Arnold, John M., William C. Summers, Daniel L. Gilbert, Richard S. Manalis,
Nigel W. Daw, and Raymond J. Lasek (1974) *A Guide to Laboratory Use of the Squid Loligo Pealei.* Woods Hole, MA: Marine Biological Laboratory, 74pp.

III. Reproductive Behavior

Although frequently the subject of much anthropomorphic voyeurism in the classroom, the reproductive behavior of *Loligo pealei* has been seriously studied only in cursory fashion. What follows here is an attempt to briefly summarize the sequence of events in the courtship and spawning behavior, more to aid the observer than to attempt to analyze or interpret the finer points of technique. The interested reader is referred to the studies made by L. Tinbergen (1939) on *Sepia,* by Tinbergen and Verwey (1945) on *L. vulgaris,* by Arnold (1965b) on *Sepioteuthis sepioidea,* by Fields (1965) on *L. opalescens* and by Drew (1911, 1919) and Arnold (1962) on *L. pealei* for such detail. However, in this case, as in many analogous situations, there is no substitute for first hand experience. At M.B.L. it is best to make such observations in the bottom tank of one of the fiberglass sea tables because this gives the animals room to maneuver. The males and females should be approximately equal in size and number, freshly caught, and in good condition. Disturbances in the laboratory will frequently prevent the animals from displaying any activity. Most often the animals mate in the evening of the day they are captured if the proper conditions are maintained and if they are stimulated as described below. The following comments apply primarily to *Loligo pealei,* but other species in this genus seem to behave quite similarly.

The sexes can be easily distinguished in the living animals because the males tend to be larger and slimmer, and when the animal is pale the testis is quite evident as a long white mass between the fins. The females tend to be shorter and thicker through the posterior portion of the mantle. On the ventral surface of females in breeding condition the accessory nidamental glands can be seen through the mantle as an orange to reddish spot midway along the mantle. At the apex of the female's body the eggs have a characteristic color even when viewed through the mantle. Figure three shows both sexes with open mantles.

The male reproductive tract is composed of a large white median testis which lies behind the hearts. It empties into the ciliated funnel end of the vas deferens through which the sperm pass. The vas deferens leads to a much specialized, convoluted and coiled tubule; the spermatophoric organ. Blancquaert (1925) described how the sperm is first twisted into a dense spiral and then covered by the various tunics, membranes and accessory structures. Austin, Mann and Mann (1964) estimated that there were between 7,200,000 and 9,600,000 spermatozoa per spermatophore. The 8 to 10 mm long spermatophores are stored in the spermatophoric reservoir ("Needham's sac") which is continuous with the spermatophoric organ, and are eventually passed to the penis. The muscular walls of the penis gather several spermatophores into bundles which are picked up by the male during copulation. These spermatophores can easily be pressed out by running the tines of a pair of forceps down either side of the penis. If placed on a microscope slide in a little of the fluid from the mantle cavity they can be examined in the unexploded state.

Drew (1911) and Austin, Mann and Mann (1964) have described in detail the structure and ejaculation of the spermatophore. Essentially there are three major components: the sperm mass in which the spermatozoa are highly oriented; the tunics, membranes, and fluid filled spaces; and the ejaculatory apparatus and its associated cement body. The ejaculatory apparatus has a spiral filament which functions to keep the lumen open, not as a coil spring as its shape would so strongly suggest.

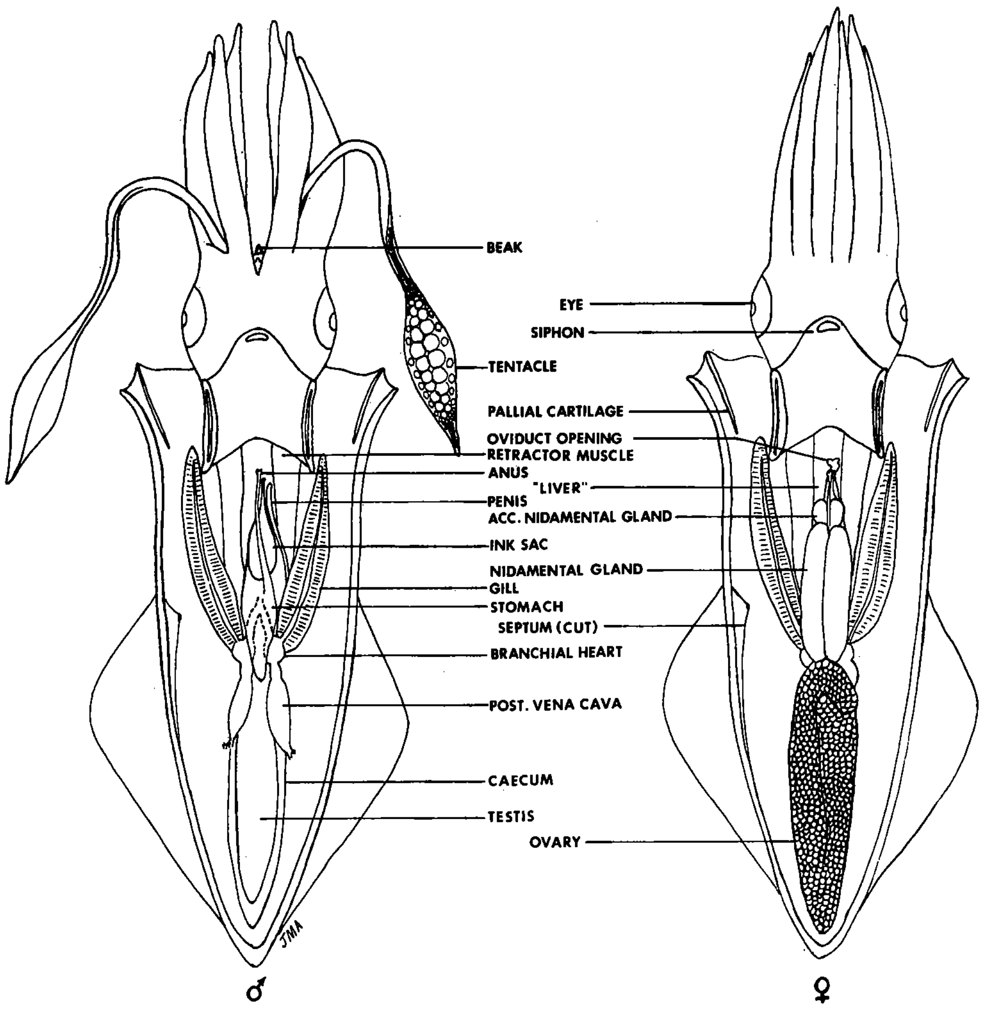

FIGURE 3

Adult male and female squid with their mantles opened ventrally. See figures 11 and 15 for details of the axon and synapse preparation. The terms anterior, posterior ventral, and dorsal are used in the functional sense of the adult animal. In development, the animal pole of the egg actually becomes the apex of the mantle, so embryologically speaking, the embryo's anterior becomes the adult ventral surface.

In ejaculation the hydrostatic pressure from the fluid-filled spaces and the elastic nature of the outer tunic cause the sperm mass and the cement body to be everted from the ejaculatory apparatus. A sperm reservoir is formed from the membranes and the now burst cement body attaches it to any substrate. The spermatozoa are slowly released from the open end of the sperm reservoir and begin to swim about actively. The entire process can be initiated by pulling the "cap thread" which is attached to the end with the ejaculatory apparatus. Putting spermatophores in onequarter saturated $MgCl_2$ slows down the reaction for easier observation. The sperm are quite large with the tail inserted to one side of a long filament which extends backward from the head.

The female reproductive tract also begins with a large median gonad located between the fins in the coelom ventral to the pen. There is a single ovary, single oviduct mainly on the left side, paired nidamental glands, and smaller paired accessory nidamental glands. The oviduct is divided into two portions: a proximal thin-walled large tubule and the distal thick-walled, glandular anterior portion which ends in the oviducal funnel. During the breeding season the nidamental glands are prominent white longitudinal masses on either side of the ventral midline and the subspherical accessory nidamental glands are orange to red mottled with white at the anterior end of the nidamental gland.

The eggs are ovate, very yolky and large, measuring about 1.6 mm x 1.0 mm. They are surrounded by a clear chorion with a micropyle at the smaller end where the blastodisc will develop. When the eggs are mature they are shed from the opaque follicle cells and are almost clear. In the gonad and oviduct they tend to have a depression in one side which later disappears during cleavage. The eggs are stored free in the thin-walled portion of the oviducts until they are spawned. At this state they can be removed from the oviduct and artificially fertilized. During mating the eggs are passed into the thick-walled glandular portion of the oviduct where they are enclosed in a fold of egg jelly. The jelly fold is spiralled by the oviducal funnel, covered with membranes of jelly, and formed into a finger-like egg capsule (= egg string). About 180 eggs are found in each egg capsule; however, nearly spent females frequently will deposit capsules with no embryos in them. Interestingly, the egg jelly suppresses ciliary action in a variety of invertebrates (Atkinson and Granholm, 1968, Atkinson, 1973). The egg jelly stiffens on contact with sea water and as the embryos grow the capsules increase in size from 8-10 cm x 3-3.5 cm to 12-18 cm x 3.5-5 cm. This is mainly due to the swelling of the chorions away from the embryos as they develop. The egg jelly appears to be a mucopolysaccharide.

The earliest recorded observations of mating behavior in cephalopods were made by Aristotle on *Sepia officinalis, Octopus sp.* and an unidentified squid. Like other reports of sexual behavior of another species during that time this work was interesting but suffered in interpretation. There is close agreement between the observations made on mating behavior of most of the decapods, but since I am most familiar with *L. pealei* my remarks pertain to that animal.

Typically, captive, healthy, unmated squid swim about side by side in a small school moving back and forth more or less in synchrony. The males and females move about without any apparent regard to one another. However, if a mass of egg capsules is introduced into the tank this tranquil scene is disturbed. The squid "break formation" and orient toward the egg mass, form their arms into a cone and flush the egg mass with spurts of water from their siphons. This is followed by individual animals darting up to the egg mass, extending their arms into the egg capsules and "groping" around among the egg capsules with the tips of their arms. The males display this behavior more readily than females, but both sexes usually participate. The males become increasingly excited and dart about quickly challenging other males and attempting to separate females from the group by swimming between the female and the other squid. Apparently there is some subtle difference in appeal of the females because all of the males seem to favor particular ones and challenge one another by special displays, but they rarely combat.

When a male has selected a female he swims beside her raising one of his median arms upward in a characteristic "S"-shaped curved and expands the chromatophores in front of his eyes to form a dark brown patch of varying intensity. If another male approaches, the mated male raises his median arm even higher, the color pattern becomes more intense, and he may dart at the challenger. Frequently, they bump their bodies together and several dark spots of expanded chromatophores appear along the edge of the fin toward the opposing male. Infrequently, they grapple with their arms and rarely bite the mantle or head and arms of each other. If the challenger is unsuccessful he withdraws and attempts to find another female; if successful, the formerly mated male withdraws, usually with a less intense display. In this way a social hierarchy is established with the largest or healthiest males selecting the most desirable females. This hierarchy tends to remain relatively stable but if a mated female high in the "peck order" is removed the now unmated male will displace a subordinate male.

During all of the male display the females remain passive or weakly attempt to avoid the disturbance. Males persist in following their chosen mates about the tank and occasionally a female will jump out of the tank in an attempt to avoid the advances of a male. Frequently a large aggresive male will pursue a subordinate male until he jumps out of the tank. Since this usually occurs at the corners of the tank triangles of cardboard should be placed at the top of the tank in each corner to avoid the loss of animals and the subsequent mess of sea water and ink that is spurted around in a dying gasp of a grounded squid.

Copulation and spawning follow establishment of the pairs and the social hierarchy but frequently each of these steps in the mating sequence overlaps one another. Before copulation the male swims beside and slightly below his mate and undergoes a few brief flashes of chromatophores. He then grasps her with his arms from slightly below about the mid-mantle. By a series of quick manipulations he pulls himself forward so his arms are close to the opening of her mantle. At this point he reaches into his own mantle with his modified arm, the hectocotylus, and picks up a bundle of spermatophores from the penis. In one quick motion the spermatophores are ejaculated and cemented to the inside of the mantle or around the oviduct simultaneously. The male then releases the female and they resume their paired swimming. The entire process is consumated in five to twenty seconds and usually alternates with egg laying. During copulation the female remains passive or occasionally will resist the male by attempting to avoid him or by pushing his arms away when he grabs her mantle.

In spawning the female passes an egg capsule from her oviduct to the funnel and then picks it up with her arms. The jelly is penetrated by the sperm pouring out of the many sperm reservoirs around her oviduct and they can be seen swimming in the jelly for several hours after egg laying. The sperm tend to congregate in a small cloud at the micropyle of each egg, suggesting chemotaxis. Once the egg capsule is in her arms, the female attempts to attach it to a preexisting egg mass or to any object that resembles an egg mass (see below). One end of the egg capsule is devoid of eggs and the jelly there is quite sticky. She holds the egg capsule in her arms, sticky end forward, and swims up to the egg mass, hurries into it with her arms and head, and actively twists and entwines the end of the egg capsule into the common center of the egg mass. As the jelly hardens by exposure to sea water it becomes firmly attached. The female then darts rapidly away from the egg mass, flushing it with water from her siphon, and is joined by the male. While the female has an egg capsule in her arms she will not allow the male to copulate. If she drops the egg string during her attempt to deposit it she continues as though she still held it.

As is implied above it is possible to initiate reproductive behavior and spawning by placing an egg mass in the tank with healthy squid (Arnold, 1962). It is most convenient to tie 10 or 20 egg strings from an egg mass, not more than three days old, to a weight (large glass stoppers work well). Older egg strings will induce the investigative behavior and some copulation but the animals tend not to deposit new egg capsules on it. Since the egg capsules harden and swell with age apparently the squid notice the difference and avoid forming composite egg masses which are embryos too widely separated in age. Removal of the egg mass from the tank stops the entire behavioral pattern, but the established pairs seem to remain intact. It is possible to obtain squid eggs on demand when needed for experimental work in this way. The stimulus of the egg mass is obviously visual because it can be duplicated with an artificial egg mass made of dialysis tubing or sheet polyethylene tubing filled with sea water. The animals are reluctant to lay on such an artificial egg mass but will usually do so if given enough time. Frequently they will lay among loose egg capsules that accumulate at the drain of the tank. Only rarely will they form a new egg mass attached to objects in the tank. The question of the initial stimulus of spawning to start the first egg mass of the season obviously arises. In nature the egg masses are most frequently found attached to seaweeds like *Fucus* or to the arms of a dead squid, both of which somewhat resemble an egg mass. This *in vacuo* response needs further study. Interestingly, in the closely related genus *Sepioteuthis sepioidea* where the eggs are laid under rocks and shells the egg mass does not stimulate egg laying.

To observe this mating behavior in the lab three or four pairs of animals of roughly equal size should be obtained from the Supply Department and kept quiet in a large tank for several hours. The initiation of mating works best in the evening hours. The egg mass should be placed in the center of the tank since the animals tend to "forget" it if it is out of sight in one corner. External noise and confusion (such as the shouting of analogies or the popping of flashbulbs) will inhibit the squid and prevent them from spawning. Each female can lay about 20 to 30 egg capsules which contain roughly three to seven thousand embryos.

Eggs surgically removed from the oviduct can be fertilized in shallow dishes by breaking spermatophores in the sea water with them. Several spermatophores should be used to insure a fair degree of successful fertilization and the sperm should remain in contact with the eggs for 15 to 30 minutes. Even with this exposure typically only 10 to 20% of the eggs are fertilized. The eggs should then be washed carefully in several changes of fresh sea water and separated into other dishes so they are not crowded. A few hundred eggs per 9-inch finger bowl is about maximal. Although the embryos will cease development at stage 11 or 12 (Arnold, 1965a), cleavage can be easily observed in these artificially fertilized embryos without the inconvenience of the egg jelly. Apparently, the egg jelly is necessary for further development because without it chorions do not swell away from the embryos and they die. Making a suspension of egg jelly in sea water by mechanically grinding up egg capsules will cause the chorions to swell slightly and the artificially fertilized eggs to continue development for a longer period, but eventually these eggs also die.

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