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Factors Affecting Fecundity and Larval Distribution in the Squid, *Illex illecebrosus*

by

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ABSTRACT

A second year's observation of mating and spawning by captive Illex illecebrosus suggests that actual fecundity may be much below the potential of about 5 x 10^5 ova present in each female. When fully mature, males may initiate spawning by mating with less than fully mature females. Not all fully developed eggs are necessarily spawned. Less than half of the egg mass survived the incubation period. The proportion of ova fertilized in a mass may be drastically lowered when males distribute spermatophores to too many females.

The neutral or positive bouyancy of egg masses previously reported seems to occur only when ambient water density increases. Thus, the movement of egg masses is probably less significant than previously suggested and the likelihood of locating bottom spawning sites greater.

INTRODUCTION

A large fishery for I. illecebrosus has developed in eastern Canadian waters in recent years, with catches steadily increasing. Historical data (Mercer, MS 1973) suggest that large cyclic fluctuations in the population may occur; but given the short, 12- (Squires, 1967) to 18- (Mesnil, 1977) month life cycle of the species, there is at present no possibility of predicting stocks more than a few months in advance of the fishing season. Mature adults disappear from the continental shelf in late fall and immature animals of a new generation return next spring. Although larvae and juveniles were found in the Gulf Stream in February and March (Amaratunga, 1980), no field information is available for the breeding period. During the last four years, however, I. illecebrosus held captive in the Aquatron Laboratory (O'Dor et al., 1977) have reached sexual maturity and in the last two

years they have spawned there. Observations on these captives suggest that some characteristics of the emigrating breeding stock in the fall could be important determinants of fecundity. Durward et al. (1979) placed an upper limit on fecundity from total egg counts in captive mature females at 5×10^5 eggs per female. The present document describes observations of fertilization and spawning which suggest that this number is about ten times higher than the viable egg count and of social interactions which could make the average fecundity for the species far less.

MATERIALS AND METHODS

Techniques for collection and maintenance of squid have been previously described (O'Dor et al., 1977 and in 1979). Conditions in the 15 m pool in 1979 differed little from those used in previus years to accelerate sexual maturation (16-hour light/8-hour dark cycle, ad libitum feeding once per day, etc.) in that water temperatures were slightly lower this year and frozen prawns were added to the diet to supplement the live mummichogs (Fundulus spp.) normally used.

Groups of squid were brought into the Laboratory on 25 June (A), 23 July (B), and 5 September (C); only groups B and C were held to maturity. Morphometric data (Amaratunga and Durward, 1979) and data on reproductive conditions were collected for 35 to 100 specimens taken initially from each group, and for each animal that died during the maintenance period and the occasional animal sacrificed for examination. Deaths were usually attributable to skin damage (in the first three weeks), gill infections (five to ten weeks) or, ultimately, to post-spawning mortality. The presence, and usually the number of spermatophores in Stage 3 males and fertilized females, was noted.

RESULTS

Mating

Initially, 73 animals from group B were placed in the pool. Of these, 42% were males. Only 14 of these remained, four of which were males, when 53 animals from group C were introduced. Group C had only three males (males made up only 6% of group C; low male to female ratios are common in our late season inshore samples) and were easily distinguished from group B males which had large, white testes visible through their mantles. Figure 1 shows the summary of reproductive conditions of the females during the experiment.

Figure 1 shows the maturity of group B females and that they were distinctly more advanced than those of group C at any given time. Also indicated in Figure 1 are females with spermatophores implanted inside their mantles at death, the death dates of males, and the dates on which new egg masses were noted. With the exception of one animal 28 IX (this number corresponds to the animal recorded on this date in Figure 1), all of the fertilized females had begun vitellogenesis (Stage 4). With the exception of five females probably fertilized by male 30 IX, all had finished ova (approximately 0.90 x 0.65 mm) in their oviducts. This included 12 IX, the smallest (224 g) animal with finished eggs that we have seen. This female had 526 spermatophores attached to the inside of the mantle; other females contained from 16 to 700 spermatophores, with 300 to 500 being the normal range. Males 20 IX and 22 IX contained 311 and 610

spermatophores respectively. The other three males were virtually completely spent with only a few spermatophores remaining. Usually the death of a spent male was associated with a cluster of deaths of fertilized females and the appearance of egg masses. Although male 30 IX appears to have been unusual in mating with several relatively immature females from group C when more mature group B females were present, it is clear that males do mate more than once and generally do so only with females of advanced maturity.

Some displays and interactions between individuals which resembled the mating behaviours of other squid (Drew, 1908) were observed, and on one occasion a pair was seen in a posture probably associated with fertilization; however, such incidents must have been very rare, very subtle, or restricted to periods of darkness. Pairing and mating activities in schools of mature Loligo pealii observed in the pool in an earlier experiment were much more common and obvious.

Spawning

At least ten large (50 to 100 cm in diameter) and several small egg masses were deposited in the pool. The small masses could have resulted from small layings or fragmentation of larger ones; hence, an actual count was difficult. Individuals produced more than one mass, but this may have been because they were interrupted during spawning. On two afternoons, animals were observed while spawning. A position similar to the "resting" posture (Bradbury and Aldrich, 1969) was assumed, but the chromatophore pattern of transverse stripes gradually shading from light to dark normally seen in resting animals was replaced by a pattern of almost pure white (chromatophore fully contracted) with a very sharply contrasting dark band at the mantle opening, a very dark region at the fin tip and the tips of the anterior arms (see Figure 2). In this posture on the bottom, the animals made very strong, rapid mantle contractions (about 42 per minute compared to 35 per minute seen in the normal resting posture). These contractions did not move the animal and were presumed to be made with the funnel closed. Such mechanical activity probably squeezed eggs from the oviducts, sperm from the spermatophores, and jelly from the nidamental glands to be mixed with water and exuded slowly into a large gelatinous mass which formed in front of the animal. In one case, when disturbed by lights used in an attempt to film the process, a startled female jetted away, leaving a strand of jelly (Figure 3) which provides some evidence that the gelatinous mixture is produced and mixed inside the mantle.

Characteristics of the Egg Masses

As in 1978 (Durward et al., MS 1979), the egg masses were typically 75 to 100 cm in diameter and either unattached or only incidentally attached to a substrate. Several ropes, buckets, etc. were made available and in only one case was a bit of rope included in the mass. The assumption of the resting posture and other spawning behaviours suggest that the masses are always formed on the bottom, but no particular bottom features appear to be required. Gentle currents roll the masses around and masses that did not move tended to go black and become anoxic on the side in contact with the bottom. Also, as in previous years, some masses became neutrally or positively bouyant and rose in the water column; however, all such events this year were clearly asssociated with an influx of colder (by about 1°C), more saline (by 0.5 to 1.0‰) water into the pool. Apparently, the gel retains the low density, allowing the mass to float in the higher density water.

Attempts to observe complete embryonic development in situ in the masses failed but gave a number of insights into the properties and functions of the gel mass. When fragmented, the masses tend to disintegrate rather rapidly. Several techniques were tried to position intact mass for microscopic observation. It was relatively easy to catch an intact mass in a plankton net as the gel tends to plug the fine mesh completely, but all efforts to transfer the masses into glass-sided containers shredded them (an 80 cm mass weighs nearly 300 kg and collapses under its own weight in air). As an alternative, a plankton net with windows was constructed, allowing intact masses to be trapped and positioned at the pool windows through which they could be photographed or observed under a dissecting microscope.

Two masses captured in this way were observed and photographed for up to two weeks. Survey photos such as the one shown in Figure 4A allowed gross developmental changes to be monitored. Conditions for microscopic observation of embryonic development were not ideal, but there was sufficient resolution to stage development if it had occurred. In fact, the surveys showed that unfertilized eggs (chorions not expanded) predominated (approximately 60%) and that very few of the eggs advanced beyond Stage 21 (Hamabe, 1962). The most advanced eggs seen this year were only in Stage 30. The reasons for the lack of successful development are unclear. Many of the eggs became infected and developed large "halos" of bacterial or fungal growth (Figure 4B), while many simply stopped developing.

An attempt was made to artificially fertilize eggs removed from the oviducts of dead females to avoid the problems of contamination. Eggs were given a variety of treatments, including sterile seawater and antibiotic washes followed by artificial fertilization with sperm released mechanically from washed spermatophores. Fertilization (chorion expansion) occurred in nearly 100% of the eggs in these experiments, but development always stopped at about Stage 16. High concentrations of sperm in the experiments may have resulted in polyspermy, a problem common in many molluscan eggs which leads to early developmental failures. When such eggs were transferred to flowing non-sterile seawater, they developed a complex microfauna on their surfaces and were eventually invaded by a variety of protozoans and crustaceans.

The importance of the large quantity of gel for protection of the developing eggs was more apparent this year. At the outside edges of the mass a host of organisms accumulated, ranging in size up to copepods several millimeters in length. These were presumably feeding on bacteria and fungi growing on the gel and the eggs. Even the largest copepods could not swim freely through the gel, but they did penetrate it a few centimeters. This apparently created tears which were then invaded by smaller organisms. The outer surface of the gel thus collapsed at a rate of a few centimeters per day. This was seen in free masses as well, which blackened and eroded at the surface until after two to three weeks very little remained. If full development requires one to two weeks (Durward et al., MS 1979), less than half of the original eggs would remain in the mass.

DISCUSSION

It is not clear whether such a mix of mature and immature squid would ever exist in nature, nor indeed such dramatic

difference in male and female numbers in a spawning stock. However, their interactions in the pool provided clues about the characteristics and conditions necessary for mating. The observations bring to question two aspects of the pattern of reproductive activity proposed by Durward et al. (MS 1979):
i) Which sex controls the onset and success of spawning?
ii) What is the site of spawning and distribution of larvae?

The data suggest that males control the onset of spawning. The observations support the suggestion that the presence of spermatophores in Needham's pouch is not equivalent to maturity. In fact, it appears that when males reach full maturity they may mate. They may mate with one or with several females even if the females are not completely mature. Although most of the fertilized animals in 1978 and 1979 had finished ova in the oviducts, they had far more unfinished eggs remaining in the ovary than females kept without males in 1977. If males normally fertilize females before they have finished all of the developing ova, the potential fecundity is markedly reduced. There is some evidence that females retain intact spermatophores (females 23 XI had spermatophores with motile sperm five days after the last male died); and, while there may be a sufficient lag to allow for some continued ova development after mating, most of the captive females spawned and died within a short time of the death of a spent male. Placement of spermatophores thus still appears to be the likely stimulus for spawning; and for captive females, at least, the rigors of spawning appear to be lethal.

Another factor which could reduce fecundity is the low fertilization rate found in the egg masses. Since about 100% fertilization was achieved by artificial insemination (when ova from the same females that produced the masses were mixed with sperm from their implanted spermatophores), the low success in the masses was presumably related to a failure of spermatophores to reach the ova. One cause could be the low numbers of spermatophores in each female resulting from the large number of females serviced by each male. If this is the case, male:female ratios in the natural population could be important determinants of spawning success.

A few observations made during *in vitro* fertilization experiments suggest that the rate of sperm release from spermatophores and sperm mobility both decrease with temperature. This could also affect fecundity and perhaps be a factor in limiting the time and location of successful spawning. Further tests of this effect would be easy to carry out and might provide clues to spawning sites.

The probability of finding egg masses in specific spawning sites now seems higher than the observation of floating masses initially suggested (Durward et al., MS 1979). Since the masses appear only to float when ambient water density increases and since spawning seems unlikely in mid water, floatation would occur only if there were an influx of higher density water at a spawning site. Some movement of masses on the bottom and lifting of masses to mid water by density changes may occur, but unless spawning all occurs in a rather carefully selected spot, the broadcasting of masses by currents seems unlikely.

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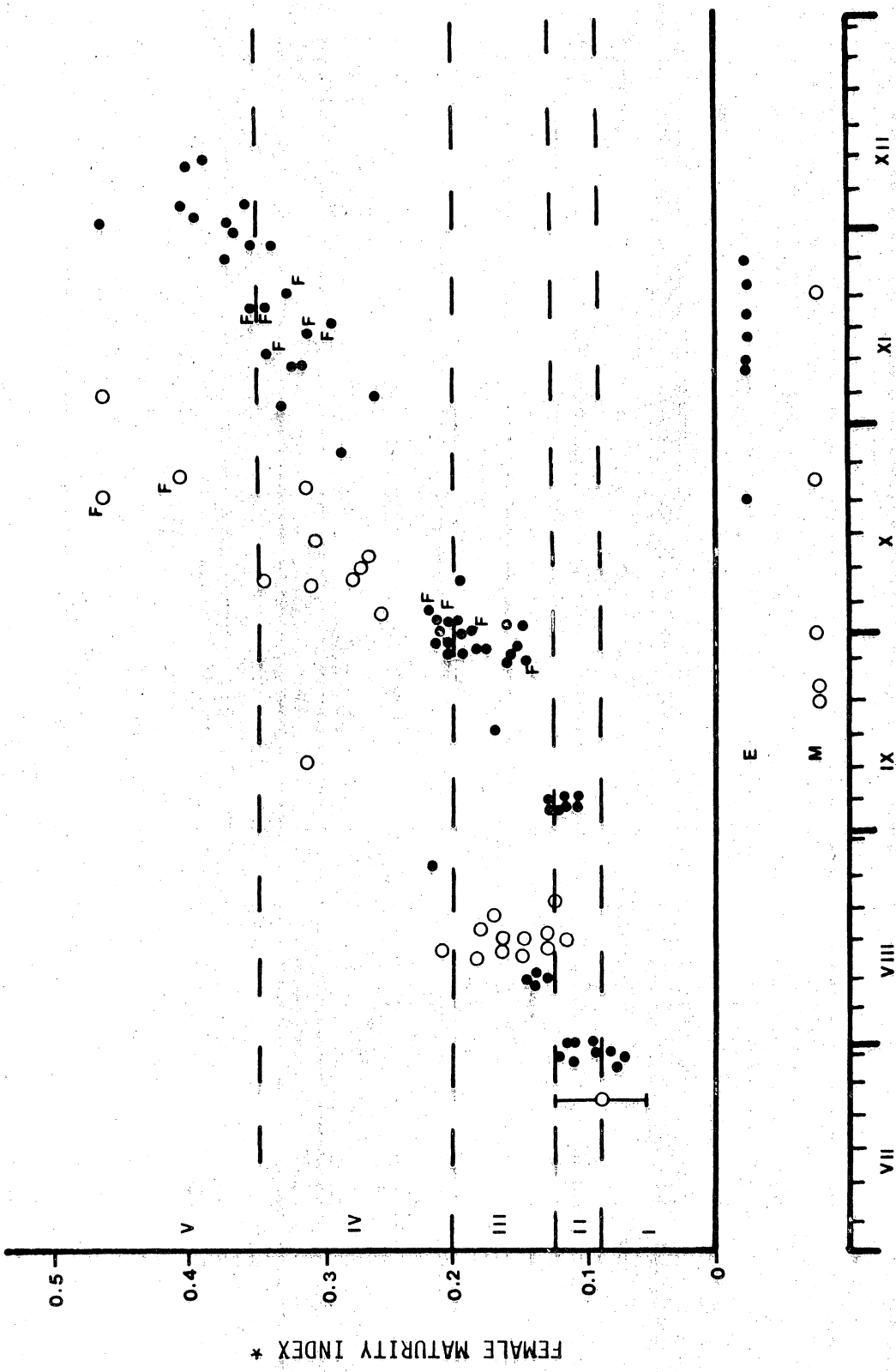

FIGURE 1. The progress of female sexual maturation and related events in two groups of captive squid during 1979. Group B, ● ; Group C, ○. The latter F denotes females with spermatophores in their mantles at death. Only the lower lines M indicate the death date of a mature male and E the date of appearance of an egg mass.

*Female maturity index is based on nidamental gland length ratio (Durward et al., 1979).

Fig. 2. Photograph of a squid in the resting posture. The same posture is assumed during spawning, but the chromatophore pattern is one dark band at the front edge of the mantle and dark patches at the fin tip and tips of the anterior arms.

Fig. 3. A strand of gel left by a female as it jetted away from the egg mass being deposited in the lower left.

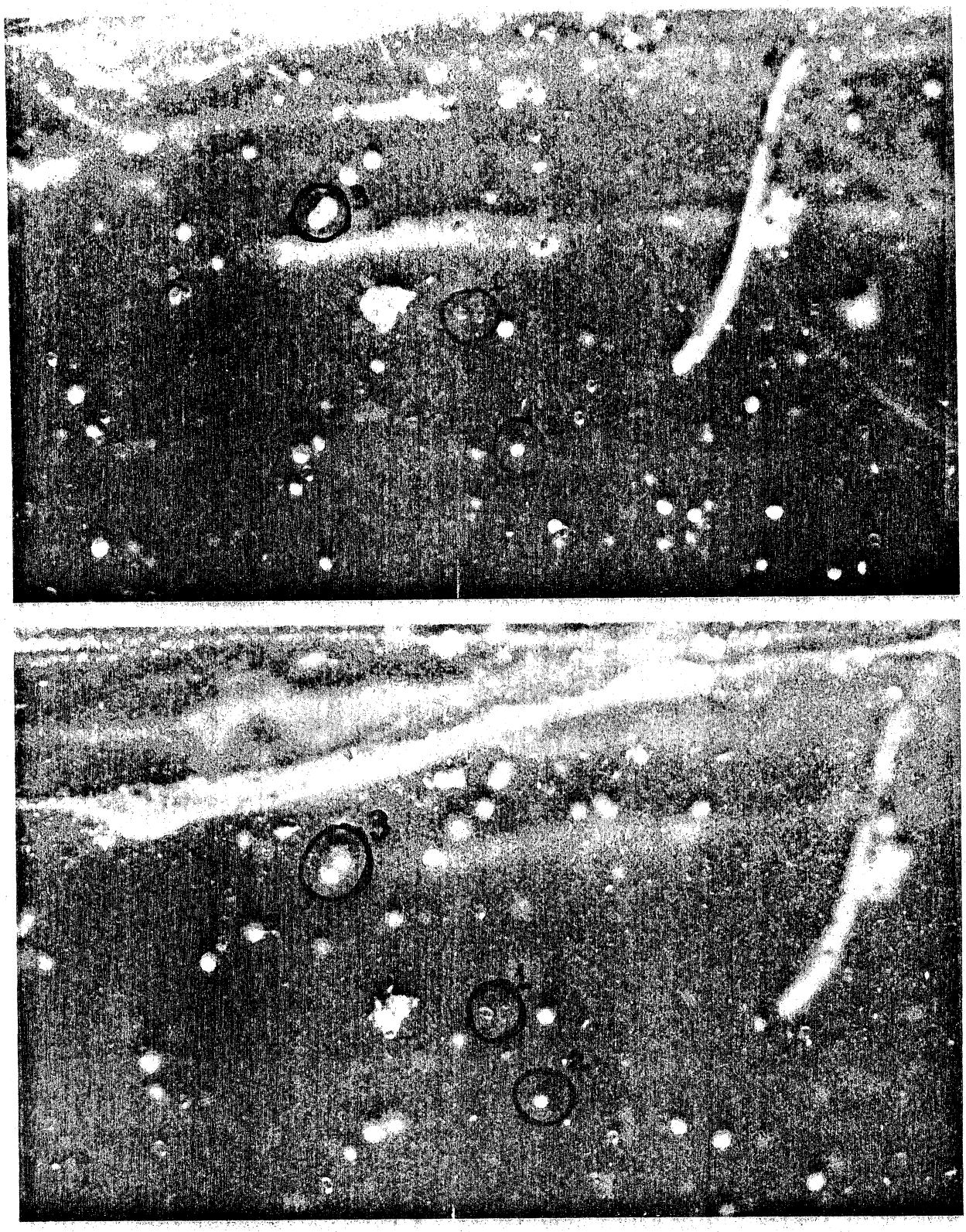

Fig. 4. Photographs of eggs suspended in the gel (A) three days after deposition and (B) four days after deposition. (1) denotes an unfertilized egg; (2) a fertilized egg with extended chorion; and (3) a "halo" of microbial infection.