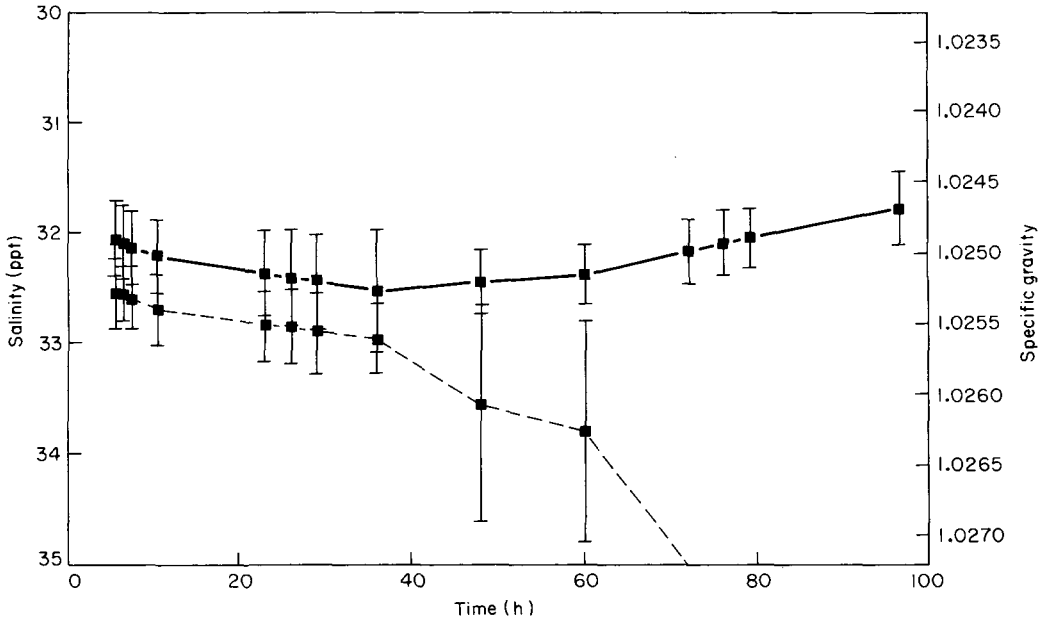

Figure 1. Changes in the mean specific gravity, and the corresponding salinity of neutral buoyancy, of fertilized and unfertilized plaice eggs from the time of fertilization (0 h). The vertical axes have been inverted so that, for example, a negative slope indicates a decrease in buoyancy. Standard deviations are indicated by the vertical bars. — — — = unfertilized, —— = fertilized.

became less distinct and irregular structures progressively appeared within it. After 30–35 h the perivitelline space increased in size and after 48 h the perivitelline membrane in many eggs had collapsed completely. At this time, the fertilized eggs were commencing gastrulation (Stage IA/IB).

Sole

None of the "globular" eggs nor those containing a single cell progressed to first cleavage. They were, therefore, judged to be unfertilized. The changes in those eggs in which a single cell had been formed by the time of collection were similar to those observed in plaice eggs. An additional feature was the migration of the aggregations of oil globules (which are not present in plaice eggs) from a peripheral position to one overlying the germinal disc. This movement appeared to correspond to the normal orientation of the oil globules along the advancing edge of the periblast during epiboly in fertilized eggs. As in plaice eggs, the collapse of the perivitelline membrane occurred when fertilized eggs from the same collection had reached early gastrulation (Stage IA/IB). The appearance of the globular eggs changed little until the collapse of the perivitelline membrane. Again, this occurred when fertilized eggs were undergoing early gastrulation.

Specific gravity

Changes in the specific gravity of fertilized and unfertilized eggs from captive stocks of plaice and sole are shown in Figures 1 and 2 respectively. All batches of fertilized eggs followed a similar pattern of an initial increase followed by a gradual decrease in specific gravity. The inflection occurred at around the time of gastrulation. At the time of collection, the salinity of neutral buoyancy of the fertilized sole eggs ranged from about 31.0 to 32.5‰.

In each batch of eggs examined the mean specific gravity of the unfertilized eggs was greater than that of the fertilized eggs, though the difference was not significant ($p < 0.05$). The specific gravity of the unfertilized eggs increased gradually at first, in parallel with that of the fertilized eggs, but increased sharply after 30–60 h for plaice or 15–30 h for sole. In both species this coincided with the time of the pronounced increase in the size of the perivitelline space.

Changes in the specific gravity of fertilized sole eggs collected at sea followed a similar pattern to those originating from the laboratory stocks (Fig. 3). These eggs had a higher specific gravity than those in the laboratory, but were positively buoyant in the water into which they had been spawned (34‰).

Incidence of unfertilized sole eggs at sea

Both types of unfertilized eggs identified in the laboratory samples were found at sea, i.e. "globular" and "1-cell" eggs. The total number of eggs caught during the three 48-h surveys are shown by developmental stage in Table 1. Data for each haul are given in a separate paper on the daily periodicity of spawning (Child *et al.*, in prep.).

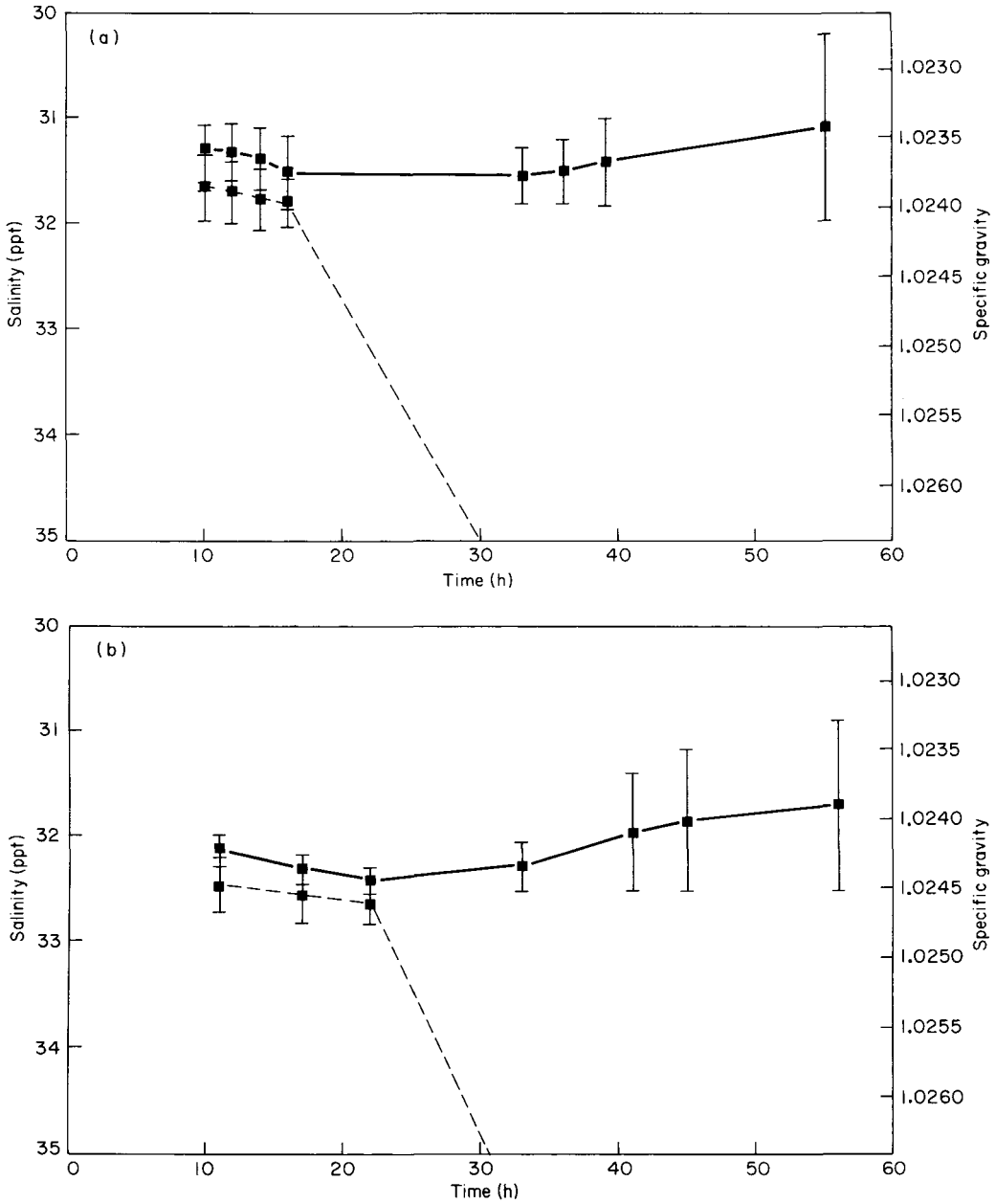

Figure 2. Changes in the mean specific gravity, and the corresponding salinity of neutral buoyancy, of two batches (a and b) of fertilized and unfertilized sole eggs from the estimated time of spawning (0 h). The eggs were spawned naturally by a captive stock. The vertical axes have been inverted so that, for example, a negative slope indicates a decrease in buoyancy. Standard deviations are indicated by the vertical bars. — — — = unfertilized, —— = fertilized.

In all, over 16 000 eggs were examined and of these only 38 were judged to be unfertilized, having failed to develop the normal features of fertilized eggs after at least 5 h incubation. In addition, a total of 458 opaque, non-buoyant eggs occurred in the samples. This represented less than 3% of the total number of eggs caught, though their incidence in the catches varied widely between 0 and 86%. There was no significant correlation ($p > 0.05$) between the numbers of opaque and undifferentiated fertilized eggs, the presence of the latter being indicative of the proximity of spawning and showing a marked daily periodicity (Child *et al.*, in prep.). The opaque eggs were excluded from the calculation of fertilization rate but their significance is discussed later.

In the laboratory, unfertilized eggs retained their buoyancy only until the time that fertilized eggs of the

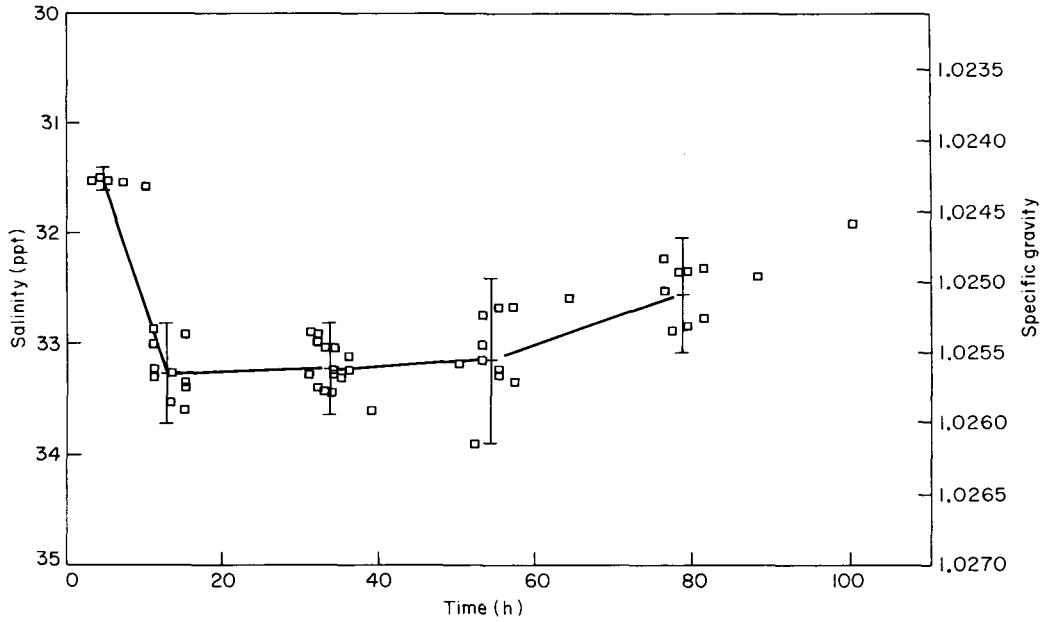

Figure 3. Changes in the mean specific gravity, and the corresponding salinity of neutral buoyancy, of fertilized sole eggs from the estimated time of fertilization (0 h). The eggs were taken from plankton samples and the time from fertilization was estimated from known developmental rates at the prevailing temperature (Riley, 1974). The vertical axes have been inverted so that, for example, a negative slope indicates a decrease in buoyancy. Standard deviations are indicated by the vertical bars.

Table 1. The total numbers of fertilized and unfertilized eggs caught during five 24 h surveys in the Thames estuary.

Status	Number of eggs
Unfertilized	38
Fertilized	
prior to first cleavage	273
Stage IA	5808
Stages IA/IB to IV	10 020
Opaque (dead)	458
Total live eggs up to and including stage IA	6120
Total unfertilized	38
Unfertilized %	0.6

same age had reached the early stages of gastrulation. The ratio of the total number of unfertilized eggs to the total number of eggs up to and including those with a well-developed blastula therefore provides an estimate of the incidence of unfertilized eggs at the time of fertilization. This value was calculated to be about 0.6% (Table 1).

Discussion

The contribution of fertilization failure to egg mortalities has been a matter of speculation for some time, but despite this there have been surprisingly few attempts to quantify fertilization rates in natural populations. Markle and Waiwood (1985) review the evidence for fertilization failure and discuss the difficulties of making such estimates in their recent study of fertilization failure in gadoids. One of the major problems they encountered was the damage that may be caused to eggs during capture. About 40% of the eggs they examined were damaged and were predominantly at an early stage of development. Such high levels of damage would not only reduce the material available but if, as seems probable, the susceptibility of eggs to mechanical damage decreases with development, any estimate based on undamaged eggs would be strongly biased. A further difficulty is that of assessing the fertilization status of undeveloped eggs, a problem exacerbated by the loss of structural detail in preserved material.

The validity of an estimate of fertilization rate will depend on effective sampling and recognition of fertilized and unfertilized eggs. In addition, in order to compute fertilization rates at the time of fertilization from relative abundances, information on the longevity of unfertilized eggs and development rates of fertilized eggs is required.

In this study, slowly hauling a ring net vertically from a freely drifting ship proved to be an adequate method of catching eggs in sufficient numbers and in good condition. In this respect, the choice of a comparatively sheltered area was clearly advantageous, not only facilitating the gentle capture of eggs but also the examination of the live eggs on board ship.

The examination of live eggs avoided the problems associated with the use of fixatives and those of distinguishing between unfertilized and fertilized eggs before characteristic features had developed. In some species, such as the cod, the persistence of the cortical alveoli and the accompanying lack of a perivitelline space (Davenport *et al.*, 1981) in unfertilized eggs could possibly be used as criteria for fertilization status in eggs showing no other signs of development. In the sole, however, neither feature was readily detectable and apart from resorting to histological methods for the detection of sperm or its genetic material, a reliable identification could only be made by incubating eggs until recognizable development had occurred. This proved to be a satisfactory procedure. Eggs of uncertain identity almost invariably continued to develop in vials of filtered sea water even when subjected to repeated examinations.

A curious feature of unfertilized eggs was that some were "activated" and produced a germinal disc (1-cell eggs) whereas others showed no development (globular eggs). Both these types were observed in field samples as well as in batches from the captive stocks. There is no obvious explanation for the existence of these two types of unfertilized egg. It may be that those showing no development were eggs which had not been expelled from the ovary during the spawning following their ovulation and that when ultimately released they had lost the capacity to be activated. Alternatively, it could be argued that those that developed a germinal disc had been fertilized but were incapable of further development. This, however, clearly could not have been the case for the unfertilized plaice eggs, all of which developed a germinal disc.

Changes in the specific gravity of fertilized eggs followed a similar pattern to that described for other species (e.g. Coombs, 1981 and Coombs *et al.*, 1985). Conclusions regarding the buoyancy of unfertilized eggs at sea from the laboratory studies of specific gravity were complicated by the fact that all batches of fertilized eggs were non-buoyant in the water in which they were fertilized. The specific gravity of eggs is known to be influenced by salinity at or before the time of spawning (Solemdal, 1971; May, 1974) and at certain salinities eggs may sink (May, 1974). The results suggest that in eggs of plaice and sole the adjustment to their specific gravity necessary to retain buoyancy was not possible at the salinities prevailing at the time of the observations (28–30‰). Initially, in all batches studied, the specific gravity of unfertilized eggs was slightly greater than their fertilized counterparts. This difference was no greater than a salinity equivalent of 0.5‰. Since the salinity of neutral buoyancy of the early stage eggs caught at sea was at least 1‰ less than the salinity of the water from which they were caught, unfertilized eggs would be expected to be buoyant and have the same vertical distribution as their fertilized counterparts.

Laboratory observations indicated that unfertilized eggs rapidly lost their buoyancy at about the time when fertilized eggs of the same age were commencing gastrulation. Thus, the incidence of unfertilized eggs at the time of release was assumed to approximate to the ratio of the number of unfertilized eggs to the total number of eggs up to and including those which contained a well-developed blastula, i.e. Stage IA eggs. This assumes no difference in the mortality rates of fertilized and unfertilized eggs over this period, such that their initial proportionality was maintained.

This estimate of fertilization rate assumes that at the time of release all unfertilized eggs were buoyant and therefore effectively sampled by the procedure adopted. It is possible, however, that the fish also released overripe non-buoyant eggs which are common in, for example, hand-stripped batches from captive stocks of turbot (Howell and Scott, 1989). These may, however, be a consequence of hand-stripping, a procedure which may not only mechanically damage eggs but may also be less effective than the natural mechanism in expelling all ovulated eggs from the ovaries. Unevacuated eggs would rapidly overripen, become opaque, and lose their buoyancy. The extent to which the presence of opaque eggs in the field samples was indicative of a similar phenemonon in natural stocks is uncertain, though the lack of an association between their abundance and that of recently spawned eggs does not support that contention. It is more likely, perhaps, that the opaque eggs were damaged eggs and that their incidence depended on conditions during capture. Although the aim was to sample from a freely drifting ship, variable conditions of wind and tide often prevented the ship from being stationary relative to the water so that the net did not descend vertically. The resultant increased flow of water through the net may have been sufficient to damage some of the eggs.

This study does not provide evidence to support the contention that fertilization failure may significantly contribute to egg mortalities, though it does not preclude the possibility of significant levels of fertilization failure under different circumstances. Nevertheless, a different result would perhaps have been surprising since fish have evolved a variety of strategies for ensuring successful fertilization of their eggs (Breder and Rosen, 1966). In the sole, the testes are small but the low production of milt is evidently adequately compensated for by the complex prespawning behaviour patterns (Howell, unpubl. obs.) which ensure synchronous and adjacent gamete release to maximize the opportunity for fertilization.

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