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Measurement of Spawning Frequency in Multiple Spawning Fishes

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ABSTRACT

Methods for estimating the frequency of spawning in natural fish populations using histological criteria are described using the northern anchovy, *Engraulis mordax*, as an example. Illustrations and descriptions are provided of oocytes in mature ovaries, postovulatory follicles, and atretic follicles. Postovulatory follicles are used to estimate frequency of spawning, and atretic follicles (which may be confused with postovulatory follicles) are useful in estimating size at first maturity. Postovulatory follicles of four common commercial fishes, *Sardinops sagax*, *Merluccius gayi*, *Scomber japonicus*, and *Euthynnus lineatus*, are also described and illustrated. Methods for estimating spawning frequency other than ovarian histology are also discussed, as is testing of the assumption of determinate annual fecundity in multiple spawning fishes.

INTRODUCTION

The egg production method of biomass estimation requires an estimate of spawning frequency. This requirement exists because the method was developed for fishes, such as the northern anchovy, which have indeterminate annual fecundity. In such, the standing stock of advanced oocytes gives no indication of annual fecundity because new spawning batches are recruited from small, unyolked oocytes during the spawning season. Thus, the rate of egg production can be calculated only from estimates of spawning frequency and batch fecundity made during a survey period. On the other hand, in some multiple spawning boreal fishes, including haddock, *Melanogrammus aeglefinus*, and whiting, *Merlangius merlangus* (Hislop 1975; Hislop et al. 1978; Hislop[1]), and other fishes (Yamamoto 1956), the stock of oocytes destined to be spawned in a season is identifiable at the beginning of the season. In such fishes, annual fecundity may be considered to be determinate even though the fishes may spawn repeatedly during the season and the standing stock of advanced oocytes at the beginning of the season is considered to be equivalent to the annual fecundity. Hence, estimates of spawning frequency are unnecessary in fishes with determinate annual fecundity as long as the biomass survey includes the entire spawning period and nearly all of the stock of advanced oocytes are actually spawned. In some cases, not all the standing stock of oocytes are spawned and, consequently, potential annual fecundity is not realized during the spawning season. Documentation of the existence of determinate annual fecundity, and of its total utilization during the spawning season, is essential if the standing stock of oocytes is used to estimate spawning biomass. This is rarely done satisfactorily, and in a number of cases we believe the presumption of a seasonally determined fecundity is probably false which may lead to serious errors in biomass estimation. Estimation of the frequency of spawning and batch fecundity is one way to test the assumption of determinate annual fecundity.

The objective of this paper is to describe the methods for estimating frequency of spawning in natural fish populations. We begin with a brief discussion of histological methods, followed by a general description of the oocytes in an active ovary of northern anchovy, *Engraulis mordax*. We then describe the estimation of spawning frequency using aged postovulatory follicles (employed in the current method for biomass estimation of northern anchovy) and briefly discuss other methods. In the second section of the paper we discuss ovarian atresia, because histologists must be able to distinguish postovulatory follicles from atretic follicles, and knowledge of atresia is also necessary to separate postspawning females from immature ones. In the final sections we discuss tests of the assumption of determinate fecundity and future applications of histological classification of ovaries.

Most of the descriptions and all of the data in this paper are for the northern anchovy. Nevertheless we have tried to bring out important differences that may exist among species and to mention methodologies other than the ones we use for northern anchovy.

HISTOLOGICAL METHODS

All the techniques discussed in this paper involve, at least to some extent, histological examination of fish ovaries. In this section we briefly discuss the histological techniques used and comment on the

[1]John R. G. Hislop, DAFS Marine Lab., P.O. Box 101, Victoria Road, Aberdeen AB9 8DB, Scotland, pers. commun. 25 Oct. 1983.

procedures and time required to use histological classification for fish population work.

Histological preparation of tissues

Almost all northern anchovy ovaries histologically examined for postovulatory follicles were fixed in 10% neutral buffered Formalin (NBF), embedded in Paraplast, and 6-μm serial sectioned slides were made and stained by Harris' hematoxylin followed by Eosin counter stain. This procedure gave good results, and specific steps can be found in Luna (1968), Preece (1965), or most any histotechnique handbook. It is also possible to use resin embedding, sectioning on a rotary microtome, and Lee's Methylene blue-basic fuchsin staining (JB-4 Embedding Kit, Data Sheet No. 123, Polysciences, Inc., 1982). This technique may be preferable for small ovaries or small pieces of ovaries, although it is more difficult to get many serial sections and may not be practical for larger ovaries.

In addition to the instructions given in standard histotechnique texts, we found the following procedures and precautions to be useful in preparation of Hematoxylin and Eosin (H&E) sections of northern anchovy ovaries.

Fixation—Bouin's fixative gave better results than 10% NBF, but it required transferring ovaries into 70% ethanol within 1-2 d or the ovaries become hard and brittle. Transferring is possible in the laboratory but at sea it is often impractical. It is important to fix ovaries quickly and thoroughly, otherwise lysis or poor differentiation may occur. We insured proper exposure to fixative by slitting the abdomen of fish before fixing. In larger fish it is best to remove the whole ovary from the fish and preserve it separately. It is even better to cut a 1-cm section from the midsection and preserve it in ample fixative. Initial freezing of whole fish or ovaries is not recommended; fixation after freezing results in poor-quality H&E sections in which postovulatory follicles cannot be consistently identified, thus producing substantial errors in the estimation of spawning frequency. In addition, the process of thawing may cause lysis of hydrated oocytes.

Infiltration—Hardening of ovaries may occur if time spent in xylene or toluene is not kept at a minimum or if temperature of paraffin is not kept below 60°C.

Sectioning—It is necessary to have 10-20 good serial sections per slide for histological analyses. Cold (4°C) blocks and knives usually improve sectioning. If shattering occurs, it is possible to soak blocks (prefaced-off) in ice water for a short time (1-15 min) to improve sectioning with little or no shattering.

Mounting—Wiping slides with a very fine coat of Mayer's Albumen Affixative and drying mounted slides are often necessary to keep sections attached to slides. By mounting as many sections as possible on a slide, it may be possible later to microscopically trace questionable structures.

Staining—The standard procedure for Harris' Hematoxylin and Eosin staining is quick and easy, and results in good staining of postovulatory follicles. Initially a trial run is necessary to find out the optimal amount of time spent in each stain. Other stains, i.e., Mason's Trichrome, periodic acid-Schiff reagent, or Heidenhain's iron hematoxylin, can also be used except they often require more time or more elaborate techniques. These stains may be preferable for study of structures other than postovulatory follicles, for example, nuclear changes in oogenesis.

Rates of Processing and Classification

The production of slides depends largely upon the capacity of the automatic tissue infiltrator and the number of histotechnicians. In small research laboratories (infiltrator capacity = 30 samples per batch) production of 100-150 H&E slides (one ovary per slide) per week for one person is close to the maximum rate of production. Thus, for production work it is preferable to use commercial histological laboratories where H&E slides can be produced more quickly at a cost of (in 1983) about $4.00/slide.

The time required to classify each slide of ovarian tissue (about 10 sections per slide) depends upon the amount of detail recorded. If only spawning frequency is estimated (ageing postovulatory follicles and identification of hydrated oocytes), one person can classify about 1,000 slides in 25 d (8 h/d). Two to three times as much time is required if all other significant histological characteristics of the ovary are recorded, including the presence and abundance of alpha through delta stages of atretic yolked and unyolked oocytes and the relative abundance of oocyte classes. We have employed this detailed system over the years to provide a complete record of the condition of the ovary. However, our analysis indicates that the system can be simplified by identifying only hydrated oocytes, two age classes of postovulatory follicles, and by classifying ovaries into three atretic states (defined in a subsequent section). This reduces classification time per ovary to nearly the equivalent of that required to estimate only spawning frequency, yet the ability to identify major changes in atretic condition, and to separate postspawning females from immature females, is still maintained.

HISTOLOGICAL CHARACTERISTICS OF OOCYTES

The northern anchovy is a multiple spawning fish (Hunter and Goldberg 1980) with asynchronous oocyte development, i.e., oocytes in many stages of development occur simultaneously in reproductively active ovaries (Wallace and Selman 1981). During the spawning season, oocyte development is a continuous process involving all stages of oocytes, with a new spawning batch maturing every week to 10 days in peak spawning months (Hunter and Leong 1981). The average female anchovy spawns at least 20 times per yr, yet within the ovary only 1-3 potential spawning batches of yolked oocytes exist and fewer than 10 potential batches exist when all oocytes >0.1 mm are included (Hunter and Leong 1981). Oocytes ≤0.1 mm constitute the reservoir of oocytes that are present in ovaries year-round in inactive as well as active ovaries. Little or no atresia occurs among oocytes in this size range. Thus, in anchovy spawning, batches must be recruited from the reservoir of oocytes ≤0.1 mm to account for the observed frequency of spawning. This means the fecundity of northern anchovy is clearly seasonally indeterminate.

Oocyte development and maturation in teleosts, reviewed recently by Wallace and Selman (1981), has been frequently subdivided into many stages (Yamamoto 1956; Lambert 1970b), but a simpler histological classification system seems appropriate for the purpose of this manual. We have combined the stages of past authors into four oocyte classes (unyolked, partially yolked, yolked, and hydrated), and we describe below for the northern anchovy the histological characteristics of each class.

Unyolked Oocytes

This class includes all oocytes without yolk that are between about 0.04 and 0.35 mm (Fig. 1a, b). Oocytes <0.04 mm are excluded because they consist mostly of "oogonial nests," have no true follicle layer, and appear not to undergo degeneration (Fig. 1b). The smaller oocytes within this class (0.04-0.15 mm) are spherical, have a large nucleus, and have cytoplasms that are narrow, homogeneous, and very densely stained with hematoxylin (Fig. 1b). A very thin, single layer of elongated, spindle-like cells (the beginning of the granulosa layer) surrounds these small oocytes. The large oocytes in this class are oval; their cytoplasm stains faintly with hematoxylin and has a cloudy, mottled appearance (Fig. 1d). The oval nucleus of these oocytes contains several nucleoli and is surrounded by a granular perinuclear zone. In these larger oocytes a thin, definite, hyaline membrane which has a faint eosinophilic stain (percursor of the zona radiata) appears between the oocyte and the growing follicle. The follicle consists of a narrow, single, inner layer of cuboidal granulosa cells and a single outer layer of flat elongated thecal cells with some blood capillaries. The larger oocytes also may have some small vesicles in the periphery of the cytoplasm. These vesicles are at times difficult to distinguish and they seem to disappear in yolked oocytes. No oil vacuoles exist, as northern anchovy eggs do not contain oil droplets.

Partially Yolked Oocytes

Oocytes in this class are in the early stages of yolk deposition (vitellogenesis) and range in size from 0.3 to 0.5 mm (major axis) (Fig. 1d, g). The class includes oocytes in the initial stage of yolk deposition up to and including those in which yolk granules or spherules extend 3/4 of the distance from the periphery to the perinuclear zone. Yolk deposition starts at the periphery of the oocyte cytoplasm as small eosinophilic staining granules and then subsequently spreads internally until it nearly reaches the finely granular perinuclear zone. Usually by this time the granules have become small spherules. The oval-shaped nucleus of oocytes in this class contains several nucleoli. At the time yolk appears in the oocyte, delicate striations appear on the hyaline membrane between the oocyte and follicle layer and it is henceforth referred to as the zona radiata. As maturation proceeds, the follicle layer becomes wider due to an increase in the width and proliferation of the granulosa cells. The thecal cells do not increase in size but remain elongated, flat cells with occasional blood capillaries, and form a thin outer covering to the follicle. The thecal cells do not change until hydration, when they become even flatter and have a stringy appearance.

Yolked Oocytes

Oocytes in this class range from 0.45 to 0.80 mm (major axis) and all contain yolk spherules or globules throughout the region between the periphery of the oocyte and the perinuclear zone (Fig. 1c, d). As vitellogenesis continues, the yolk varies from spherules in the smaller oocytes to large globules in the larger ones. Just prior to spawning (<24 h) the globules fuse to form yolk plates (Fig. 1h). Such oocytes are excluded from this class, since this characteristic is diagnostic of the last (hydrated) class of oocytes. The nucleus of oocytes in the yolked class is oval with numerous nucleoli and is centrally located until just before hydration, when it migrates to the animal pole. The granulosa cells have a wide rectangular shape in cross section and a large oval nucleus; their walls are clearly evident in sagittal section where they form polyhedrons. The zona radiata is a wide, striated, eosinophilic band until hydration, when it stretches thin and the striations seem to disappear.

Hydrated Oocytes

These oocytes range in size from 0.75 to 1.2 mm (major axis) (Fig. 1g, h). Hydration (rapid uptake of fluid by the follicle; Fulton 1898) begins at about the time the nucleus has completed its migration to the animal pole (Fig. 1e, f) and yolk globules have begun to fuse, forming yolk plates. The nucleus of a hydrated oocyte is not visible because after the nucleus has arrived at the animal pole the nuclear membrane disintegrates, dispersing its contents into the cytoplasm. During hydration, all yolk globules fuse into plates and the oocyte expands greatly, stretching the granulosa and thecal cell layers. At this time the granulosa cells in cross section appear as long, thin rectangles, the thecal cells are extremely flat and have a string-like appearance, and the zona radiata is very thin and lacks striations. Hydrated oocytes are the most ephemeral of all oocyte classes since this stage lasts for less than a day, whereas the other stages are always present in reproductively active anchovy ovaries. Migratory nuclei may be seen as early as 24 h before ovulation, but hydrated oocytes in which all globules are fused to form yolk plates do not occur earlier than 12 h before spawning. We have observed no atresia in hydrated oocytes; apparently, in northern anchovy, nearly all hydrated oocytes are ovulated.

The hydrated oocyte stage in northern anchovy begins when the nuclear membrane disintegrates and ends at ovulation. As this was an arbitrary decision, the duration of this stage could be increased by including the period of nuclear migration. This broader definition of the hydrated stage might be useful when sampling occurs before complete hydration or if hydration occurs very rapidly, as might be the case in tropical fishes.

ESTIMATION OF SPAWNING FREQUENCY

Postovulatory Follicle Method

In northern anchovy, each hydrated oocyte is surrounded by a thinly stretched follicle of an inner, epithelial layer of granulosa cells and a single, outer connective tissue layer of thecal cells with some blood capillaries. At ovulation, the fully hydrated oocytes are released from their encompassing follicles. The follicle does not fragment and pass out of the ovary with the hydrated oocyte but retains its integrity. The follicle collapses away from the opening formed for the release of the hydrated oocyte into the lumen and remains in the ovary as an evacuated follicle, or postovulatory follicle. Spawning occurs simultaneously with ovulation or in <60 min after ovulation. Initially, the postovulatory follicle is a distinct structure, but it rapidly deteriorates and is resorbed. In northern anchovy, by 48 h after ovulation postovulatory follicles can no longer be accurately discriminated from the intermediate stages of atretic oocytes.

To use postovulatory follicles for estimation of spawning frequency, it is necessary to divide the deterioration and resorptive processes of the follicle into a series of distinct histological stages, each with an assigned age (time from spawning). This requires a series of ovary samples taken at regular intervals from the time of spawning and can be accomplished by spawning fish in the laboratory (Leong 1971)

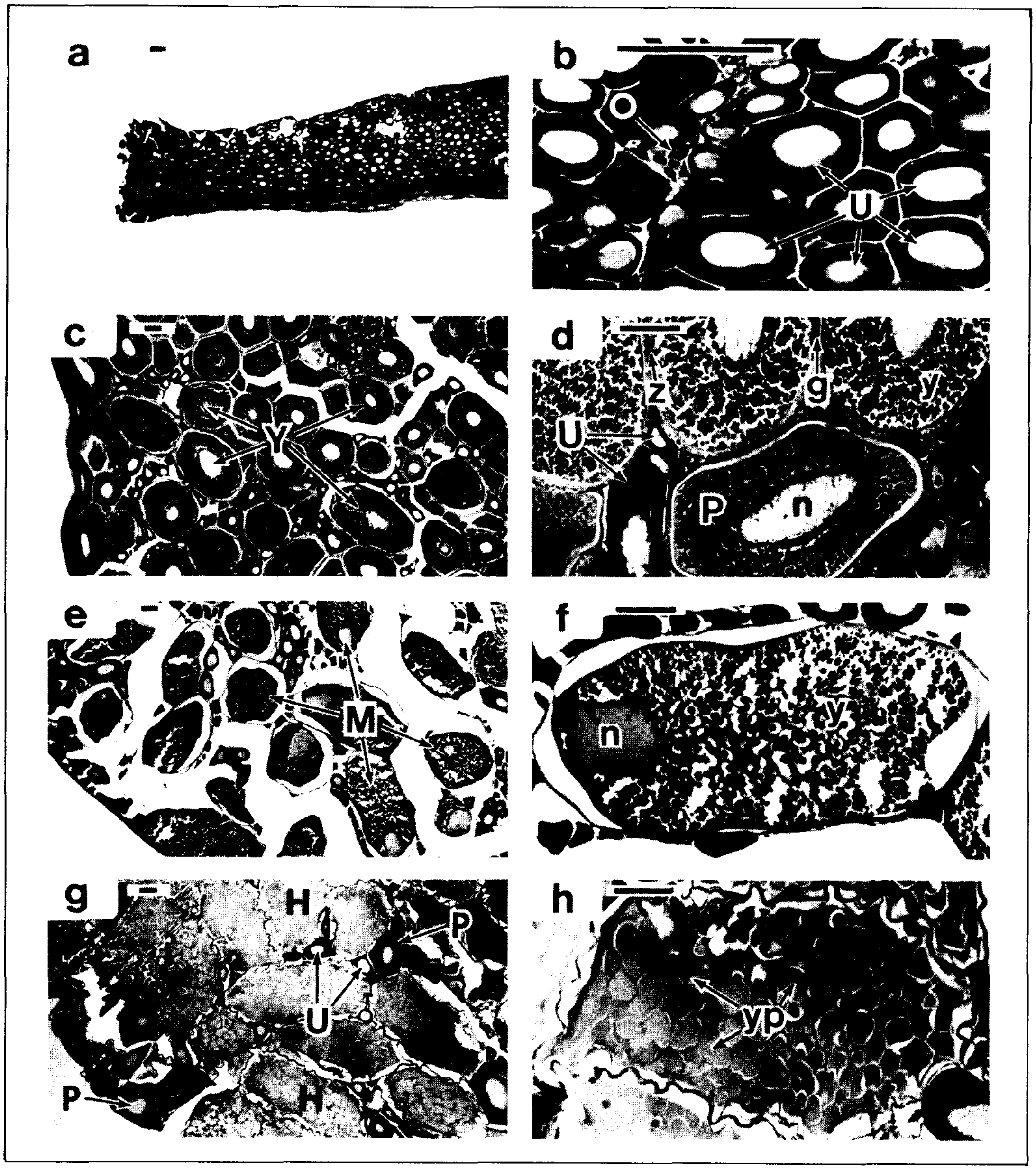

Figure 1.—Development of northern anchovy ovary at various magnifications. Stain = H&E; bar = 0.1 mm.

a) Immature ovary consisting of unyolked oocytes and no atresia.

b) Enlargement of (a) showing small, spherical, unyolked oocytes (U) with a large central nucleus and "oogonium nests" (o).

c) Normal, mature ovary with many fully yolked oocytes (Y).

d) All stages of oocytes: unyolked (U), partially yolked (P), and yolked (Y), are present in normal mature ovaries. (g = granulosa epithelial cell layer; z = zona radiata; n = nucleus; y = yolk globules.)

e) Prespawning ovary showing oocytes in the migratory nucleus stage (M).

f) Enlargement of a migratory nucleus oocyte (M). (n = nucleus; y = yolk globules.)

g) Imminent (<12 h) spawning ovary with hydrated oocytes (H) still within their follicle layers. (U = unyolked; P = partially yolked.)

h) Enlargement of a hydrated oocyte. Note that the yolk globules have fused into yolk plates (yp), and there is no prominent nucleus due to disintegration of the nuclear membrane.

or by sampling spawning schools over a 24-h period in the sea (Goldberg et al. 1984; Alheit et al. 1984). The latter approach requires estimating the average time of spawning for the population and has somewhat greater uncertainties than the preferable laboratory approach. At moderate temperatures (∼16°C) we recommend taking samples of at least 10 females with postovulatory follicles at 4-h intervals for 3-4 d. At higher spawning temperatures, e.g., 28-30°C, sampling at 1-2 h intervals might be required. Once the classification criteria and stages are defined, it is necessary to conduct a blind classification of ovaries that include females with all stages of postovulatory follicles as well as those without these structures. The classification system we employ for northern anchovy is described below, and the results of the original blind classification are given in Hunter and Goldberg (1980), although our level of accuracy has considerably improved since that initial test.

Age "0 Day" postovulatory follicles in anchovy—Included in age 0-day postovulatory follicles are those new, remnant, evacuated follicles that are from 0 to 23 h old. In our surveys, however, we include in this age those postovulatory follicles about 0 to 5 h old since we trawl for northern anchovy only during the nightly spawning period (1800-0300 h). In laboratory-spawned females sacrificed 0-4 h after spawning, the new postovulatory follicles show no signs of degeneration but they may undergo some structural changes. At ovulation, while the follicle is collapsing, the follicle cell layers, appearing cord-like, form loose folds or loops. The granulosa cells, which had been extensively stretched during hydration, appear elongated and narrow with a large prominent nucleus in the center (Fig. 2a); the thinly stretched thecal layer thickens and becomes more noticeable. After spawning, the fully collapsed postovulatory follicle is a much more tightly folded or looped structure. It is relatively large, irregular in shape, with an irregular lumen which frequently contains eosinophilic granules of uncertain origin. The granulosa cells of this new postovulatory follicle (age-0 day) are characteristically columnar or cuboidal and in some cases have hypertrophied slightly; these cells are arranged orderly along the edge of the lumen with their cell walls usually evident and possessing prominent nuclei. The nucleus of the granulosa cells may be located at either the apex or base of the cell. In field-caught fish, the follicles with apical nuclei appear to occur in fish taken near the time of spawning and those having basal nuclei somewhat later (Fig. 2b, c). After spawning, the thecal cell layer is more clearly defined, adheres closely to the granulosa layer, and contains blood capillaries.

We use the above characteristics of the follicle plus no signs of follicle degeneration as the diagnostic characteristics of postovulatory follicles of age 0-day. Our surveys occur only at about the time of spawning, about 1800 to 0300 h, an insufficient period for significant follicle degeneration to occur. If sampling times were extended 6-8 h or longer after the peak hour of spawning, early degenerative follicle characteristics would have to be included as a characteristic of age 0-day postovulatory follicles. Degeneration appears to start 6-8 h after spawning in northern anchovy. The first sign of degeneration is the presence of a few pycnotic nuclei in the granulosa cells, followed shortly by the migration of a few lymphocytes into the postovulatory follicle; finally a few vacuoles appear in the granulosa layer. In northern anchovy, all of these events occur before the postovulatory follicle is 12 h old.

Age "1 Day" postovulatory follicles in anchovy—Degeneration is pronounced in postovulatory follicles examined about 24 h after spawning. The characters we use to classify postovulatory follicles as age 1-day are those that appear between 19 and 28 h after spawning, since our surveys occur only near the time of spawning. We describe follicles of age 19-28 h and subsequently discuss identification of older follicles.

By 19-28 h after spawning, the regressing postovulatory follicle is greatly shrunken and has fewer folds, hence a less irregular form than a new postovulatory follicle. The lumen, which is much reduced, may contain some granular material, although not as much as occurs in the lumen of 0-day postovulatory follicles. The granulosa cells of the follicle no longer have the orderly alignment characteristics of age 0-day postovulatory follicles, although some pattern in arrangement of cells can still be seen. Some of the nuclei of the granulosa cells are pycnotic, vacuoles are common, and only few of the cell walls are intact. The underlying layer of thecal cells is present, although less distinct, than in an age 0-day postovulatory follicle (Fig. 3).

Postovulatory follicles older than 28 h in anchovy—Classification beyond 28 h becomes difficult as the follicle continues to degenerate, and by 48 h it may easily be confused with the beta stage of atretic follicles (see section on atresia). A postovulatory follicle 48 h old is one-half to one-fourth the size of 24-h-old follicles, the lumen is much reduced or absent, and no eosinophilic granules are present. Cell walls are absent in the remaining granulosa layer tissue, and a few vacuoles or pycnotic nuclei may be seen. The theca is present but is often indistinct as it becomes incorporated into the ovarian connective tissue stroma. The number of postovulatory follicles in the ovary appears to be reduced by 48 h after spawning. Fewer follicles may be present because they were resorbed or, alternatively, fewer may be seen per section because of the growth of the larger oocytes. We believe that by 3-4 d after spawning all postovulatory follicles have been resorbed, although they might produce a structure that is indistinguishable from delta-stage atretic follicles. However, delta-stage atretic follicles are far less numerous in the ovary than new postovulatory follicles.

Postovulatory follicles in other fishes—The structure of ovaries among teleosts is relatively similar to anchovy especially in species that spawn unadorned pelagic eggs. As far as we can ascertain, the postovulatory follicles of such fishes differ only in minor details from the descriptions we have provided for the northern anchovy. The postovulatory follicles of the Peruvian anchovy, *Engraulis ringens*, is identical in all respects to that of the northern anchovy, regardless of follicle age, and hence will not be discussed further. We illustrate and briefly describe postovulatory follicles of four other species to indicate the variation in these structures among common commercial fishes that spawn unadorned pelagic eggs of about 1 mm diam. These fishes include Pacific sardine, *Sardinops sagax*, Chilean hake, *Merluccius gayi*, chub mackerel, *Scomber japonicus*, and black skipjack, *Euthynnus lineatus*.

The new postovulatory follicle (near the time of spawning) of these four species has the following characteristics in common with northern anchovy: a very convoluted shape with many folds or loops; a lumen containing some granular or particulate material; a definite granulosa epithelial cell layer lining the lumen; linearly arranged granulosa cells of cuboidal or columnar shape which contain a prominent nucleus; a definite thecal connective tissue layer with blood capillaries; and, most importantly, no degeneration of the follicle. Various minor differences from anchovy in the appearance of a new postovulatory follicle are mentioned below. The new postovulatory follicles of chub mackerel seem to differ from anchovy only in the presence of fewer granules in the lumen just after spawning (Fig. 4a, b). In the Chilean hake, the granulosa layer at the time of spawn-

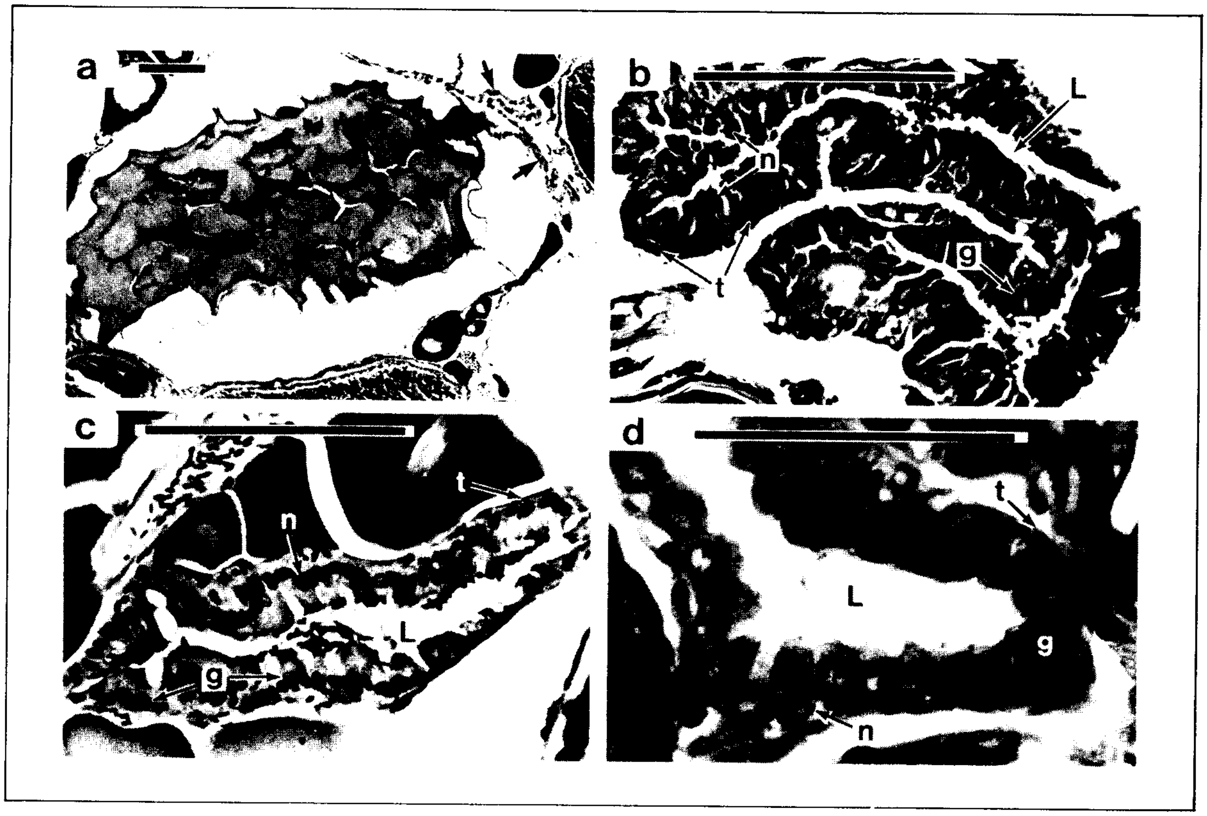

Figure 2.—Postovulatory follicles of age 0-day; elapsed time from spawning about 0-6 h. In a, b, and c, bar = 0.1 mm; in d, bar = 0.05 mm; g = granulosa epithelial cell layer; t = thecal connective cell layer; and L = lumen of follicle.

a) At ovulation the postovulatory follicle (arrow) collapses away from the hydrated oocyte (H).

c) Follicle with the nuclei (n) at the base of the granulosa cells (g). Elapsed time from spawning is 1-6 h.

b) Newly collapsed follicle with nuclei (n) at the apex of the granulosa cells (g). Elapsed time from ovulation <1 h.

d) Enlargement of follicle age 0-day (from Hunter and Goldberg 1980).

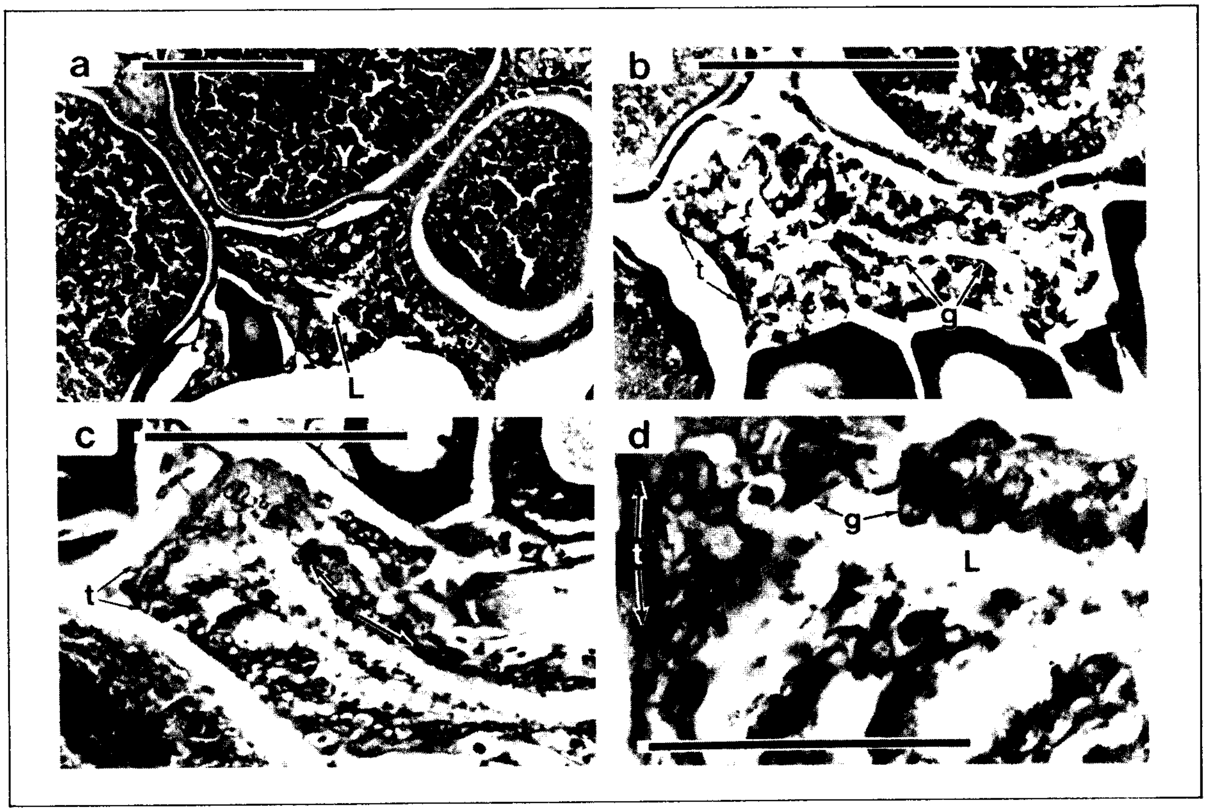

Figure 3.—Postovulatory follicles of age 1-day; elapsed time from spawning about 24 h. Degeneration of the follicle is pronounced by 24 h. In a, b, and c, bar = 0.1 mm; in d, bar = 0.05 mm; g = granulosa epithelial cell layer; t = thecal connective cell layer; L = lumen of follicle.

a, b) Follicles from sea-caught anchovies; the next batch of yolked oocytes (Y) is evident.

c, d) Follicle from anchovy spawned in the laboratory (from Hunter and Goldberg 1980).

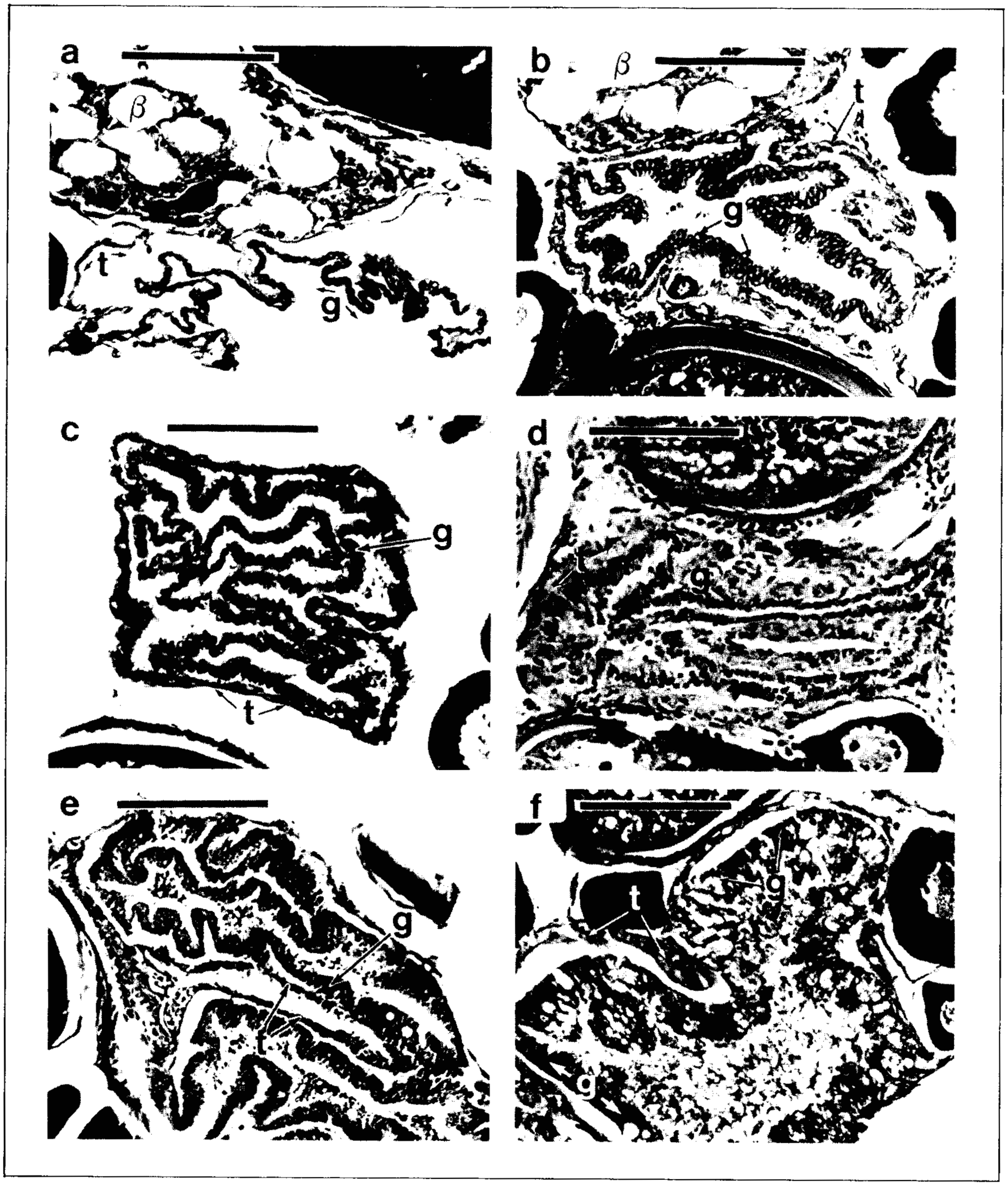

Figure 4.—New postovulatory follicles of four species of marine fishes. Bars = 0.1 mm; β = beta atresia; g = granulosa epithelial cell layer; t = thecal connective cell layer.

a) Chub mackerel, *Scomber japonicus*, at ovulation, and

b) 1 h after spawning (laboratory-induced with hormones). In both sections (a) and (b), a single beta-stage atretic follicle (β) is present; in (a), the atretic follicle contains 12-13 vacuolated areas which may be remnants of the oil droplet.

c) Chilean hake, *Merluccius gayi*. Histological material from S. R. Goldberg, Whittier College, Whittier, CA.

d) Black skipjack, *Euthynnus lineatus*, at the time of spawning (hydrated oocytes were in lumen of the ovary). Histological material from K. Schaefer, IATTC, La Jolla, CA.

e) Pacific sardine, *Sardinops sagax*, from Peru after spawning at sea. Histological material from S. R. Goldberg, Whittier College, Whittier, CA.

f) At ovulation (laboratory-induced with hormones).

ing appears to be coiled up inside a less irregular thecal layer covering, and the lumen is less distinct than anchovy and contains many fewer granules (Fig. 4c). The follicles of black skipjack differ from anchovy only by the presence of a thicker thecal layer that adheres to the granulosa cells (Fig. 4d). The only differences between sardine (Goldberg et al. 1984) and anchovy in the appearance of new postovulatory follicles are the slightly longer columnar granulosa cells, the occurrence of more particles in the lumen, and a few vacuoles in the granulosa cells of the sardine (Fig. 4e). During ovulation the sardine follicle may have quite a different appearance from the anchovy as the granulosa layer contains numerous vacuoles and the cells are more strongly hypertrophic (Fig. 4f). These differences in the appearance of new postovulatory follicles seem trivial relative to their overall similarities, indicating that the new postovulatory follicles of most fishes should be easy to identify using the general characteristics we have described.

We now consider the characteristics of degenerating postovulatory follicles in these four species and how they compare to the descriptions for the northern anchovy. The four species have the following characteristics in common with anchovy: an irregular shape that is smaller and much less convoluted than a new postovulatory follicle; the presence of a lumen, although the size may be greatly reduced; degeneration of granulosa cells (pycnotic nuclei, few cell walls, vacuoles usually present, and lack of alignment of nuclei); and an identifiable thecal layer. The degenerating postovulatory follicle (about 24 h old) of chub mackerel differs from the anchovy because a few red blood cells may occur among the granulosa cells and in the lumen and because very few vacuoles occur in the granulosa layer (Fig. 5a). Bara (1960) noticed red blood cells in the lumen of a degenerating postovulatory follicle of the Atlantic mackerel, *Scomber scombrus*, indicating this may be a common characteristic of *Scomber*. In the degenerating postovulatory follicles of the Chilean hake, some of the pycnotic granulosa cells appeared to have been sloughed into the lumen, a characteristic not seen in anchovy ovaries, and the granulosa cells contained very few vacuoles relative to the anchovy (Fig. 5b). In addition, the thecal layer in the hake now appears to be in closer contact with the remaining granulosa layer than it is in a new postovulatory follicle. The degenerating follicles in black skipjack differ from anchovy by the presence of a much thicker thecal layer surrounding the granulosa layer (Fig. 5c). The degenerating follicle of the sardine (age about 24 h) differs from the anchovy and from a new sardine postovulatory follicle by having a considerably thicker thecal layer and by the separation of the granulosa layer from the thecal layer. In addition, the degenerating granulosa cells of the sardine may be enlarged slightly and may be sloughed into the lumen (Fig. 5d). These differences among degenerating postovulatory follicles indicate minor differences in oocyte structure and perhaps differences in the pattern of resorption among species. However, our descriptions for northern anchovy can be used as a general guide for identification of postovulatory follicles in these and presumably other species. After identification of postovulatory follicles, it is best to age them by laboratory experiments or round-the-clock sea samples (see previous section).

A cautionary note on ageing postovulatory follicles—The persistence of postovulatory follicles in the ovary of the northern anchovy and the Peruvian anchovy is the same, and it appears that Pacific sardine may have a similar duration. These species spawn at moderate temperatures, 13-19°C. In tropical species that spawn at high temperatures, spawning frequency, resorption of postovulatory follicles, and ovarian maturation all may be accelerated. For example, the dragonet, *Callionymus enneactis*, spawns daily at 28-30°C, and postovulatory follicles are not seen 15 h after spawning and were only clearly distinguishable up to 3 h after spawning (Takita et al. 1983). Clearly in such a rapidly maturing ovary, postovulatory follicles would be highly transitory in nature. Thus, the duration of postovulatory stages must be newly estimated for each species, and an assumption that the duration of these stages in a new species is similar to northern anchovy is highly speculative.

Anatomical Maturity Scales and the Gonosomatic Index

Systems for grading ovaries according to macroscopic characteristics have long been used in fishery research; they include the Hjort scale (Bowers and Holliday 1961) and numerous others (see, for example, Holden and Raitt 1974; Macer 1974; Foucher and Beamish 1977; and Robb 1982). Typically, these systems include an immature stage, several stages for maturing ovaries, one for active ovaries, 1-2 stages for hydrated ovaries ("ripe" or "running ripe"), and various stages for "spent" ovaries. At present only the hydrated oocyte stage or stages have sufficiently distinct macroscopic characteristics (large ovary size, large hyaline oocytes) to be of value for estimating spawning frequency.

The terminology used in maturity scales to identify "spent" ovaries is confusing, as "spent" may refer to ovaries with new postovulatory follicles or to atretic postspawning ovaries. Macer (1974) used the terms "spent" and "partially spent" to make this distinction. At present the anatomical stages that seem to be equivalent to postovulatory ovaries are too ambiguous to be used to estimate spawning frequency. It might be possible using a laboratory calibration to identify some macroscopic characteristics that could be used to identify postovulatory ovaries for a short time after spawning. On the other hand, hydrated ovaries and postovulatory ovaries (<24 h old) have similar sampling biases. Thus, the need to develop such anatomical criteria is not great unless postovulatory ovaries could be detected macroscopically 24 h after spawning, which seems unlikely.

The gonosomatic index (GSI)—the ratio of ovary weight divided by fish weight or the equivalent (Davies 1956)—can be used to detect hydrated ovaries since the wet weight of hydrated ovaries is two to four or more times that of other maturity stages. However, the GSI has the inherent problem that dividing by an expression of body size usually does not compensate completely ("normalize") for the effects of fish size (Davies 1956; Vlaming et al. 1982). For the same reproductive state, small fish usually have a lower GSI than do larger fish, and this effect increases with maturation of the ovary (Vlaming et al. 1982; Hunter and Goldberg 1980). In other words, ovary weight increases faster with fish length than does somatic weight. The assumption underlying the GSI is that ovary weight/fish weight relation has the same slope for different maturity stages; this is clearly not the case. Thus for accurate detection of hydrated ovaries, regression analysis or other techniques are probably preferable to the simple ratio. Gonad weight has the same ambiguities as do the maturity scales when used to detect other maturity stages. Postovulatory ovaries differ little in weight from the earlier stages of postspawning (atretic) ovaries.

In summary, the traditional methodologies for assessment of reproductive condition other than histology (anatomical maturity scales, and GSI) can be used to identify females with hydrated ovaries. The incidence of such females with hydrated ovaries might be used to provide at least a crude index of spawning frequency (see

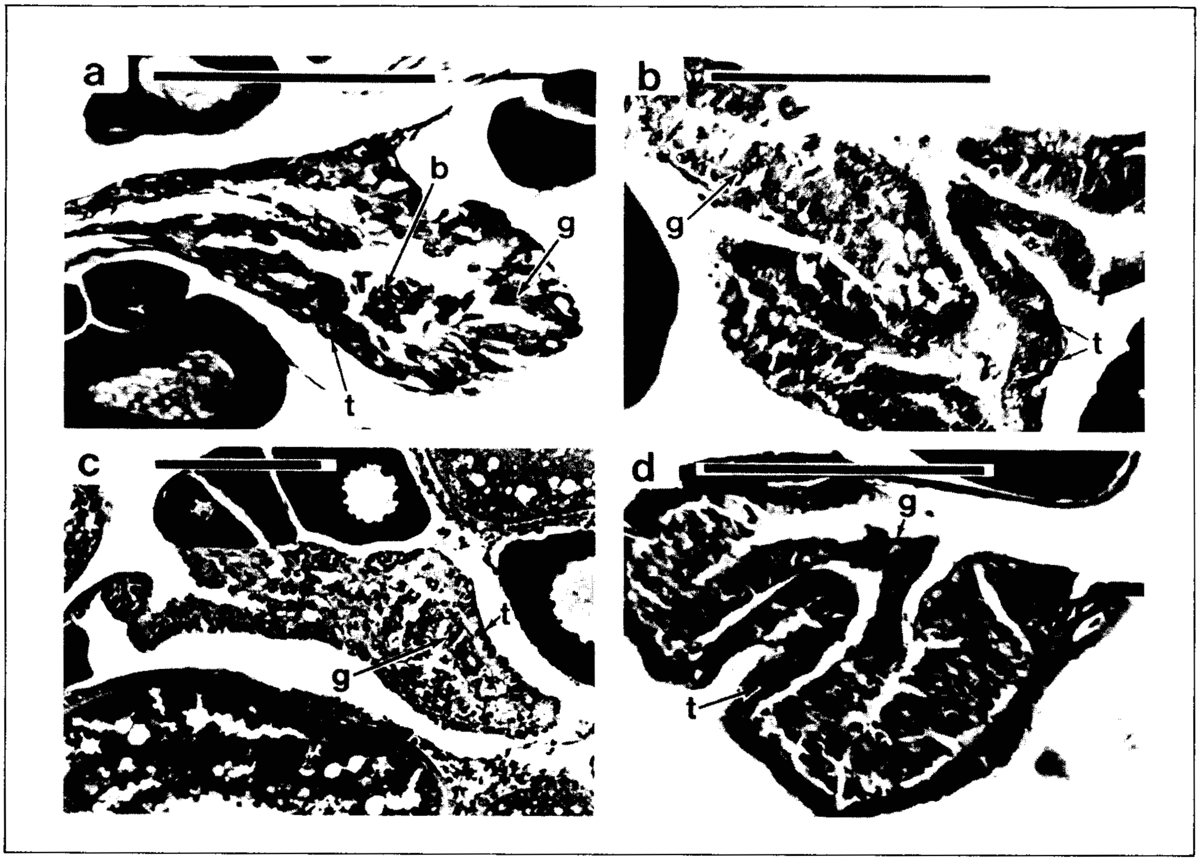

Figure 5.—Degenerating postovulatory follicles in four marine fishes. Bars = 0.1 mm; g = granulosa epithelial cell layer; t = thecal connective cell layer; b = red blood cells.

a) Chub mackerel 24 h after spawning (laboratory-induced with hormones).

b) Chilean hake. (Histological material from S. R. Goldberg, Whittier College, Whittier, CA.

c) Black skipjack. Histological material from K. Schaefer, IATTC, La Jolla, CA.

d) Pacific sardine from Peru about 24 h after spawning. Histological material from S. R. Goldberg, Whittier College, Whittier, CA.

below). These methods are useful, however, for estimating the duration of the spawning season, and they could possibly be used to provide a size or age-specific index of the relative duration of the season. At present these methodologies alone cannot be used to accurately identify in multiple spawners either postovulatory females or post-spawning females, but it is possible that the GSI or maturity scale could be calibrated using histological detection of spawning frequency. Such a calibration would be worthwhile where a long historical record of GSI or maturity-scale determinations existed, because little is known of the interannual variation in spawning frequency in marine fish stocks. The technique of estimating spawning frequency using anatomical detection of females with hydrated ovaries is discussed in the next section.

Hydrated Oocyte Method

The incidence of females with hydrated oocytes can provide a quantitative estimate of the frequency of spawning in natural populations. Using this technique, DeMartini and Fountain (1981) found that the percentage of female queenfish, *Seriphus politus* (Family *Sciaenidae*), spawning per day averaged 13.6% over a 4-5 mo spawning season indicating that the mean interval between spawnings was 7.4 d. The chief advantage of the hydrated oocyte method is that spawning frequency estimates can be made using gross anatomical examinations of ovaries, and extensive histological processing and analysis are not required. Two preliminary steps required for estimation of spawning frequency using this method are outlined below.

1. **Verification of gross anatomical criteria**—The anatomical criteria used to identify hydrated ovaries must be verified using standard histological criteria, although advanced hydrated ovaries are relatively easy to identify from anatomical criteria or their weight (see Hunter et al. 1985; DeMartini and Fountain 1981). In addition, it is important to examine histologically the hydrated ovaries from females captured over the entire hydration period to determine the range of stages of hydration that are anatomically detectable.

2. **Estimation of the optimal time for sampling**—To estimate spawning frequency using the incidence of females with hydrated ovaries, females must be sampled prior to the onset of spawning, but at a time sufficiently close to the onset of spawning, so that all females destined to spawn are detectable using gross anatomical characteristics of the ovary. In addition, the duration of the period of hydration must be established and it must be documented that nearly all hydrated oocytes are spawned in less than 24 h. These determinations require that a series of samples of females be taken at regular intervals at sea over 24-48 h (see DeMartini and Fountain 1981). The optimal time for sampling females with hydrated ovaries can be determined from this series and biases caused by the onset of spawning or by the failure to detect hydration can be avoided.

The chief advantage of the hydrated ovary method over the use of the postovulatory follicles is that histological examination is not necessary after the preliminary work outlined above. On the other hand, our data on northern anchovy indicate three actual or possible disadvantages. First, sampling for the incidence of females with hydrated ovaries can be done only during a very limited portion of each day. In anchovy this period is probably 6 h or less, whereas females with day-old postovulatory follicles can be sampled at any time of day. The second possible disadvantage is that females with hydrated ovaries may be more vulnerable to trawls and perhaps other fishing gear than females in other reproductive states. This may bias an estimate based on hydrated ovaries. We find that spawning frequency estimates based on the number of female northern anchovy with hydrated ovaries and new postovulatory follicles can as much as double those estimates based on the incidence of day-old postovulatory follicles. However, this bias may be the result of the trawl oversampling the depth strata where hydrated females are abundant rather than a lack of avoidance by hydrated females. The third disadvantage of the hydrated ovary method is that incidence of females with hydrated ovaries is contagiously distributed among fish samples whereas females with day-old postovulatory follicles are not (Hunter and Goldberg 1980; DeMartini and Fountain 1981). This increases the variances of the estimate and demands a larger sample size. The contagion is probably caused by the separation of fish prior to spawning into groups composed mostly of males and hydrated females and other groups with fewer males and non-hydrated females.

Despite its actual and possible disadvantages, the simplicity of the hydrated ovary method is appealing. It seems a useful initial approach, especially since past estimates of spawning frequency were based on various speculative arguments (number of modal groups of oocytes in the ovary, or standing stock of yolked oocytes) and were in error by a factor of ten or more. The hydrated ovary method certainly provides a useful first approximation of spawing frequency at a relatively low cost.

ATRESIA

In northern anchovy, as well as in most other seasonal spawning fishes, a low incidence of oocyte atresia (degeneration of oocytes) occurs throughout the spawning season but becomes marked as the spawning season closes and the remaining advanced oocytes in the ovary are resorbed. The incidence of atresia is not used directly in the estimation of spawning biomass using the egg production method, but it is important for several other reasons: 1) A general knowledge of atresia is required to age postovulatory follicles because some stages of follicular atresia are very similar in appearance to late-stage postovulatory follicles; 2) it is a key histological marker for the cessation of spawning, and as a consequence can be used to determine whether or not a cruise period is optimal for biomass estimation (estimates near the end of the season have less precision); 3) it is necessary for accurate estimates of size at first maturity; and 4) it is essential for separating immature females from those in postspawning condition, a distinction required by the egg production method.

Atretic Stages

Oocyte degeneration or oocyte atresia has been divided into four or more sequential stages. We use the nomenclature and general characteristics defined by Bretschneider and Duyvene deWit (1947) and Lambert (1970a), but details of the descriptions of individual stages are based on our examination of northern anchovy ovaries (Hunter and Macewicz 1985).

During the initial stage of the atretic process, alpha (α), the entire oocyte is resorbed, including the yolk if present, by the hypertrophying granulosa cells of the follicle. In the second stage, beta (β), the major degeneration and resorption of the follicle (granulosa and thecal cells) occurs. In the third, gamma (γ), and fourth, delta (δ), atretic stages, regression of the theca and granulosa cells continues, greatly reducing the size of the follicle, and a yellow-brown pigment appears in H & E sections. The histological characteristics used to identify these stages in northern anchovy are outlined below.

Alpha stage atresia—In the alpha stage of atresia the oocyte is resorbed, leaving only the follicular layers. The early phase of alpha stage atresia is characterized by disintegration of the nucleus, evident by an irregular shape and granular, dark, basophilic staining, and the disintegration of some of the yolk globules, indicated by less refractive globules, fused globules, or globules expanded and of less regular shape (Fig. 6a, b, c). The zona radiata slowly dissolves as indicated by the loss of striations and uneven diameter (Fig. 6b). In the subsequent phase of alpha atresia, granulosa cells enlarge and upon rupture of the zona radiata invade the degenerating oocyte (Fig. 6d). Yolk adjacent to the invading granulosa cells liquifies (loses all structural integrity and appears as a homogeneous eosinophilic area), and becomes phagocytized by the granulosa cells as indicated by the presence of yolk in the vacuoles of these cells. The basophilic staining cytoplasm is also resorbed by the granulosa cells. In the alpha stage of atresia, blood capillaries and vessels are numerous in the thecal connective layer which does not proliferate or invade the oocyte but remains as a thin layer covering the granulosa cells. The alpha stage ends when resorption of the oocyte is complete (all cytoplasm and yolk are gone). The resulting structure (beta stage) is usually much smaller than the original oocyte. The subsequent atretic stages (beta-delta) are steps in the resorption of the remaining follicle and the structure at this point is called an atretic follicle, the term atretic oocyte being reserved for only the alpha stage of atresia.

In unyolked oocytes, the alpha stage process is similar but without yolk (Fig. 6e, f). The nucleus disintegrates, the thin pre-zona radiata (if present) dissolves, and the granulosa cells enlarge and with only a slight proliferation phagocytize the unyolked oocyte. When resorption is complete, all that remains is the atretic follicle.

Beta stage atresia—Initially the beta-stage atretic follicle is a compact structure composed of numerous disorganized granulosa cells surrounded by a thin thecal and blood vessel layer. The nucleus of some of the granulosa cells is pycnotic and many of the cells contain intracellular vacuole(s) that may be empty or contain amorphous particles. Occasionally one or more large intercellular cavities may exist among the granulosa cells (Fig. 7b, d). Preovulatory beta-stage atretic follicles containing such cavities may easily be confused with postovulatory follicles (older than 48 h), and as a consequence we do not age postovulatory follicles older than 48 h (Hunter and Goldberg 1980). In addition, small (older) beta-stage atretic follicles from yolked oocytes (Fig. 7c, d) are indistinguishable from beta-stage atretic follicles from unyolked oocytes. Thus, we do not identify the original oocyte type undergoing atresia in beta or subsequent atretic stages; such distinctions are made only for alpha-stage atretic oocytes.

Three different patterns of atresia may occur at the conclusion of the beta stage: 1) The follicle may follow the classic pattern outlined by Bretschneider and Duyvene deWit (1947) and pass through subsequent gamma and delta stages (both characterized by increased pigmentation, see below); 2) the follicle may be completely resorbed during the beta stage, leaving no histological characteristics that can be identified; and 3) the follicle may pass directly from a beta stage structure to a delta structure without passing through the intervening gamma stage. In northern anchovy, either the duration of the gamma stage is very short or few follicles pass through the gamma stage into the delta stage, because in regressing ovaries the incidence of gamma stages is very low compared to those of either beta or delta stages.

Gamma stage atresia—The gamma-stage atretic follicle is usually much smaller than the typical beta stage follicle (Fig. 7e). The granulosa cells contain flocculent material of light-yellow hue, and have nuclei of very irregular shape. The granulosa cells are surrounded by many fewer thecal cells and blood vessels than occur in the beta-stage atretic follicles. Occasionally we see an atretic follicle of quite different appearance in anchovy ovaries which we classify as a gamma-stage atretic follicle; they are included in the gamma stage because they also contain flocculent material of light-yellow hue. In this case, the flocculent yellow material is extracellular rather than intercellular and the material is encapsulated by a layer of granulosa and thecal cells. It is possible that the extracellular flocculent material is produced by the disintegration of granulosa cells.

Delta stage atresia—The diagnostic characteristic of this stage is the presence of a dark yellow-brown, finely granular pigment in the granulosa cells (Fig. 7f). The delta-stage atretic follicles are normally very small structures typically composed of 2-20 granulosa cells in the connective tissue stroma. Thecal cells and blood vessels no longer directly encompass the granulosa cells because they have been absorbed into the ovarian connective tissue stroma.

Atretic States

For population work it may be impractical to grade every ovary for the abundance of each of the four atretic stages. To simplify the assessment of atretic condition of the ovary, we have defined four atretic states which are based on some of the atretic stages described in the previous section. The duration of these states was estimated for anchovy in the laboratory and then assessed using starvation to trigger resorption of the ovary (Hunter and Macewicz 1985). In addition, the spawning potential of sea-caught females that were classed in these states was estimated by calculating the incidence of females with postovulatory follicles and hydrated eggs within each atretic state ($N > 5000$); their results are summarized below.

Atretic state 0—Females classed as atretic state 0 have yolked oocytes present and no alpha atresia of yolked oocytes. Beta stage atresia may be present but is not considered, as it cannot be separated with certainty from late-stage postovulatory follicles (>48 h old). Female anchovy in this state have a high potential of spawning, with spawning occurring at a frequency of every week to 10 d.

Atretic state 1—Females with yolked oocytes in which <50% of the yolked oocytes are in the alpha stage of atresia. Frequency of spawning of females classed in this state is less than half of that in females of atretic state 0. Thus, atretic state 1 is an index of a decline in spawning rate (Table 1). Females appeared to persist in this state under natural conditions for extended and probably variable periods, but the state persisted in the laboratory under induced starvation for only 5-7 d. Atretic state 1 is the most common atretic condition during peak spawning periods, and it can be used to detect differences in spawning between length classes; it appears to be a more sensitive index of differences in reproductive rate among length classes than is the incidence of postovulatory follicles (Hunter and Macewicz 1985).

Atretic state 2—Females with yolked oocytes in which ≥50% of the yolked oocytes are in the alpha stage of atresia. Females in atretic state 2 persisted for about 9 d in the laboratory, and it seems to have a similarly short duration in natural populations. It is the best measure of the absolute rates of ovary resorption in the population

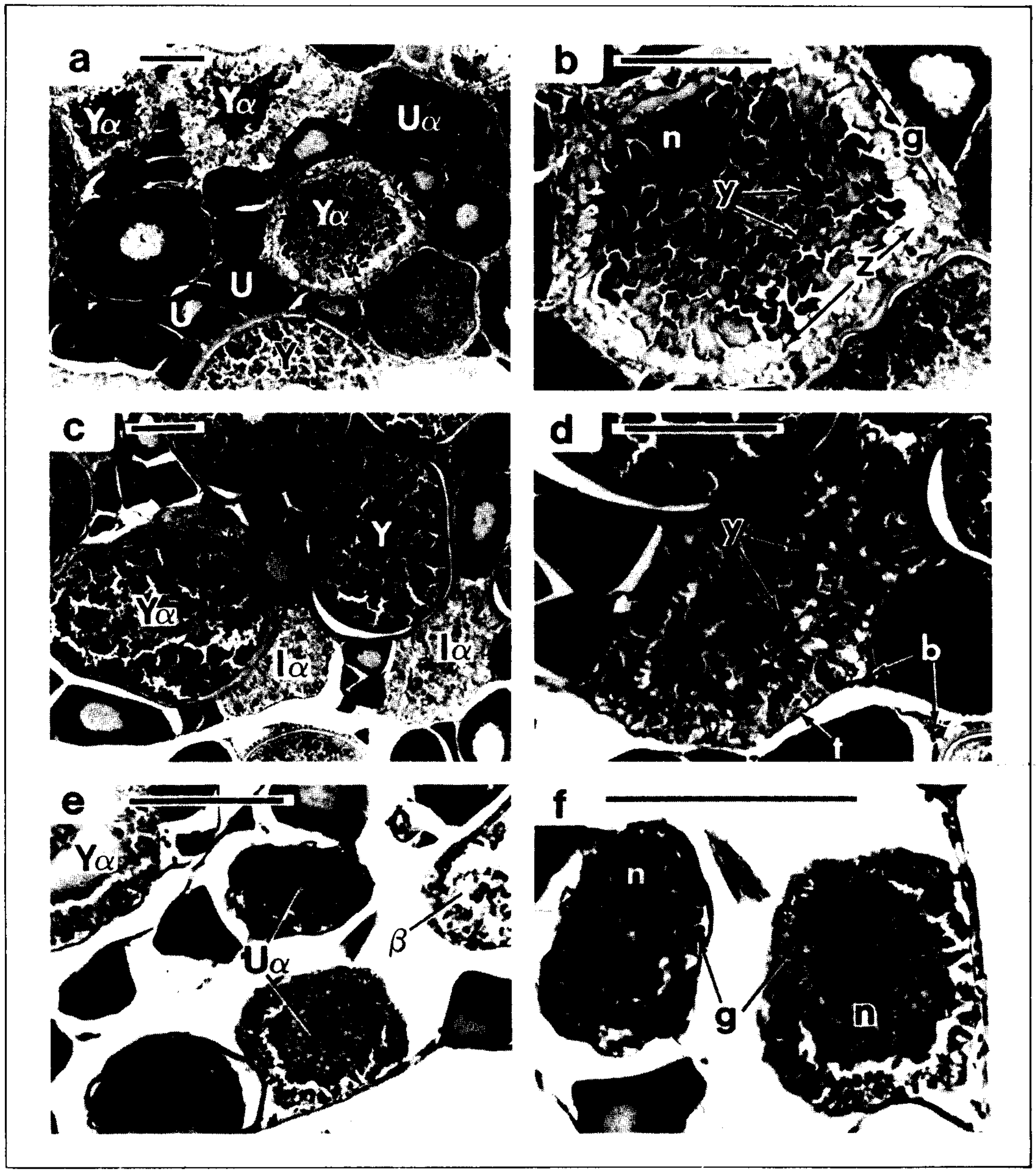

Figure 6.—Alpha (α) stage atresia in yolked (Y) and unyolked (U) oocytes. Bar = 0.1 mm.

a, b) Yolked oocyte undergoing alpha atresia (Yα). Notice dark irregular nucleus (n), uneven dissolving zona radiata (z), hypertrophic granulosa cells (g); and alpha atresia of a large unyolked oocyte (Uα).

c, d) Only remnants of yolk material (y) remain among the invasive phagocytizing granulosa cells in this late phase of alpha atresia (Iα). Note also the thecal layer (t) and the closely associated red blood cells (b). Y = yolked oocyte; Yα = alpha yolked atretic oocyte.

e, f) Unyolked oocytes in the alpha stage of atresia (Uα). Note enlargement of granulosa (g) and disintegration of nucleus (n). Yα = alpha yolked atretic oocyte; β = beta atretic follicle.

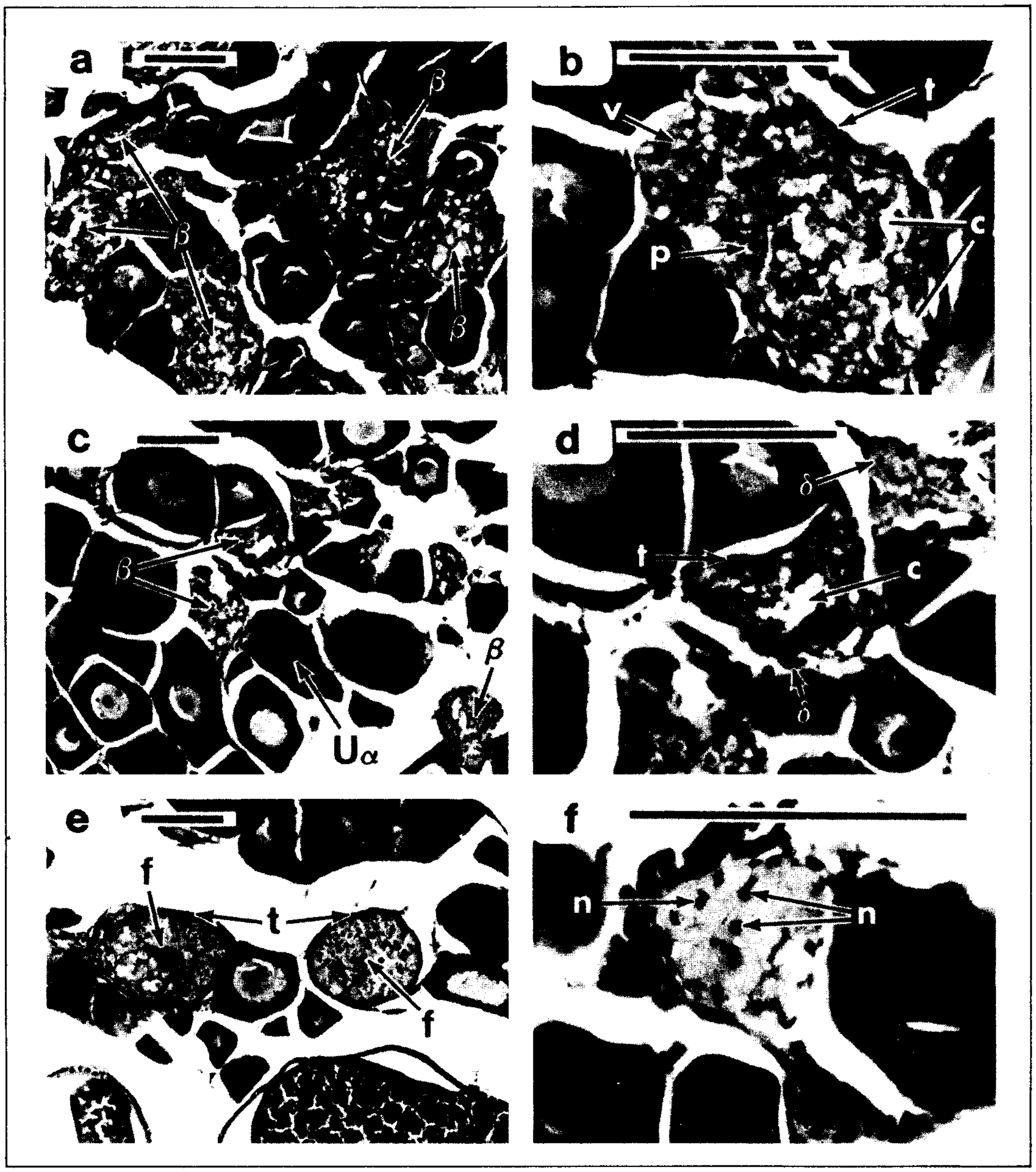

Figure 7.—Stages of atresia following complete yolk absorption. Bar = 0.1 mm.

a, b) Typical beta (β) stage atresia. Note the disorganized granulosa cells with some pycnotic nuclei (p) or intracellular vacuoles (v). t = thecal connective cell layer; c = intercellular cavities.

c. d) Disintegration of granulosa continues in these older beta (β) atretic follicles. Note the large intercellular cavity (c) and the prominent, contracted thecal cell layer (t). Also present is an unyolked oocyte in early alpha (Uα) stage and several delta (δ) stage atresia cells.

e) Two types of gamma (γ) atresia seen in northern anchovy ovaries. Note flocculent material (f) and the thecal connective cell layer (t).

f) Delta (δ) stage atresia characterized by dark-yellow, fine, granular pigment and an irregular nucleus (n).

Table 1.—Histological classifications[1] of northern anchovy ovaries and the reproductive implications. PO = postovulatory.

Ovary	State	Key histological characteristics	Reproductive implications
Spawning States			
Active	Hydrated	Hydrated oocytes present	Spawning will occur in >12 h[2].
	Age 0-day PO	Postovulatory follicles <24 h old.	Spawning occurred <24 h ago[3].
	Age 1-day PO	Postovulatory follicles 24-48 h old.	Spawning occurred 1 d ago[3].
Atretic States			
Active	0	Yolked oocytes present; no alpha-stage atretic yolked oocytes.	High probability of spawning within 1-10 d[4].
	1	Alpha stage atresia of yolked oocytes; ≥50% of yolked oocytes are affected.	Probability of spawning is half that of atretic state 0[5].
Inactive	2	Alpha stage atresia of yolked oocytes; ≥50% of yolked oocytes are affected.	Probability of spawning = 0; may have had an active ovary 1-20 d ago (average 8 d)[6].
	3	No yolked oocytes present; beta stage atresia present.	Probability of spawning = 0; may have had an active ovary ≥16-30 d ago[6].
	Immature	No yolked oocytes present; no beta atretic follicles.	Probability of spawning = 0; no evidence of past reproduction within last 30 or more d. May develop an active ovary within ≥31 d[7].

[1]Ovaries are classed into both spawning and atretic states, but the reproductive implications are for a single-state classification and not for a combination of atretic and spawning states.
[2]Hunter and Macewicz 1980.
[3]Hunter and Goldberg 1980.
[4]In peak spawning months the average female spawns at 1-10 d intervals (Hunter and Leong 1981); hence females with active ovaries are likely to spawn within 1-10 d depending on maturity of the ovary.
[5]Based on the fraction of females in spawning states within atretic state 1 (Hunter and Macewicz 1985).
[6]Hunter and Macewicz 1985.
[7]Hunter and Leong 1981.

and could be used to forecast the end of reproduction near the end of the spawning season. A high incidence of females in this state indicates that cessation of spawning in the population is imminent.

Atretic state 3—Females without yolked oocytes in which beta stage atresia is present. This state identifies females in late postspawning condition. The state persisted for about 30 d in the laboratory, and it may last much longer under natural conditions while the numerous small oocytes are resorbed. (The laboratory data indicate that the duration of this state could be increased if definitions were redefined to include gamma + delta stages of atresia which have a longer life in the ovary than does the beta stage.) This state is used to separate females in postspawning condition from immature females (females with no previous reproductive history). Consequently, identification of females in this state is essential for accurate estimates of age or size at first maturity and for an accurate definition of spawning biomass using the egg production method.

TESTING THE ASSUMPTION OF SEASONALLY DETERMINATE FECUNDITY

A critical assumption underlying some biomass estimates from ichthyoplankton data and many estimates of annual fecundity in fishes is that the annual fecundity in a species is determined at the beginning of the spawning season. Validation of this assumption of determinate fecundity requires proof that 1) all oocytes destined to be spawned in a season are identifiable at the beginning of the season and no new spawning batches are recruited from the reservoir of small unyolked oocytes that exist in the ovary the year round, and 2) the identified standing stock of yolked oocytes that constitute the maximum potential annual fecundity are in fact spawned and only a negligible quantity of these oocytes are resorbed at the end of the season.

The traditional evidence for determinate fecundity is the presence of a major gap in oocyte maturity stages or size classes between the oocytes matured for the season and the reservoir of immature oocytes present year-round in the ovary (Yamamoto 1956). The presence of such a gap in oocyte classes in females taken at the beginning of the season seems to be adequate proof that the standing stock of oocytes is a measure of maximum annual fecundity, as long as the gap is not between a batch of hydrated oocytes and other yolked oocytes. The absence of such a discontinuity in oocyte classes is evidence for indeterminate fecundity. In some cases, however, determinate annual fecundity is believed to exist despite the fact that oocyte size classes are continuously distributed in the ovary. In such cases, spawning frequency and batch fecundity for the species must be estimated over the season and their product (fecundity × frequency) compared to the standing stock of mature oocytes at the beginning of the season. This was done for whiting and haddock in the laboratory by Hislop (1975) and Hislop et al. (1978).

For northern anchovy, Hunter and Leong (1981) compared field estimates of spawning frequency with standing stocks of oocytes. They showed that oocytes as small as 0.1 mm must be included in the annual fecundity to account for 10 spawnings per yr and estimated that anchovy spawn an average of 20 or more times per yr. Their analysis clearly documents the existence of indeterminate fecundity in anchovy. However, anchovy have a continuous oocyte distribution, and so indeterminate fecundity is expected in this species.

In species for which determinate fecundity is inferred because of a discontinous oocyte distribution, it is also necessary to document that the maximum annual fecundity estimated from the standing stock of oocytes is in fact realized in the sea (see above). To validate this assumption requires an analysis of rates of atresia of yolked and partially yolked oocytes. The key atretic state in such an analysis would be one similar to our atretic state 2 where many oocytes are in the alpha stage of atresia. It should be borne in mind that this state may have a short duration.

In anchovy the mean duration of this state is only 9 d and the maximum only 20 d (Hunter amd Macewicz 1985). Consequently, sampling to determine the extent of resorption of oocytes must be done over a relatively short period near the end of the season; otherwise, significant oocyte resorption may not be detected. Substantial oocyte resorption has been observed in the Pacific hake, *Merluccius productus*, which seems to have a discontinuous oocyte distribution (Foucher and Beamish 1977). They conclude that the standing stock of yolked oocytes is very likely in excess of the number of oocytes that will be spawned, except in exceptional years.

In conclusion, an assumption of determinate fecundity requires extensive work to prove, and in some cases may be only wishful thinking. The most conservative assumption is that seasonal fecundity is indeterminate for multiple spawning fishes and that estimates of batch fecundity and spawning frequency are required. The documented cases of determinate fecundity appear to be restricted to boreal or cold temperate climates where spawning seasons are short. Thus in most of the world's oceans indeterminate fecundity and multiple spawning are the rule for epipelagic spawners, and estimates of spawning frequency are essential both for biomass estimation using ichthyoplankton data and for estimates of annual fecundity.

FUTURE APPLICATIONS OF HISTOLOGICAL CLASSIFICATION OF OVARIES

An important future application of the histological classifications we have described is the study of reproductive processes in multiple spawning fish populations. This work requires not only histological classification but sufficient laboratory calibration and analysis of sea data to be able to specify the reproductive implications and duration of each class. Sufficient data exist on northern anchovy to make a relatively accurate interpretation of the reproductive significance of each spawning and atretic state (Table 1). Reproductive state and its approximate duration can now be related to the physiological state of the female (age, size, fat content, biochemical composition, instantaneous growth rate from otoliths or RNA/DNA ratios, and environmental conditions). In this way it may now be possible to identify the factors controlling the 2-3 fold variation in batch fecundity (Hunter et al. 1985), the potentially large variation in spawning frequency and duration of the spawning season, and, most importantly, the functions regulating partitioning of energy between reproduction and growth.

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UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
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April 16, 1987

MEMORANDUM FOR: USERS OF THE EGG PRODUCTION METHOD FOR ESTIMATING SPAWNING BIOMASS OF PELAGIC FISH.

FROM: REUBEN LASKER

SUBJECT: ERRATA FOR NOAA TECHNICAL REPORT NMFS 36; "AN EGG PRODUCTION METHOD FOR ESTIMATING SPAWNING BIOMASS OF PELAGIC FISH: APPLICATION TO THE NORTHERN ANCHOVY".

A number of printing errors have been discovered by Dr. Sachiko Tsuji in the published account of the egg production method. These are important and warrant this memo. Please make these corrections in your copy.

p. 5, Abstract, 4th line should read:
"be estimable and spawning rate constant over the field sampling interval."

p. 12, in equation 8, $\hat{\beta}$ should be β.

p. 17, Table 1. on the January line +3.5 should be -3.5.

p. 20, two lines under the formula in the second column, "sample size" should be "sample scale" and σ_i should read σ_i^2. Five lines under the formula "larger observations" should be "bigger scales."

p. 22, 1st para., No. 3 last line should be simulation, not stimulation.

p. 23. 1st para., line 7. "Table 9" should read "Table 6."

p. 44. Temperature table in second column on the page.
The temperatures read
13.9
13.5
16.2

The correct temperatures are
13.9
15.2
16.2.

p.45. Second column, $y_{i,t,k}$ should read $y_{i,t}$.

p.46 1st Para., line 7, change the word "spawning" to "tows, $\hat{T}$".

p.49. Table 5d. Strike out the words "within or" in the second line of the heading.

p.55. 9th line from the bottom, x_1 should be x_i.

p.56. First. para. second column, sixth line, 26 should read *25.*

p.63. Under "Preservation" $Na_2H_2PO_4$ should be Na_2HPO_4.

p.93. In table 1, atretic state e, change > to <.

p.97. In the! formula after the second para. change < to >.

p.98. In the formula in the first column change -Zt to $-Zt_h$.