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Properties of Illex illecebrosus Egg Masses Potentially Influencing Larval Oceanographic Distribution

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ABSTRACT

Visual observations and video-tape records of the spawning of captive Illex illecebrosus show that this species and probably other oegopsid squid can produce gelatinous egg masses up to 1 m in diameter while swimming in open water. Measurements of the density of the eggs and the changes in water density necessary to lift egg masses indicate that the masses have densities about 0.005 % greater than the water used to make the ge1, while the eggs are more than 5% denser than typical seawater. The gel thus appears to function as a buoyancy mechanism preventing eggs from sinking. Measurements of rates of temperature equilibration between egg masses and the surrounding water suggest that complete density equilibration requires many days under most conditions. Many common oceanographic situations where density increases with depth, due either to decreasing temperature (e.g. North Atlantic Central Water Mass) or increasing salinity (e.g. the Gulf Stream), should suspend egg masses spawned near the surface in the mesopelagic zone. This mechanism could retain oegopsid eggs in a zone relatively free from predators where temperatures are adequate to allow embryonic development and helps to explain why there are so few records of oegopsid eggs in nature.

INTRODUCTION

Since there are to date no observations of the egg masses of <u>Illex</u> <u>illecebrosus</u> in nature, our understanding of this critical life history phase comes from scanty information on egg masses of other oegopsid squid and from laboratory observations on captive populations of <u>Illex</u> itself. Although there are a few records of egg masses of oegopsids found floating on the surface of several of the world's oceans (Clarke, 1966), these occur too rarely to account for the large number of oegopsids that exist. Floating egg masses of <u>Todarodes pacificus</u>, for example, have been reported (Okiykama, 1965); but the other evidence suggests that they are normally demersal and are either attached to the bottom or deposited in cavities (Hamabe, 1962; 1963).

During the 1981 studies of captive squid in the Aquatron Laboratory pool tank, observations were made of the extrusion of an egg mass by a female while swimming, and the complete process was video-taped. This report examines data on changes in seawater density required to render egg masses neutrally buoyant and the rates at which temperature and salinity equilibration would allow the masses to change their density. Based on these observations, conditions in which open ocean spawning and mid-water development could be key features in the reproductive biology of <u>lllex</u> and other oegopsids are examined.

MATERIAL AND METHODS

Squid were held to sexual maturity in the 15 m diameter, 3 m deep pool at the Aquatron Laboratory under conditions previously described (O'Dor et al., 1977). Reports on the characteristics of the egg masses produced have appeared elsewhere (Durward et al., 1980).

The spawning sequence was recorded on 31 October, 1981 with a hand held TV camera (RCA TC 2011/N) from the surface. Prints were prepared from 35 mm photographs of the video monitor with the Sony SLO-323 Recorder in pause mode taken at f4 and 1/60 sec. with Ilford XP-1 film.

Estimates of the density of egg masses are based on incidents where an increase in the density of the water in the pool occurred which caused masses resting on the bottom to "lift off". These events usually are the result of wind-forced advection of colder, higher salinity water to the intakes of the seawater system. Daily measurements of temperature and salinity of seawater in the tank were used to calculate the density changes associated with "lift-offs" which were noted in daily records of egg masses throughout the spawning season. Usually by the time masses were noticed they had been lifted to the surface. In a few cases masses were seen suspended in mid-water and the temperature and salinity differences above and below were noted using a Beckman RS-5 salinometer or Niskin samplers.

Estimates of egg density were made by dropping eggs into a series of sodium chloride solutions of increasing known densities until they would no longer sink. Series of progressively narrower density ranges were used to refine estimates.

During the 1982 season techniques were developed to remove intact egg masses

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from the pool and to incubate these masses at controlled temperatures. A long-handled triangular sheet-metal funnel, 1 m on a side at the outside edge, was used to "scoop" a mass off the bottom and direct it into a bag 0.5 m in diameter and 1 m long made of black hylon window screen. The bag was attached to the funnel with Velcro strips; once a mass had been raised near the surface it was detached and the open end sealed with the Velcro. A polyethylene drum liner (45 gal) was lowered beneath the enclosed mass, and an entire mass, still suspended in water, could be lifted out using a crane. For studies of egg development rate an enclosed mass was left suspended in the liner and a gentle flow of constant temperature water introduced.

The same system was used (often on the same egg masses) to measure the heat diffusivity of the gel. Before starting the flow of heated water a thermal probe was carefully inserted into the center of an enclosed mass from the surface and the core temperature continuously recorded as it approached the incubation temperature. In some cases unfertilized masses were subjected to several cycles of heating and cooling to provide additional data.

RESULTS

Spawning

During the experimental program in the fall of 1981, several spawned egg masses were formed; and in one instance a female was observed during the spawning activity. The female squid shown in Figure 1 had previously mated, and spermatophores present inside her mantle cavity were visible through her translucent mantle. Just prior to the scenes depicted, she had spent about 15 minutes slowly circling the pool away from the rest of the school. The egg mass extrusion began while she was swimming. Frame (a) shows the small translucent spherical egg mass beginning to be formed and held within the arms. Outlined against one of the black grid lines on the pool bottom, the mass is clearly visible. The extrusion progressed rapidly and in approximately 15 seconds the sphere had expanded and become sufficiently tenuous that it is no longer visible directly (frame (c)); however, the contined extension of the arms mark the outline of the expanding mass in frame (d). During the spawning process the fins beat powerfully at frequencies up to 90 beats per minute. This is 2 to 3 times the frequency in normal swimming, but despite this the squid and mass sank steadily. Frame (e) was taken just as the mass touched bottom (near a water-inlet port of the tank) and shows the normal arm cone reforming as the arms withdraw from the mass of gel. The sequence covered a period of 2 min.

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The mass formed in Figure 1 was only 30 to 40 cm in diameter, but clearly illustrated the process by which masses are formed in mid-water. The females apparently release 10⁴ to 10⁵ eggs into a concentrated gel from the nidamental glands which is mixed with sperm released from spermatophores and/or intact spermatophores broken loose from the mantle wall (Durward et al., 1980). After some delay, possibly to ensure fertilization, the gel mixture is moved into the funnel and a large volume of water is mixed into the gel by the mantle pump. The process is similar to blowing up bubbble gum, except that it results in a relatively uniform mix of gel and eggs. During the preparatory period, the animal can continue to use its jet propulsion system and swim normally, but during the period that the gel is being extruded, fin movements provide the only method of propulsion. The squid apparently cannot maintain position this way and so sinks to the bottom of the pool. In the open ocean, however, the small amount of sinking which occurs would be of little consequence.

Buoyancy

The individual eggs have a specific gravity of about 1.10, and thus the masses must always be slightly denser than the water they contain which makes up most of their volume. A typical, spherical mass 50 cm in diameter has a volume of 65.5 L. A typical 400 g female squid produces about 100 g of eggs and spawns half of these in several separate masses. If four masses received 12.5 g of eggs each, it would yield masses containing about 1 egg/m1, which is typical. If made of water with a density of 1025.000 kg/m³ the density of the masses would be increased by less than 0.02 sigma-T units to 1025.014 kg/m³ by the added eggs. The gelling agent from the nidamental glands could potentially increase the density further, but not greatly, since the total weight of the glands is only 20 to 30 g.

Measurements of water density changes which lifted masses like the one shown in Figure 2 off the bottom are given in Table 1 and confirm the very slight negative buoyancy of the masses relative to the water in which they are spawned. Naturally these differences often exceed those required to lift the masses and can only give an upper limit. A change of as little as 0.05 sigma-T will prevent masses from sinking, but the calculations above suggest that the real minimum may be considerably less.

Since the difference in density between the mass and the surrounding water depends primarily on the temperature and salinity of the water in the egg mass, the rates of temperature and ionic equilibration determine how long a mass will stay suspended after an influx of denser water. Direct observations of masses show that they continue to float in the pool for a week or more, but the measured rates of heat transfer allow reasonably accurate estimation of equilibration times under various conditions. Figure 3 shows actual data for central temperatures in an intact 50 cm egg mass, initially at 10.5 C, after transfer to 25 C water. The curve was generated using the equation for heat flow in a sphere (Ingersoll et al., 1954),

$$\frac{T_{c}-T_{s}}{T_{c}-T_{c}} = 2(e^{-x} - e^{-4x} + e^{-9x} - \dots)$$

where T_c is the central temperature, T_o is the initial mass temperature, T_c is the surface temperature and $x = \pi^2 At/R^2$.

In the latter equation t is in seconds, R is the radius of the mass in centimeters, and A is the thermal diffusivity, chosen to approximate the data at 0.0036 cm²/s. This value is 2.5 times the diffusivity constant for water, suggesting that heat transfer in the mass is not purely by conduction but that the gel severely restricts mixing and transfer by convection. At least part of the difference is probably attributable to damage to the gel structure by the thermal probe and to the fact that since the probe was not truely a point, it over-estimated central temperature. With time, under the conditions in the laboratory the structure of the masses degenerates and the apparent diffusivity increases. The mass in Figure 3 was in good condition and gave one of the lowest diffusivities recorded, but its rate of equilibration is still probably higher than that of an undisturbed mass in nature.

DISCUSSION

Our studies of captive <u>1</u>. <u>illecebrosus</u> in the Aquatron Laboratory pool tank (O'Dor et al., 1977; Durward et al., 1980), have enabled us to make observations of nearly 50 egg masses since 1978. Although no prior observations of the actual egg-laying process had been made, the behaviour of mature females resting on the bottom of the tank suggested that the species might be a demersal spawner. This view was reinforced by the fact that most egg masses were first observed on the bottom of the tank and by the limited observations on other species. Although several masses had been seen floating at the surface or in mid-water, this was usually explained by changes in the density of the water in the tank or by the formation of air bubbles in the egg mass gel caused by supersaturation resulting from warming of the water. The present observations do not rule out bottom spawning, but it is now clear that I. illecebrosus can spawn pelagically.

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Whether this is the only or most common spawning mode and hence what the role of this type of spawning may be can only be determined by direct observations in nature. However, since there have been no observations of <u>I. illecebrosus</u> egg masses in nature some speculation may help in the search. Water temperatures above 13 C are important for successful embryonic development (O'Dor <u>et al</u> ., 1982), and, since surface waters are commonly warmer than waters at depth, pelagic spawning near the surface may ensure appropriate temperatures. Squid leaving the feeding grounds in Newfoundland in late autumn would have to travel over 2000 km to find suitable temperatures if spawning were to occur on the bottom, but need only swim a few hundred kilometers to reach such temperatures in the Gulf Stream. Spawning in the Stream could also have important consequences for larval distribution as discussed by Trites (1983). Given the details of the physical properties of egg masses reported above it should be possible to predict the behaviour of a mass in various oceanographic regimes. Three such scenarios follow. In each case it is assumed that:

1) The mass has a sigma-T 0.03 higher than the water it contains.

2) The thermal diffusivity is $0.0036 \text{ cm}^2/\text{s}$, which means that if the temperature of the surrounding water changes, the average temperature in the mass will be 90% equilibrated in about 10 hours (Ingersoll et al., 1948).

3) The diffusion of ions into the mass occurs at a rate proportional to the ratio of the diffusivity constants for heat and sodium chloride in water (i.e. Salinity differences between the mass and the environment take about 130 times as long to equilibrate as temperature differences, on the order of 50 days). This is probably conservative since the "skin" around the mass may be much more of a barrier to ions than to heat. Since the life of a mass is generally less than 16 days, this effect is probably negligible.

4) The mass behaves hydrodynamically like a rigid sphere. The terminal velocity of a 0.5 m egg mass sinking in water of constant density and having a density 0.03 sigma-T higher than the water can be estimated at 1 m/ min. using standard equations and assuming a drag coefficient of 0.4 which is relatively constant for rigid spheres over the appropriate range of Reynold's numbers.

North Alantic Central Water Mass

This water mass is relatively stable throughout the year and Fuglister (1963) records a typical profile for $33^{0}00'$ N and $52^{0}27'$ W for April with surface temperatures of about 20 C decreasing at about 1 C/100 m and reaching 13 C at about 700 m. The combination of temperature and salinity changes produce a sigma-T

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gradient of about 0.15 units/100 m. Actual calculations of the sinking rate in such a gradient would be extremely complex, given the changing drag coefficients, rates of temperature equilibration and temperature and salinity gradients, but it is fairly simple to demonstrate an upper limit by assuming a stepwise descent. An egg mass produced at the surface would sink about 20 m before reaching an isopynchic point. At an average velocity of 0.5 m/min. this would require only about 40 min. At this point the water temperature would have decreased by 0.2 C. The average change in sigma-T with temperature over the range from 20 to 13 C is 0.16 per degree, so 90% temperature equilibration would return the mass to its original relative density in about 10 h. If this cycle repeats every 20 m it would take about 16 days for the mass to sink to waters below the 13 C limit. Since the development time for the eggs is 16 d at 13 C (and only 12 d at the average temperature of 16 C), a mass spawned in this area appears to have a reasonable prospect of hatching. Recent studies of the vertical swimming abilities of newly hatched larvae suggest that they would have no difficulty returning to the surface (0'Dor et al., 1984). It is not clear, however, what they would eat when they arrived or how they would get to the northern edge of the Gulf Stream where most juveniles seem to be found.

It is interesting to note that similar calculations for the sinking rate of individual eggs give a terminal velocity of 1.2 m/min., but without the equilibration time this would put them below the 13 C limit in only 10 h. Whether or not the slow sinking rates of egg masses under these conditions play an important role in the life history of <u>I. illecebrosus</u> it seems likely that the production of similar gelatinous masses is a critical adaptation for other oegopsids living in the open ocean.

Gulf Stream

An oceanographic regime with a subsurface salinity maximum would seem to be ideally suited to reducing the sinking rate of egg masses. In the western North Atlantic such a feature can be found in two areas. One is the Shelf/Slope front where cold, low salinity Shelf water often extends seaward over warmer, higher salinity Slope water. However, in the months when spawning presumably occurs (January-February) the temperatures of the water masses are too low for egg development. The second area is the Gulf Stream where there are consistently high temperatures and where there appears to be a salinity maximum at 100-200 m. We have found little discussion of this feature in the literature, but Table 2 records a number of examples. An egg mass made of surface Gulf Stream water would sink and

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become neutrally buoyant at the observed salinities at the subsurface maximum. The much slower equilibration of salinity would allow it to remain there for many days and perhaps weeks allowing adequate time for development. In addition, this would have important consequences for transport since it would essentially trap the mass in the fastest moving part of the Stream, and therefore result in egg mass distributions similar to the larval distributions discussed by Trites (1983). It could also be an asset for development since recent studies have shown that <u>I.</u> <u>illecebrosus</u> eggs develop well in as little as 6 d at temperatures up to (and perhaps above) 26 C (O'Dor et al., 1984). In fact larvae incubated at higher temperatures appear to be more fully developed at hatching and may have a better chance of survival. If adults spawn in the Stream south of Cape Hatteras this would provide rapid delivery of prime larvae to appropriate sites on the northern edge of the Stream where food supplies would be adequate and where larvae and juveniles have, in fact, been found (Amaratunga et al., 1980).

Gulf Stream/Slope Water Front

The complex shear zone between the Gulf Stream and Slope Water, where features such as shingles, eddies and rings are common and where biological productivity is high, also suggests itself as a potential site for spawning. This zone is often characterized by relatively cool, low salinity water overlying warm, high salinity water which would trap egg masses. To illustrate this, Fig. 4 presents two salinity transects, with temperature superimposed, taken across the Gulf Stream/Slope Water front south of Halifax in April 1979 by Amaratunga et al. (1980). Six out of eight such transects showed subsurface salinity maxima. In addition, early stage I. illecebrosus were consistently present in the frontal zone. However, these features are much more variable than those in the Stream or North Atlantic Central Water. On the other hand, this zone presents a relatively sharp boundary to which adults migrating away from the shelf can cue in order to trigger spawning. Even spawning in Slope waters north of Cape Hatteras may be less risky than it appears since there is some evidence that low salinity water is entrained into the surface waters of the Gulf Stream (Ford et al., 1952). Presumably any egg masses in such low salinity surface water would also sink to isopyncnic positions within the Stream. An additional safeguard for masses spawned in this boundary region is the fact that bottom temperatures above the 13 C minimum exist here even if they sink completely (Trites, 1983). In general most of the transport mechanisms discussed by Trites would be equally applicable to larvae from masses spawned in this area.

Of the three areas discussed, the North Atlantic Central Water Mass would seem the least suitable for <u>I. illecebrosus</u> since it presents a rather diffuse signal to adults that would have to migrate across the Stream, it is relatively poor in potential food items, and it presents a problem for larvae which would have to perform a substantial horizontal migration to reach the Slope water area where they are most commonly observed.

For oegopsids in general, if egg masses normally become distributed mesopelagically, it would explain why they are rarely seen or collected. The gel is too tenuous to be recovered by trawls, and plankton nets would be little better. Even in the pool tank, it is difficult to catch masses in plankton nets since a small pressure wave will push them aside. The occasional appearance in nature of masses on the surface might result from entrainment in water moving to the surface from storm-induced turbulence or from upwelling processes such as those associated with the edge of the Gulf Stream (Yoder et al., 1981; Lee et al., 1981). In general, the mesopelagic zone seems a safer place for eggs to wait out their development period than either the surface or the bottom, and the behaviour of larvae is consistent with their return to the surface.

Determining which, if any, of the strategies outlined above are used by <u>I.</u> <u>illecebrosus</u> will require recovery of egg masses in nature. However, further studies of the environmental factors which induce spawning in adults or of the temperature and salinity preferences of mature adults might help to narrow the search. If the strategy used proves to be one of the more risky ones, it could explain the high variability in population size. For example, spawning in or on the edge of the Gulf Stream at a site involved in the formation of a cold core ring could transport large numbers of larve south of the Stream, thus reducing the probability of subsequent return to the shelf area.

Since egg mass recovery in nature using nets will be difficult, the most promising method for making observations may be the use of submersibles, divers or underwater television. Such techniques have been employed for underwater observations of similarily fragile gelatinous zooplankton some of which have mucous webs 2 m across. (Hammer <u>et al</u> ., 1975). However, even these techniques would have to be used with special care, particularly with respect to lighting, because the egg masses are difficult to observe even under the optimum conditions existing in the laboratory.

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	Sigma-T				
Date	Minimum	Maximum	Difference		
25 Sept. 1981	22.64	23.09	0.45		
30 Sept. 1981	22.96	23.17	0.21		
20 Oct. 1981	21.78	21.83	0.05		
8 Dec. 1982	23.33	23.68	0.35		

Table 1. Changes in seawater density in the Pool Tank which rendered egg masses positively or neutrally buoyant.

Table 2. Some reported sub-surface salinity maxima in the Gulf Stream.

Location	Number of Stations	Depth Range of Salinity <u>Maxima (m)</u>	Salinity Differences from Surface to <u>Maximum</u> (⁰ /00)	<u>Reference</u>
Off Cape Hatteras	12	63-131	0.071-0.886	1
Off Cape Hatteras	1	150	0.4	2
South of Nova Scoti	a l	75	1.0	3
South of Nova Scoti	a 11	90-190	0.028-0.342	4
Off Cheasapeake Bay	8	100	0.200-0.350	5
l) Richardson & Kna Fuglister, 1963. 5	uss, 1970.) Iseiin,	2) Lambert, 19 1936.	82. 3) Smith & Petr	ie, 1982.



the inflation of a 30 cm egg mass by a 23 cm mantle length <u>Illex illecebrosus</u>. As the time display indicates, the process requires just over 2 min. See text for details.



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Fig. 2. A 50 cm *Illex illecebrosus* egg mass spawned *in situ* and suspended on a pycnocline in the Aquatron Pool (15 m diameter by 3 m deep). (Photo by R. W. M. Hirtle, Biology Dept., Dalhousie University.)





