POSTOVULATORY FOLLICLE HISTOLOGY OF THE PACIFIC SARDINE, *SARDINOPS SAGAX*, FROM PERU[1]

The use of the postovulatory follicle as a means of estimating incidence of spawning in multiple spawning fishes was originally developed by Hunter and Goldberg (1980) for the northern anchovy, *Engraulis mordax*, from southern California. This technique has proven to be quite useful for biomass assessment using the "Egg Production Method" (Parker 1980).

As a result of this work, the postovulatory follicle has assumed new importance. In the work of Hunter and Goldberg (1980), *E. mordax* were spawned artificially in the laboratory (Leong 1971). Fish were sacrificed at different time intervals, and histological conditions of the postovulatory follicles were noted. As an alternative to this method, in the current report we have aged postovulatory follicles of the Pacific sardine, *Sardinops sagax*, from Peru by establishing the time of spawning (egg collections) and by making periodic collections of *S. sagax*.

Methods

Samples of *Sardinops sagax* were collected during September-October 1982 near Chimbote, Peru (lat 09°05′, long. 78°35′). Ovaries were preserved immediately on collection in 10% neutral, buffered Formalin[2]. Later, samples from a total of 270 ovaries were dehydrated in ethyl alcohol and embedded in Paraplast. Histological sections were cut at 6μ. Slides were stained with Heidenhain's iron hematoxylin or Harris' hematoxylin followed by eosin counterstain.

Sardine egg samples from Peru indicated 0100 h to be the midpoint of the daily spawning interval (Smith[3]). Therefore, by knowing the hour of collection and assuming that spawning occurred around 0100 h, we calculated the approximate age of postovulatory follicles.

Results and Discussion

The sardine is a multiple spawning fish (Clark 1934), and during the spawning season we typically observe a mature yolk-filled mode of eggs representing the next spawning session and a vitellogenic mode for a subsequent spawning.

Postovulatory follicle, Day 0 (0-6 h after spawning)

The new *S. sagax* postovulatory follicles (Fig. 1A, B) were striking in their strong resemblance to the age 0-day postovulatory follicles of *E. mordax* (elapsed time from spawning <24 h) (Hunter and Goldberg 1980). The newly formed follicles of *S. sagax* contained many involutions or corrugations and were composed of columnar epithelium resting on a connective tissue theca. Nuclei had a basal location. The lumina occasionally contained eosinophilic granules of unknown origin (Hunter and Goldberg 1980) similar to those reported in the newly formed postovulatory follicles of *E. mordax*.

Postovulatory follicle, Day 1 (7-30 h after spawning)

These structures showed the beginning (Fig. 1C) of a breakdown in organization in comparison to day-0 postovulatory follicles. This included a size decrease to about one-half and marked degeneration of the columnar epithelial cell lining. Many epithelial cells had irregular shapes, vacuoles, and pycnotic nuclei. The convoluted structure was not as distinct as in day-0 postovulatory follicles. The linear arrangement of columnar epithelial cells was still evident. This is important, and constitutes the chief character that should be used for distinguishing day-1 from day-2 postovulatory follicles in *S. sagax*. This linear arrangement was absent in day-2 *S. sagax* postovulatory follicles.

Postovulatory follicle, Day 2 (31-53 h after spawning)

Degeneration of the *S. sagax* postovulatory follicle was clearly more advanced (Fig. 1D) at this stage. Distinguishing them from old atretic follicles is now a critical problem. Lumina were typically occluded and contained irregularly shaped cells with pycnotic nuclei, representing the final stages in the degeneration of the columnar epithelial cells that were previously so evident (Figs. 1A, B) in day-0 *S. sagax* postovulatory follicles. Vacuoles may be present. While the greatly convoluted structure that characterized earlier postovulatory follicles is no longer pronounced, there

[1]Publication No. 11 of PROCOPA.

[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

[3]P.E. Smith, Southwest Fisheries Center La Jolla Laboratory, National Marine Fisheries Service, NOAA, P.O. Box 271, La Jolla, CA 92038, pers. commun. March 1983.

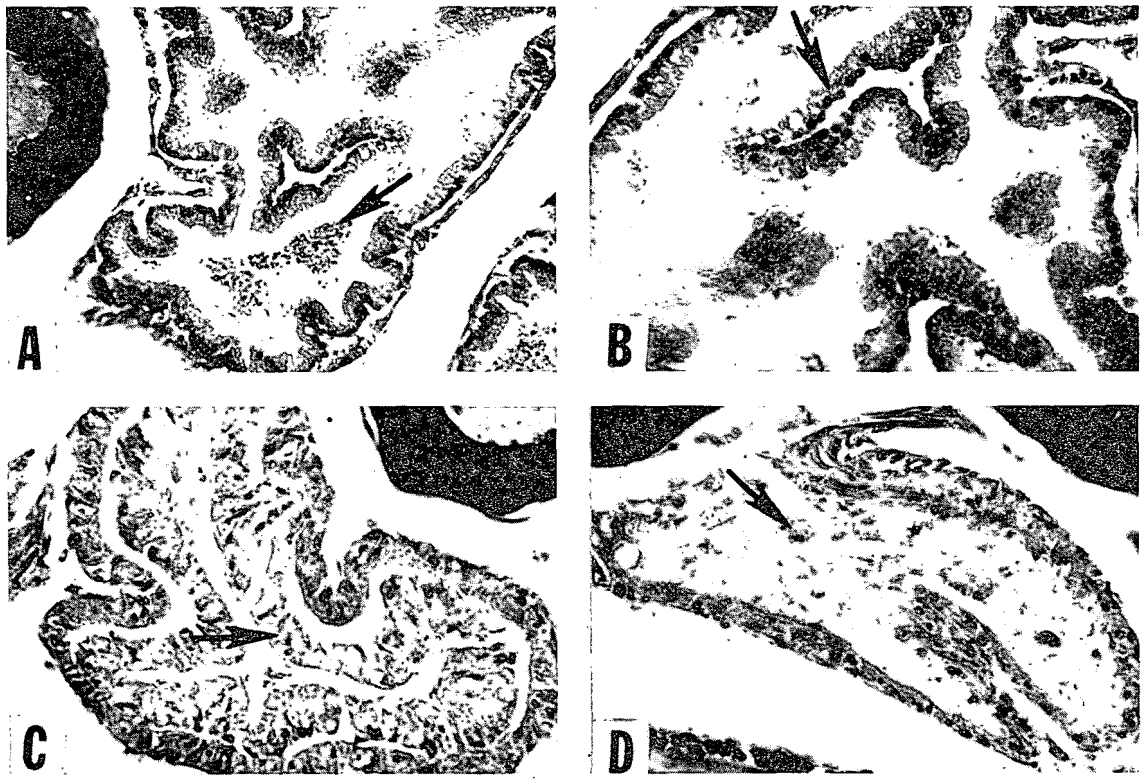

FIGURE 1.—Photomicrographs of *Sardinops sagax* postovulatory follicles. (A) Day 0, showing highly convoluted morphology. Note cluster of eosinophilic granules (arrow) in lumen (250×). (B) Day 0, showing columnar epithelial cell lining (arrow) (400×). (C) Day 1, columnar epithelial cell lining (arrow) undergoing degeneration. Underlying layer is connective tissue theca (400×). (D) Day 2, lumen contains scattered degenerated columnar epithelial cells (arrow) (400×).

should be some suggestion of it. We therefore recommend careful observation of the convoluted structure of day-0 and day-1 structures before attempting to identify day-2 structures.

A useful criterion for distinguishing day-2 *S. sagax* postovulatory follicles from advanced atretic follicles would be the presence of yellow granules (irrespective of staining) that are found in advanced atretic structures (delta atresia) (Lambert 1970). These were occasionally noted in *S. sagax*. The presence of these yellow granules which appear in nucleated clusters conclusively indicates atretic structures.

We did not use the artificial spawning technique (Leong 1971) for aging postovulatory follicles in *S. sagax*. However, we feel that estimating their age from periodic collections of fish, after the spawning time is established from collections of egg samples (as done herein), will prove to be a useful alternative method. This is particularly true in situations where facilities are lacking for laboratory-induced spawning. Laboratory-induced spawning studies using *S. sagax* will be useful to provide estimates of the accuracy of our classification scheme.

While there are numerous accounts of the occurrence of postovulatory follicles in marine fishes, there are few reports describing their longevity and subsequent degeneration. They have been described previously as being short-lived structures by Yamamoto and Yoshioka (1964) and Hunter and Goldberg (1980). More studies are needed of a wide variety of fishes before our knowledge of their histology and function is completed. Of utmost value will be investigations on how to distinguish conclusively between old postovulatory follicles and old atretic follicles.

Acknowledgments

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A NOTE ON SPAWNING OF THE PACIFIC MARKET SQUID, *LOLIGO OPALESCENS* (BERRY, 1911), IN THE BARKLEY SOUND REGION, VANCOUVER ISLAND, CANADA

In California, *Loligo opalescens* (Berry, 1911), has large spawning schools and spawn masses (McGowan 1954; Fields 1965; Hobson 1965; Cousteau and Diole 1973; Hochberg and Fields 1980). Spawns and spawning effort of this squid in the Pacific Northwest are poorly known and, to our knowledge, large spawns or spawning events have not been quantitatively described.

Loligo opalescens spawns regularly in Barkley Sound near Bamfield, British Columbia, (lat. 48°50.0'N, long. 125°07.5'W) in spring. We examined and measured portions of a spawn using scuba during early June 1982. The largest single capsule mass aggregation in our 200 × 50 m survey area was measured. Adjacent areas of smaller solitary egg capsule masses were surveyed using transects to determine overall spawn dimensions and percent cover of individual capsule masses. Dimensions of 23 typical masses were determined. Four representative masses were collected; the number of capsules in each was counted; and from each, 10 capsules were randomly selected and the number of eggs in each capsule was determined. These eggs were examined microscopically to determine the developmental stage, which was compared with the embryological stages illustrated in Fields (1965) to estimate the time of deposition.

The spawn, including areas of continuous and solitary egg capsule masses, was larger than the area surveyed, as the spawn extended below our deepest possible survey depth. Within our survey area, the largest capsule mass aggregation covered about 69.3 m^2 and averaged 0.28 ± 0.09 m (n = 4) in thickness. The mean density of the individual masses was 1.3 ± 0.1/m^2, and the mean area covered by 23 masses was 0.28 ± 0.14 m^2/mass, with a range of 0.13-0.66 m^2. The mean number of egg capsules per solitary mass was 1,937 ± 912 (n = 4), with 149 ± 35 eggs/capsule (n = 40). Thus, the total number of eggs per isolated mass was 288,000 ± 125,000. For the large areas of isolated masses, the potential number of larvae produced per 100 m^2 ranged from 19 to 58 × 10^6, with a mean of 37 × 10^6. The number of potential larvae from the single large aggregation of 69.3 m^2 ranged from 27 to 204 × 10^6, with a mean of 72 × 10^6.

Based on embryological stages observed, deposition probably occurred during the night of 31 May-1 June 1982. Small squid schools were observed spawning near the survey area on that date. None of the embryos were old enough to be deposited before 31 May, and all were of the same embryological stage.

Female squids from Californian populations deposited about 21 capsules, each containing about 200 eggs, in one night (Fields 1965); fecundity data from our region are not available. Hochberg and Fields (1980) stated that *L. opalescens* females produce 180-300 eggs/capsule. Our data indicate a lower mean value of about 150 eggs/capsule. If each female deposited 20 capsules, the large measured aggregation would be the result of about 24,000 females.