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Aspects of maturation, mating, spawning, and larval development of *Illex illecebrosus* relevant to field studies

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INTRODUCTION

Each year vast numbers of a single year class of Illex illecebrosus are present on the Scotian Shelf and Grand Banks between the months of May and November. Life cycles ranging from one to two years have been postulated (Squires, 1967; Mesnil, 1976) based principly on the progress of growth and maturity during this period (Mercer, 1973; Lu, 1973; Amaratunga et al, 1978). Few specimens have been taken during the rest of the year (although Rhychoteuthion larvae tentatively identified as I. illecebrosus have recently been taken as early as February; Roper and Lu, and Vecchione, both in press) and the few mature specimens taken during the period seem unlikely to be part of the principle breeding stock (Squires, 1967). A clearer understanding of the source of the stocks being fished and of their life cycle is needed.

This report summarizes observations on a captive population of I. illecebrosus in which precocious maturation was induced and on the fertilization, spawning and larval development which resulted. The characteristics of these processes which may influence future field studies are discussed. The pattern of maturation and larval development during these previously

unobserved phases appears consistent with a one year life cycle (February to February; Squires, 1967), similar to that of the winter population of Todarodes pacificus, the most important component of the squid fishery in Japanese waters (Araya, 1976; Kawahara, 1979).

MATERIALS AND METHODS

Approximately 300 I. illecebrosus (mean ML 19.3 cm) were collected from inshore waters (Herring Cove, Nova Scotia) on July 18, 1978, and maintained in captivity on a 15 hour light : 9 hour dark cycle for up to 100 days in the 15 meter diameter Aquatron pool at Dalhousie University. Details on the experimental conditions and growth of these animals during this time are described by O'Dor et al (1979). Additional squid were added on September 18 and October 20. These could be distinquished from the original group by their immaturity and skin condition. The sex ratio of all of the groups held in the pool was approximately 50:50.

The maturity condition of both males and females, removed periodically from the pool, was monitored and defined as described by Amaratunga and Durward (1978). A fully mature female with spermatophores attached inside the mantle was removed from the pool after producing an egg mass on day 56 (Sept. 13). Two more egg masses were found on day 69 (Sept. 26). By day 82 (Oct. 9) three more egg masses had been produced and three mated females had been found.

Fragments of these egg masses, containing 500 to 2000 ova, were taken from the pool for observation and photography. Each fragment was maintained in a 5 liter aquarium covered with a 0.3 mm mesh netting and supplied with running, ambient temperature (circa 13 C) sea water. Randomly picked ova, from each aquarium, were examined daily under a dissecting microscope and staged on the basis of Hamabe's (1962) scale of embryological development for T. pacificus. Newly hatched larvae were placed in a 500 ml container covered with a 0.3 mm mesh netting and supplied with fresh running sea water. Larvel development was monitored daily.

The early stages of embrylogical development were

observed in artifically fertilized ova. Spermatophores and ova from a mated female were mixed in a beaker of sea water, and the addition of a small amount of jelly from an egg mass resulted in the release of 'clouds' of sperm (as the spermatophores ruptured) and fertilization of the ova. Eggs from a mature, unmated female were fertilized using spermatophores from a mature male by the same technique. These ova survived only 3 days due to protozoan contamination.

RESULTS AND DISCUSSION

MATURATION: Precocious maturation of captive females occurred in about 50 days as previously described (Durwart et al., 1979). There was also an acceleration of male maturation. In the field, males begin to show signs of maturation before females; but the maturity condition in the captive males was advanced beyond that observed in the field. In July, males entered the pool in Stage 1 but advanced to maturity (Stage 3, spermatophores present in Needham's sac), as defined by Amaratunga and Durward (1978), in less than 30 days. By 30 days (late August) the captive males (250 to 280 g) contained 100 to 300 spermatophores, equivalent to the number of spermatophores observed in a sample of offshore males, of a similar weight, during October/November. Similar sized captive males, examined after spawning (56 to 82 days after entry into the pool), contained 200 to 700 spermatophores remaining in Needham's sac. Larger males contained even more. Since some of these males had mated (the mated males could not be distinguished) the higher counts are probably more representative of the total number of spermatophores produced.

Although males may begin to produce spermatophores early in the season (September), this does not necessarily indicate full maturity (spawning readiness). The results from our laboratory studies suggest that males can accumulate several hundred spermatophores which are normally held in reserve until mating is triggered by the presence of mature females, and that the maturity staging of the male needs to be extended. Spermatophore counts may be an appropriate way to assess advanced stages of male maturity, but further studies of the advanced stages are needed.

MATING The female shown in Fig. 1 contained approximately 1300 spermatophores; the other mated females contained 300 to 500 spermatophores. The spermatophores were found in 'bundles' of 100 to 200 anchored inside the mantle cavity primarily near or on the oviducal gland. In two cases, however, a spermatophore 'bundle' was found on the outside of the mantle. Immature females introduced from the field never mated.

The oral ends of the spermatophores in the 'bundle' are cemented to the tissue. Attached spermatophores are shorter in length than those found in Needham's sac, but those that we observed were apparently intact. The retension of sperm in attached spermatophores may provide for some delay between mating and fertilization. Our results agree with Hamabe's (1974) report that the use of a buccal pouch for sperm storage, common in squid (Arnold and Williams-Arnold, 1977), does not occur in I. illecebrosus.

From our observations it appears that the presence of mature females induced mating behavior by the males. Then either the mating process or the implantation of spermatophores must have induced spawning since females held to maturity in previous years in the absence of males never spawned. The jelly produced during spawning probably induces release of sperm from those spermatophores it contacts. Some of the spermatophores must break free since empty spermatophore cases were abundant in the egg masses. Fertilization of the ova may actually occur after spawning in the mass. The pattern of mating and egg mass formation is consistent with the fragmentary data on other species of Illex (I. coindetii, Boletzky et al, 1973; Mangold, 1963; I. oxygonius, Roper, personal communication).

SPAWNING AND CHARACTERISTICS OF THE EGG MASS. The egg masses were spheres of nearly bouyant jelly ranging in size from about 40 to 120 cm in diameter. In contrast to those of T. pacificus (Hamabe, 1962), the masses were not attached to substrate nor deposited in buckets provided. Although in most instances we could not be sure which female had spawned a given egg mass, a mated female was usually found dead a few days after each egg mass appeared. Since only four mated females were found after the six

egg masses were produced, it is clear that repeated spawning can occur. I. illecebrosus does not appear to be a total spawner, for in each case ova were still present in the oviducts and ovary. The nidamental and oviducal glands in the spent females were smaller than those in a mature female (Durward et al, 1979), but morphologically the spent female is difficult to distinquish from the maturing female (late Stage IV).

The largest egg mass is shown in Fig. 2. This mass contained in the order of 10^5 ova spaced at distances of about 1 cm apart in an apparently uniform jelly. There was no evidence of multiple layers of jelly as seen in the egg mass of T. pacificus (Hamabe, 1962). The jelly is quite tenuous and the masses break up when handled, which probably explains the lack of observations of the masses in the field. The largest egg mass was slightly denser than sea water but drifted with the slight current along the bottom of the pool. Some of the smaller masses had bouyancies so near neutrality that they remained suspended in mid-water.

LARVAE. The fertilized ova is ellipsoid in shape being 1 mm X 0.8 mm in diameter. During embryological development the ovum becomes spherical and the chorionic membrane swells as the embryo developes and the perivitellin space enlarges reaching a maximum diameter of 1.8 mm just before hatching. The general features of the embryological development of I. illecebrosus resemble those described by Naef (1923) for an unidentified Mediteranean Ommastrephid, and Hamabe (1962) for T. pacificus.

Hatching occurred 6 to 8 days after spawning at about 13°C. This developmental rate for I.illecebrosus is similar to that observed for I. coindetii at 20 - 22°C (6 - 7 days, Boletzky et al, 1973), L. pealei at 21 - 23°C (10 days, M°Mahon and Summers, 1971), and T. pacificus at 15 - 20°C (4 - 5 days, Hamabe, 1962). Further studies on the relationship between temperature and the rate of development in I. illecebrosus are required. Survivorship to hatching was low due to protozoan contamination; from the several thousand eggs maintained in the 5 liter tanks only 13 larvae hatched but all these survived for at least one week.

Immediately after hatching the larvae had mantle lengths of 1.1 mm and a small internal yolk sac. Their appearance after 8 days of development (the longest survivor) is shown in Fig. 3. At this stage they have a mantle length of 1.25 mm, two pairs of dorsal arms (I and II) and a developing proboscis. The proboscis is not obvious until 2 or 3 days after hatching.

The chromatophore pattern of a 5 day old larva is shown in Fig. 4. Living larvae are transparent and the chromatophores appear black in transmitted light and brownish-red, orange in incident light. When fixed the larvae become opaque and the chromatophores lose some pigment, but the basic pattern can still be observed if carefully examined. The larvae should be fixed in 10 % neutralized formalin for at least 48 hours and then transferred to 40 % 2-propanol (in sea water).

Newly hatched larvae were unable to swim free off the bottom of the aquarium, but 'scooted' randomly along the bottom. After 4 or 5 days the larvae were seen swimming free in an erratic circular motion throughout the aquarium. From our limited observations we were able to discern no consistent pattern of phototropism, but did note that intense light (as well as contact or vibration) induced withdrawl of the head into the mantle cavity. This at least suggests that strong light is a noxious stimulus.

During observation under a compound microscope using a well slide, one larva appeared to be feeding on protozoa. Populate matter in the water, including several ciliated protozoans, was swept between the dorsal arms and past the mouth in a current which seemed to result from continuous radular movements. On two occassions protozoans disappeared near the mouth and were apparently eaten. Since the early larvae are so small and lack both a large yolk reserve and means of capturing larger prey, such a mechanism would seem valuable - particularly if the larvae remain in or near the egg mass where protozoans living on non-viable ova are present in high concentrations. Given the limited swimming ability of newly hatched larvae and the size and nature of the egg mass a larva might spend several days escaping from the mass.

GENERAL DISCUSSION

Spawning is obviously critical to maintenance of I. illecebrosus stocks and its timing and location important factors in understanding population dynamics. It is now possible to describe the morphology of the species throughout its life cycle and the events which occur during it, but these events still cannot be placed either geographically or seasonally. There are two approaches to determining the site and time of spawning: one which traces the events leading to spawning by adults, and the second which uses the distribution of larvae. Since studies to date have provided only fragmentary information in either of these areas, future surveys need to extend the time and area of study. The sections below summarize the results of laboratory studies, on I. illecebrosus, which should be considered in such surveys and indicate the extent to which future work on captive squid can aid in interpretation of the field data.

MATURATION AND SPAWNING. Although the rate of maturation in captivity was accelerated by conditions (probably photoperiod) in the pool, the pattern observed seems consistent with that observed in the field. In the natural population both sexes could easily reach spawning readiness by January/February as predicted by Squires (1967) and this seems the logical time to look for spawning squid. The process of mating and spawning are probably closely linked. Although mass mating followed by immediate spawning as observed in other squid species (L. opalescens, Fields, 1965; T. pacificus, Kawahara, 1979; only to name a few) could occur, there was no indication from our observations in the pool that massive deposits of spawn resulting from the addition of more and more egg masses to the original mass are likely. If individual females spawn without mass mating, the appearance of a large homogeneous population in May or June still suggests that spawning occurs over a limited period, perhaps as a result of a photoperiodic cue. The capture of mature or mated females is probably the only good indicator of spawning time. If maturity induces mating and survival after spawning is short such specimens may be difficult to find.

Males could probably mate with more than one female, but since there appears to be a considerable degree of social interaction in I. illecebrosus the situation may be quite complex. Because of our lack of understanding of the behavioral factors in mating and our observations of incomplete spawning and low hatch rate (which indicates that fecundity estimates based only on the eggs present in mature females may be very high, Durward et al, 1979), care is needed to ensure that adequate breeding stocks remain at the end of the season.

LARVAL DEVELOPMENT. The characteristics of the larvae bred in captivity are consistent with larvae collected from the natural population and tentatively identified as I. illecebrosus (Roper and Lu, 1979; Vecchione, 1979). Although our observations confirm the link between Rynchoteuthion type 'C' (Roper and Lu, 1979) and I. illecebrosus further data are required to distiquish I. illecebrosus and I. oxygonious. Because I. oxygonious is a southern species this is probably not a problem in Canadian waters; and identification of all larval and juvenile stages of I. illecebrosus should now be possible. Even extensive larval surveys seem unlikely to give any precise localization of spawning if the egg masses drift with the currents. There may, in fact, be no specific spawning site if there are no mass matings. Attempts to identify egg masses themselves may be more valuable. Although it seems unlikely that intact egg masses will be found in trawls, fragments may be, and these are now identifiable.

Larval surveys could, however, provide information about timing. The time to look for these larval and juvenile stages depends on the time of spawning. If spawning occurs in January/ February it seems likely that the development to 50 g could occur before juveniles of this size appear in the fishing areas in June. Unless temperatures at the time of spawning are less than 6°C hatching should occur in less than two weeks. Further rearing experiments are needed to establish larval growth rates, but LaRoe's (1971) data on Sepioteuthis sepiodiea show that growth from hatching to 50 g is possible in about three months at temperatures ranging from 18° to 30°C. Although growth rates in

I. illecebrosus are temperature dependent (O'Dor et al, MS 1979) their larvae are adapted to colder waters (viz.the rapid development of embryos) and can probably perform as well at lower temperatures. Any detailed analysis of natural growth of larvae will require information on temperatures in the areas where they occur. Laboratory determined growth rates at various temperatures will be needed to relate size and distribution data to any precise projection of age.

SUMMARY

1) Precocious maturation of the male and female I. illecebrosus was induced in the laboratory in about 50 days. Maturation of females under these conditions has been previously described. Spermatophore counts in males held for the same period exceeded those of males in the field previously considered mature. This suggests that both sexes reach 'full maturity' at the same time in the field; probably before February.

2) Mating, spawning and fertilization occurred in the laboratory. Intact spermatophores were implanted in the mantle of the female and sperm is released when contacted by nidamental gland jelly. Eggs were fertilized as the egg mass forms. Females produced more than one egg mass and some eggs remained in the ovary when the females died shortly after spawning.

3) Egg masses varied in size from 40 to 120 cm in diameter, contained in the order of 10^5 ova, and each was apparently the product of an individual. Egg masses were neutrally bouyant and not attached to the substrate. In nature they probably drift in the current. Recovery in the field will be difficult since the jelly is tenuous and flows through even 3mm mesh netting when removed from the water.

4) Larvae hatched 6 to 7 days after spawning at $13^{\circ}C$ with mantle lengths of 1.1 mm. Eight days after hatching (maximum survival time) they have a mantle length of 1.25 mm. At this stage the larvae resemble Rynchoteuthion type 'C', but are probably at least two weeks younger than the earliest specimen collected from the plankton to date.

5) It is now possible to characterize all stages in the life cycle of I. illecebrosus. The characteristics of the egg masses and the larvae suggest that plankton surveys will be more useful in determining the time of spawning than its location. Recovery of mated females may be the only good indicator of spawning sites.

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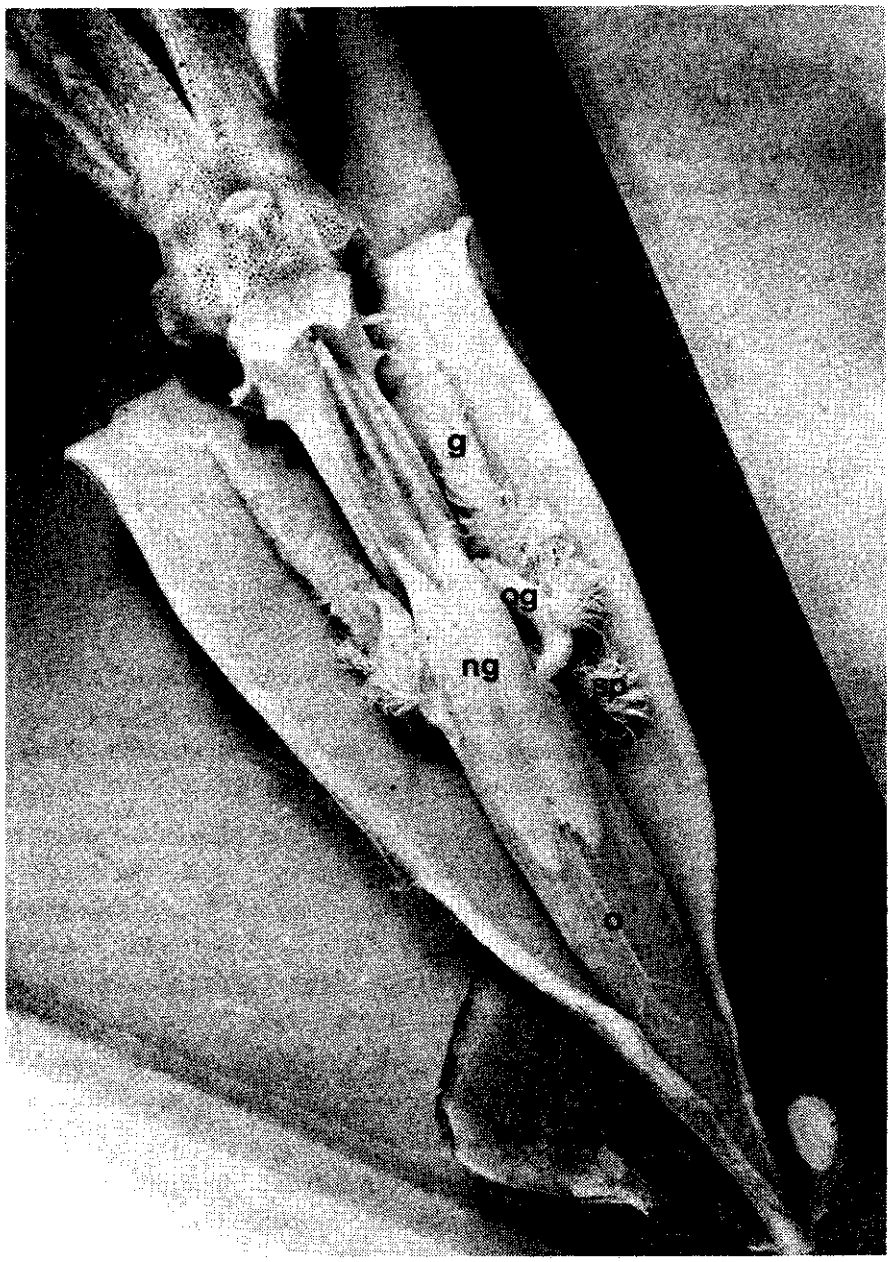

Fig. 1. Mated females (ML 23.0 cm). Note the placement of the spermatophore "bundles". See the text for details. "g", gill; "ng", nidamental glands; "og", oviducal gland; "o", ovary; "sp", spermatophore bundle. All organs have shrunk significantly due to fixation.

Fig. 2. Egg mass of *Illex illecebrosus*. This mass is approximately 120 cm x 70 cm. See text for details.

Fig. 3. *Illex illecebrosus* larva 8 days after hatching. ML is 1.25 mm.

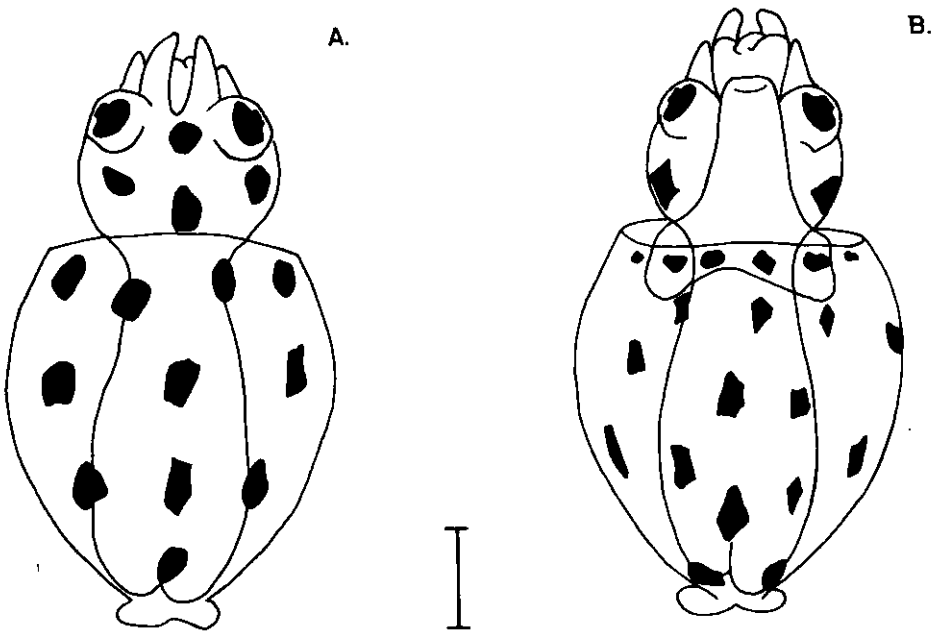

Fig. 4. Chromatophore pattern of a 5-day-old *Illex illecebrosus* larva. "A", dorsal surface; "B", ventral surface. Scale line is 0.25 mm.