RATES OF ATRESIA IN THE OVARY OF CAPTIVE AND WILD NORTHERN ANCHOVY, *ENGRAULIS MORDAX*

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ABSTRACT

The process of ovarian atresia was described for northern anchovy using a laboratory group in which atresia was induced by starvation. Atretic characteristics of the ovary were described and illustrated, atretic ovarian states defined, and the rate that the ovary passed from one atretic stage to another was measured. The ovaries of starved females regressed rapidly; 3 days after the onset of starvation the ovaries of about half of the females contained yolked oocytes undergoing resorption of yolk (alpha stage of oocyte atresia) and by 23 days after the onset of starvation no yolk remained in the ovaries of any of the females. Gamma+delta stages of atretic follicles persisted in the ovary for over a month, but their decline in abundance indicated that eventually all signs of past reproductive activity would be lost in regressed ovaries.

In the natural population, rates of ovarian atresia increased seasonally from only a few percent of the females showing some atresia in peak spawning months to over 50% near the end of the season. Females with low levels of alpha stage atresia (<50% yolked oocytes affected) spawned about half as frequently as did those with no alpha stage atresia. Spawning was rare (1% of the females) or absent in females with high levels of alpha stage atresia (≥50% yolked oocytes affected). Late in the spawning season, it may be possible to forecast the end of spawning in the populations using the frequency of females in the populations with high levels of alpha stage oocyte atresia. Throughout the spawning season atretic rates were higher in small females (standard length ≤10 cm) than in larger ones indicating that 1-year-old females spawning for the first time have a much shorter spawning season than do older females.

Four approaches commonly used to determine the reproductive state of female fishes are 1) staging of ovaries using gross anatomical criteria such as the international Hjort scale (Bowers and Holliday 1961); 2) calculation of the gonosomatic index (GSI), i.e., gonad weight divided by female weight or the equivalent (de Vlaming et al. 1982); 3) estimating the mean diameter of the oocytes in the most advanced mode of oocytes (Hunter and Goldberg 1980; Hunter and Leong 1981); and 4) classifying ovaries histologically. Histological classification is superior to all other methods. Two of its great strengths are that the frequency of spawning of multiple spawning fish populations can be accurately estimated using the presence of postovulatory follicles (Hunter and Goldberg 1980) and that regressing ovaries can be distinguished from immature and from postovulatory ovaries. The histological criteria used to identify regressing ovaries is the presence of many oocytes and follicles undergoing resorption, a process known as atresia.

The interpretive power of histological analysis could be enhanced if the process of ovarian atresia were better documented. Specifically, ovarian atretic stages need to be defined, rates of atresia and duration of stages estimated, and the relation between ovarian atretic state and the probability of spawning determined. Such information would facilitate process oriented field studies on reproductive biology, and increase the accuracy of estimates of size at first maturity and size- or age-specific duration of the annual spawning season.

This study provides the laboratory and field calibration necessary for the assessment of the reproductive state of northern anchovy, *Engraulis mordax*, using the atretic condition of the ovary. We identify a range of ovarian atretic characteristics that define the atretic condition of the ovary, estimate rates of atresia, and estimate the duration that atretic characters persist in the ovaries of starving females in the laboratory. We use this information to classify ovaries of sea-caught females and estimate the probability of spawning for females with various levels of ovarian atresia.

We know of no similar work. A large descriptive

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literature exists on follicular atresia in fishes (reviewed by Saidapur 1978) and stages of atretic oocytes and follicles have been defined (Bretschneider and Duyvene de Wit 1947; Lambert 1970a), but only the atretic rates in the guppy have been measured (Lambert 1970a). Considerable attention has been devoted to stages of atretic follicles (corpora atretica or "preovulatory corpora lutea") because of a presumed endocrine function (see reviews by Hoar 1965; Byskov 1978). The seasonal occurrence of atretic oocytes and follicles is often discussed as part of a general description of seasonal changes in the ovary of marine fish; see, for example, cycles described for the gobiid, *Gillichthys mirabilis* (de Vlaming 1972); plaice, *Pleuronectes platessa* (Barr 1963); *Paracentropristis cabrilla* (Zanuy 1977); and three species of *Epinephelus* (Bouain and Siau 1983). The proportion of females with atretic ovaries or the numbers of atretic oocytes within the ovary is given less often, but a few reports exist. For example, atresia ranged from 0 to 6% of the oocytes in female haddock, *Melanogrammus aeglefinus* (L.) (Robb 1982); corpora atretica increased to about 3% of the oocytes during the postspawning period of the dab, *Limanda limanda* (L.) (Htun-Han 1978); and atretic oocytes varied from 13% of yolked oocytes during the prespawning period to 100% during the postspawning period of the snapper, *Chrysophrys auratus* (F.) (Crossland 1977). Some attention has been given to the issue of whether or not atretic rates can account for differences in fecundity among females fed high and low rations. It appears that ration-related differences in fecundity are more closely tied to production rates of oocytes rather than atretic rates (Tyler and Dunn 1976; Wootton 1979). In summary, our literature review indicates that ovarian atresia has yet to be used for quantitative estimation of any reproductive processes in marine fish populations, although it has been used in general descriptions of the seasonality of reproduction for many years.

METHODS

Laboratory Experiment

Adult northern anchovy captured by commercial bait fishermen on 23 February 1982 were kept in a live car in San Diego Bay. Three days later about 1,000 fish averaging 104 mm SL (9.50 g) were taken to the laboratory and held in a 4.6 m diameter pool (1 m deep) at which time the first fish sample was taken. Over the first 34 d in captivity, samples of 18-24 females were taken at 3-4 d intervals with the final sample taken after 62 d in captivity. The temperature of the seawater ranged from 15.5° to 16.5°C.

The fish were not fed during the first 27 d in captivity because starvation was used to trigger the resorption of the ovary; thereafter they were fed daily. On the 27th day of starvation the ovaries had regressed from 4% of female body weight to 0.8% and feeding was resumed because we wished to learn how long the atretic characters would last once the fish began to feed.

In our calculations of atretic rates of laboratory females, we assumed that all the females at the time of capture had active ovaries without atresia, although no samples were taken until 3 d after the fish were captured. Only 3% of the 1,680 females taken in a survey conducted at the same time (28 January-8 March 1982) had atretic ovaries, and it was prominent in only 0.1% of the females (50% or more of yolked oocytes were affected). Ninety-six percent of the females in our first sample (taken 3 d after capture) had yolked eggs, and half of them had no atresia.

All females sampled during the course of the laboratory experiment were weighed and measured, and the ovary removed, weighed, and a section removed for histological analysis. Ovaries were fixed in 10% neutral buffered Formalin[2] and embedded in Paraplast. Histological sections were cut at 6 μm and stained with Harris hematoxylin followed by eosin counterstain.

Sea Data

The ovaries of northern anchovy taken in trawl surveys used for biomass estimation (Stauffer and Picquelle[3]) and various other collections from commercial seiners and midwater trawls were histologically examined. The number of females examined per catch (trawl, purse seine, or lampara net) has varied from 10 to 20. Some collections were quite small, especially those taken outside the main spawning season in the Southern California Bight; these small collections may consist of only two catches, whereas those taken during the main spawning months (February-March)

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

[3]Stauffer, G., and S. Picquelle. The 1980 and 1981 egg production estimates of anchovy spawning biomass. Unpubl. manuscr. Southwest Fisheries Center La Jolla Laboratory, National Marine Fisheries Service, NOAA, P.O. Box 271, La Jolla, CA 92038.

were large, often consisting of 100 or more catches of 10-20 females each. In all collections ovaries were classed according to atretic characteristics as well as on the basis of the presence of postovulatory follicles (age 0 and age 1 d) and hydrated eggs according to the method described by Hunter and Goldberg (1980). All but two of the collections were from the Southern California Bight or northern Baja California, the region where the Central subpopulation of the northern anchovy is concentrated (Vrooman et al. 1981). Two collections were from the vicinity of Monterey and San Francisco Bays. Fish from these areas appear to have a different spawning season from those of fish to the south so they are listed separately in our seasonal tabulations. All collections were classified using histological criteria to determine the incidence of ovarian atretic states as a function of female size, season, and reproductive state.

Histological Characteristics

We describe below the histological characteristics of four oocyte classes and four stages of atresia in the northern anchovy. These stages and classes are subsequently used to define various ovarian atretic states in laboratory and sea-caught female anchovy.

Oocyte Classes

The northern anchovy is a multiple spawning fish (Hunter and Goldberg 1980) with asynchronous oocyte development (oocytes in many stages of development occurring simultaneously in reproductively active ovaries; Wallace and Selman 1981). During the spawning season oocyte development is a continuous process involving all stages with a new spawning batch maturing every week to 10 d (Hunter and Leong 1981). Oocyte development and maturation in teleosts, reviewed recently by Wallace and Selman (1981), has frequently been subdivided into many stages (Andrews 1931[4]; Yamamoto 1956; Lambert 1970b), but our work required a simpler histological classification system. We have combined the stages of past authors into four oocyte classes (unyolked oocytes, partially yolked oocytes, yolked oocytes, and hydrated oocytes), and we describe the histological characteristics of each class below.

[4]Andrews, C. B. 1931. The development of the ova of the California sardine (*Sardina caerulea*). Unpubl. manuscr., 88 p. Stanford Univ., Stanford, CA 94305.

1) Unyolked Oocytes—This class includes all oocytes without yolk that are about 0.04 mm or larger and range upward in size to about 0.35 mm (U, Fig. 1a, b). Oocytes <0.04 mm are excluded because they consist mostly of "oogonium nests", do not have a true follicle layer, and do not seem to undergo degeneration (o, Fig. 1b). The smaller oocytes within this class (0.04-0.15 mm) are spherical, have a large nucleus with a narrow homogenous very densely staining cytoplasm (Fig. 1b). A very thin single layer of elongated, spindlelike cells (the beginning of the granulosa layer) surrounds these small oocytes. The large oocytes in this class are oval, the 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, faintly eosinophilic staining, hyaline membrane (precursor 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.

2) 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) (P, 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 three-fourths 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 they nearly reach 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. Delicate striations appear on the hyaline membrane between the oocyte and follicle layer at the time yolk appears in the oocyte. As maturation proceeds, the follicle layer becomes wider due to an increase in the width and proliferation of granulosa cells. The thecal cells do not increase in size but remain elongated, flat cells with occasional blood capillaries and form a thin outer cov-

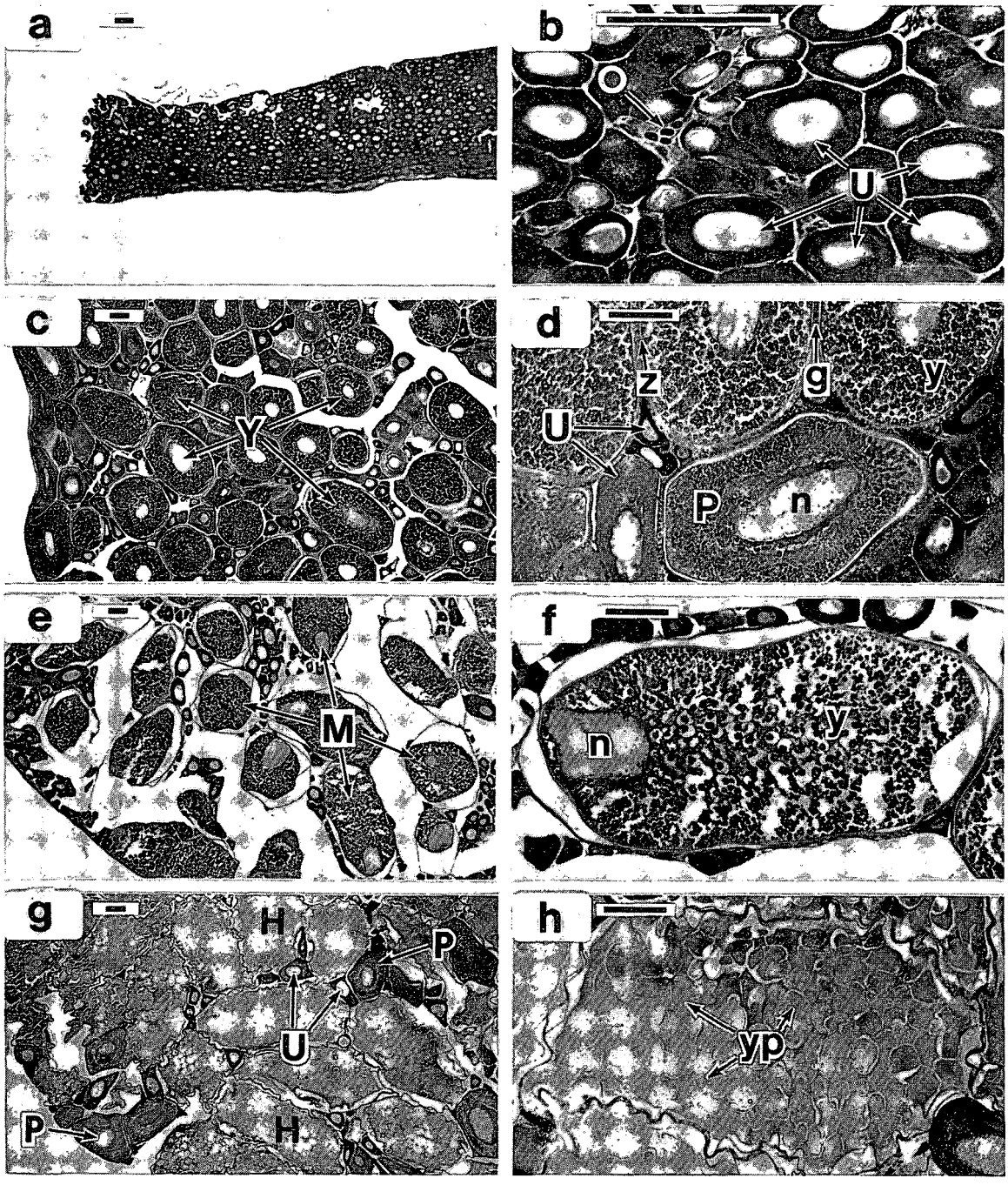

FIGURE 1.—Development of northern anchovy ovary at various magnifications (stain = H & E, bar = 0.1 mm). a) Immature ovary consisting of unyolked oocytes and no atresia. b) Enlargement of (a) showing small spherical unyolked oocytes (U) with a large central nucleus and "oogonium nests" (o). c) Normal mature ovary with many fully yolked oocytes (Y). d) All stages of oocytes: unyolked (U), partial yolked (P), and yolked (Y), are present in normal mature ovaries. (g = granulosa cell layer, z = zona radiata, n = nucleus, y = yolked globules.) e) Prespawning ovary showing migration of nucleus to the animal pole. 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 the follicle layer. (U = unyolked, P = partial 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.

ering to the follicle. The thecal cells do not change until hydration when they become even flatter and have a stringy appearance.

3) 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 (Y, 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 oocyte class, this characteristic being diagnostic of the last class (hydrated oocytes). The nucleus of oocytes in the yolked oocyte class is oval with numerous nucleoli. 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 disappear.

4) Hydrated Oocytes—These oocytes range in size from 0.75 to 1.2 mm (major axis) (H, Fig. 1g, h). Hydration (rapid uptake of fluid by the follicle, Fulton 1898) begins when the nucleus has migrated to the animal pole (M, Fig. 1e, f) and yolk globules first fuse to form yolk plates, and it ends when the hydrated oocyte is ovulated. The nucleus of hydrated oocytes is not visible except in the earliest phase because after the nucleus migrates, 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 stringlike 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 globulues are fused to form yolk plates do not occur earlier than 12 h before spawning. We have never observed atresia in hydrated oocytes; apparently, in northern anchovy, nearly all hydrated oocytes are ovulated.

Atretic Stages

The nomenclature and general characteristics used for the four atretic stages given below follow those of Bretschneider and Duyvene de Wit (1947) and Lambert (1970a). In 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 next 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. The histological characteristics used to identify these stages are outlined below.

1) Alpha (α) Stage Atresia—In the alpha stage of atresia the oocyte is resorbing leaving only the follicular layers. The early phase of alpha stage atresia is characterized by the disintegration of the nucleus, evident by an irregular shape, and a 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. 2a, b, c). The zona radiata slowly dissolves as indicated by the loss of striations and uneven diameter (Fig. 2b). In subsequent phases of alpha atresia, granulosa cells enlarge and, upon rupture of the zona radiata, invade the degenerating oocyte (Fig. 2d). 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. 2e, f). The nucleus

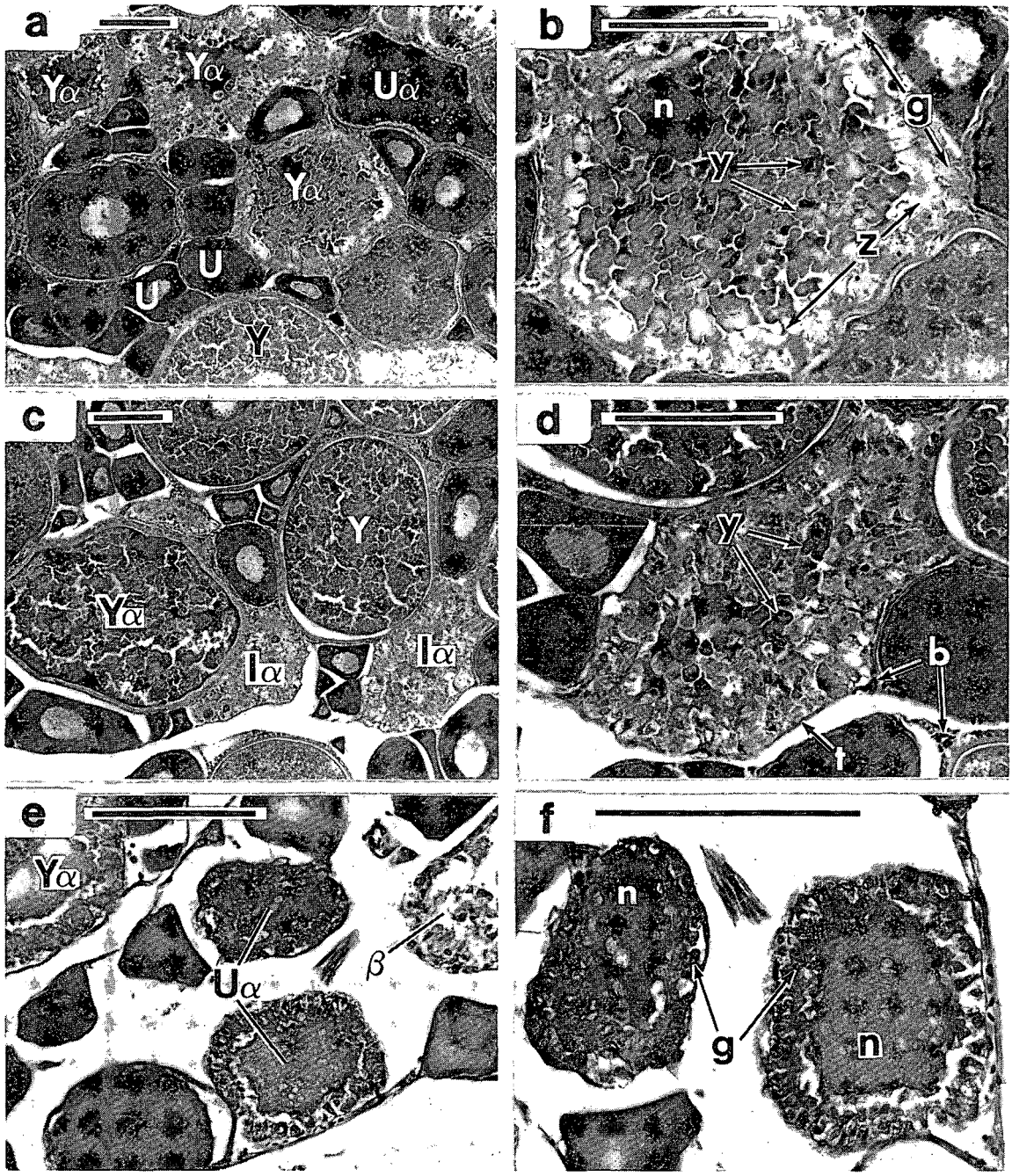

FIGURE 2.—Alpha (α) stage atresia in yolked (Y) and unyolked (U) oocytes (bar = 0.1 mm). a) and b) Yolked oocyte undergoing alpha atresia ($Y\alpha$). Notice dark irregular nucleus (n), uneven dissolving zona radiata (z), and hypertrophic granulosa cells (g); ($U\alpha$ = alpha atresia of a large unyolked oocyte). c) and d) Only remnants of yolk (y) remain among the invasive phagocytizing granulosa cells in this late phase of alpha atresia ($I\alpha$). Note also the thecal layer (t) and the closely associated red blood cells (b). e) and f) Unyolked oocytes in the alpha stage of atresia ($U\alpha$), note enlargement of granulosa (g) and disintegration of nucleus (n). ($Y\alpha$ = alpha yolked atretic oocyte, β = beta atresia.)

disintegrates, the thin prezona 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 follicle.

2) Beta (β) 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 a large intracellular vacuole that may be empty or contain amorphous particles. Occasionally one or more large intercellular cavities may exist among the granulosa cells (Fig. 3b, 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. 3c, 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 de Wit (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 stage 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 with those of either beta or delta stages.

3) Gamma (γ) Stage Atresia—The gamma stage atretic follicle is usually much smaller than the typical beta stage follicle (Fig. 3e). 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 intracellular, 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.

4) Delta (δ) Stage Atresia—The diagnostic characteristic of this stage is the presence of a dark yellow-brown, finely granular pigment in the granulosa cells (Fig. 3f). The delta stage atretic follicles are normally very small structures typically composed usually of 2-20 granulosa cells in the ovarian connective tissue stroma. Thecal cells and blood vessels no longer encompass the granulosa cells.

In our laboratory work 3-4 levels of abundance were recorded for each of three atretic classes seen in anchovy ovaries (alpha, beta, and gamma+ delta stages). The gamma and delta stages were combined since gamma stages were rare. In addition, the alpha stage atretic class was further subdivided into three groups depending on the type of oocyte undergoing atresia (unyolked, partially yolked, and yolked oocytes). In the discussion that follows we have combined some of the abundance levels and have considered only what we believe to be the most diagnostic atretic characteristics, although all atretic characteristics as originally tabulated are given in Tables 1 and 2. The system of atretic classifications was further simplified in our presentation of the analysis of sea-caught specimens, but that will be discussed subsequently.

RESULTS

Rates of Atresia in the Laboratory

The speed at which yolked oocytes were resorbed was striking. In the first sample (elapsed time from onset of starvation = 3 d) the ovaries of 11 of the 24 females (46%) had yolked oocytes in the alpha stage of atresia (Table 1). By the 13th day, half of the females no longer had yolked oocytes, and in the rest of the females 50% or more of their yolked oocytes were in the alpha stage of oocyte

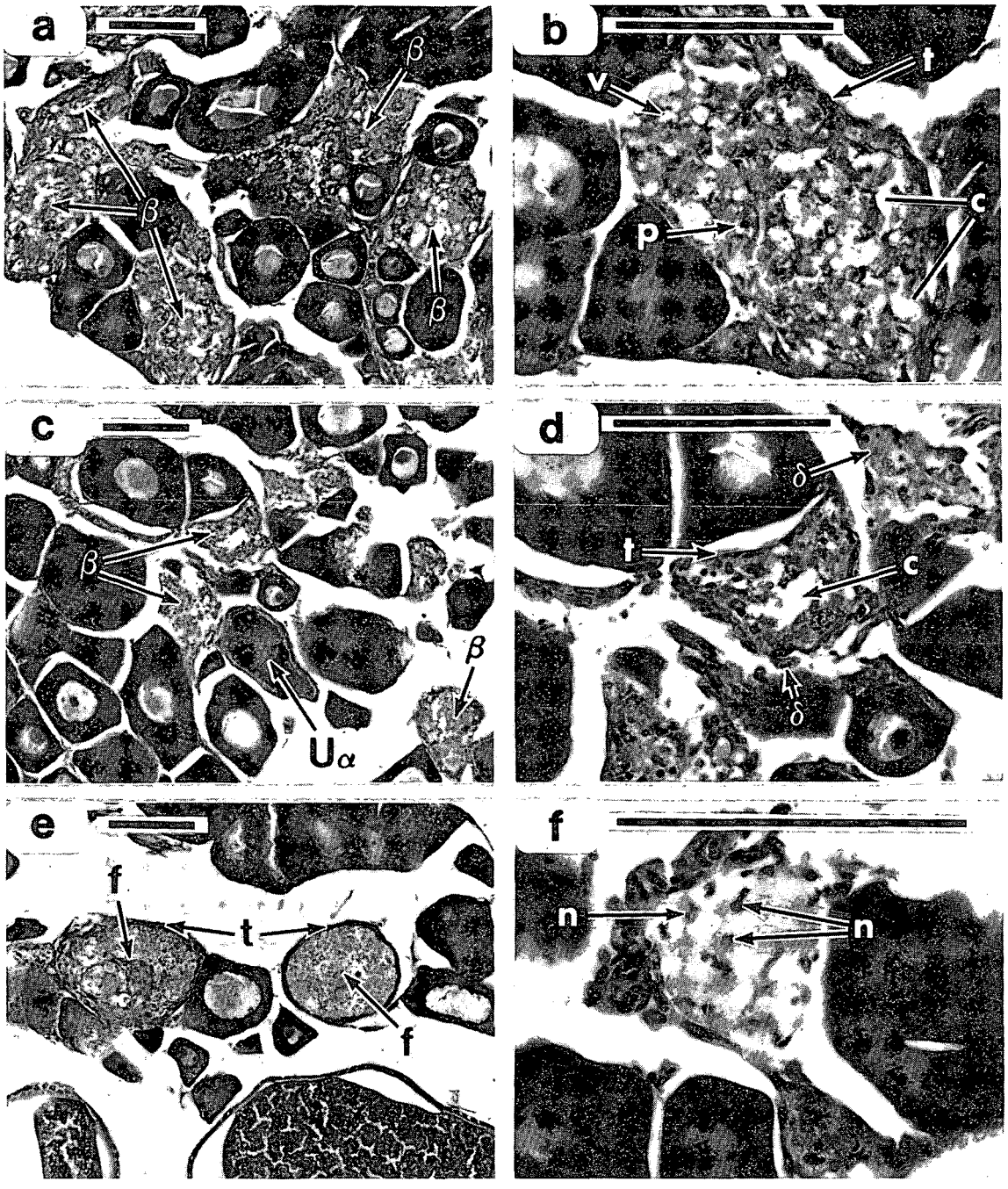

FIGURE 3.—Stages of atresia following after complete yolk absorption (bar = 0.1 mm). a) and b) Typical beta (β) stage atresia. Note the disorganized granulosa cells with some pycnotic nuclei (p) or intracellular vacuoles (v) (t = outer layer of thecal cells, c = intercellular cavities). c) and d) Disintegration of granulosa continues in these older beta (β) stage atresia. Note the large intercellular cavity (c) and the prominent, contracted thecal cell layer (t). Also present is an unyolked oocyte in early alpha ($U\alpha$) 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 layer (t). f) Delta (δ) stage atresia characterized by dark yellow fine granular pigment and an irregular nucleus (n).

TABLE 1.—Percentage of northern anchovy females with ovaries containing various levels of alpha stage atresia during starvation and after the resumption of feeding.[1]

Elapsed time from capture (d)	Feeding condition			Percentage of ovaries with levels of alpha stage atresia									
				Unyolked oocytes[2]			Partially yolked oocytes[2]			Yolked oocytes[3]			
	Starved	Fed	*N*	None	*N* ≤5	*N* >5	None	*N* ≤5	*N* >5	None	*F* ≤50%	*F* = 50-90%	*F* ≥91%
3	X		24	79	13	8	46	29	25	50	33	0	13
6	X		21	19	14	67	14	14	62	5	10	19	48
9	X		24	12	17	71	0	58	34	0	8	17	42
13	X		20	10	15	75	20	15	20	0	0	5	40
16	X		24	0	12	88	4	17	17	0	0	0	8
20	X		22	0	36	64	0	0	4	0	0	0	4
23	X		23	9	39	52	0	4	13	0	0	0	0
27	X		23	4	57	39	0	0	0	0	0	0	0
34		X	23	70	17	13	4	0	0	0	0	0	0
41		X	18	83	17	0	17	0	0	17	0	0	0
62		X	22	90	5	5	64	9	9	36	23	0	0

[1]Feeding begins on the 28th day.
[2]*N* = mean number of atretic oocytes per 6 μm section.
[3]*F* = mean percentage of atretic oocytes per 6 μm section.

TABLE 2.—Percentage of northern anchovy females with ovaries containing various levels of beta and gamma+delta stage atresia and yolked oocytes during starvation and after the resumption of feeding.[1]

Elapsed time from capture (d)	Feeding condition			Percentage of ovaries with levels of atresia									Oocyte types	
				Beta stage atresia[2]			Beta stage atresia with no yolked oocytes[2]			Gamma+delta stage atresia[2]			Yolked oocytes present	Only partial and unyolked oocytes present
	Starved	Fed	*N*	None	*N* ≤5	*N* >5	None	*N* ≤5	*N* >5	None	*N* ≤5	*N* >5		
3	X		24	71	21	8	0	4	0	92	4	4	96	4
6	X		21	24	24	52	0	5	14	71	24	5	81	19
9	X		24	4	8	88	0	0	33	88	8	4	67	33
13	X		20	0	15	85	0	10	45	60	25	15	45	55
16	X		24	0	12	88	0	12	79	16	46	38	8	92
20	X		22	0	9	91	0	9	86	9	36	55	4	96
23	X		23	0	17	83	0	17	83	13	26	61	0	100
27	X		23	0	44	56	0	44	56	26	35	39	0	100
34		X	23	35	48	17	35	48	17	4	13	83	0	100
41		X	18	88	6	6	78	0	6	0	28	72	17	83
62		X	22	82	18	0	35	5	0	0	50	50	59	41

[1]Feeding begins on the 28th day.
[2]*N* = mean number of atretic follicles per 6 μm section.

resorption (Fig. 4). None of the females sampled on the 23d day had yolked oocytes, indicating that all yolked oocytes had passed through the alpha stage of atresia by this time.

The resorption of unyolked and partially yolked oocytes began just as rapidly as did the resorption of yolked oocytes. The percentage of females with atretic unyolked oocytes in the alpha stage increased sharply from 21% on the 3d day of starvation to 90% on the 13th day. Throughout the rest of the 27-d starvation period nearly all of the females (90-100%) had some unyolked oocytes in the alpha stage of atresia, indicating a continual recruitment of atretic follicles from the unyolked and partially yolked oocyte classes. Thus, alpha stage unyolked and partially yolked oocytes are present in regressing ovaries for a much longer period than is the alpha stage of yolked oocytes. This difference probably is due to the greater number of unyolked and partially yolked oocytes in mature ovaries. Yolked oocytes constitute <1% of the total number of oocytes present in mature ovaries.

The incidence of beta stage atretic follicles also increased sharply over the first 9 d of the starvation period and followed a pattern similar to that described for the incidence of alpha atresia from unyolked eggs (Fig. 4). After attaining a high value on the ninth day the incidence of beta atresia remained high until the end of the starvation period as atretic follicles from yolked and unyolked oocytes degraded from the alpha to the beta stage of atresia. Incidence of gamma+delta stages (the third and fourth stages of follicle degeneration) increased later than did alpha and beta stages and remained high after the onset of feeding.

Once feeding resumed (day 28), rapid resorption of yolked and unyolked follicles ceased and the

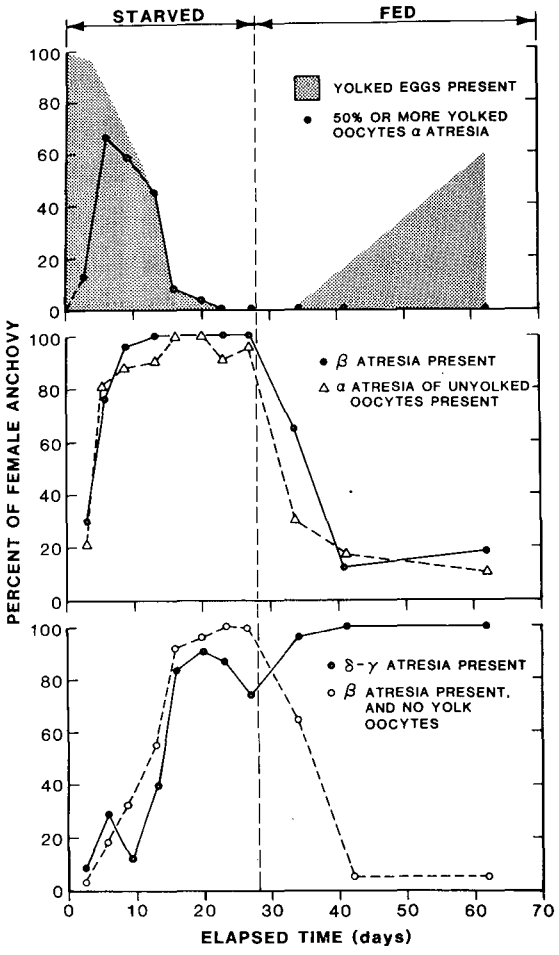

FIGURE 4.—Percentages of captive female northern anchovy with ovaries having various atretic characteristics during a 27-d starvation period and after the onset of feeding. Each percentage is calculated from a sample of 18-24 females (see Tables 1 and 2); alpha, beta, and gamma through delta stages of atretic follicles are those defined by Bretschneider and Duyvene de Wit (1941).

dominant process became maturation rather than resorption. This was indicated by sharp declines in the percentages of females with alpha stage atresia of unyolked oocytes and beta stage atretic follicles, and the reappearance of yolked oocytes (day 41). After only 1 wk of feeding the percentage of females with alpha stage atresia from partially unyolked oocytes dropped from 96 to 30%.

Some inferences can be drawn from these data regarding the duration of atretic stages. The sharp and simultaneous decline in beta stage atretic follicles and alpha stage atresia of unyolked oocytes (following the onset of feeding) indicates that alpha and beta stages must have a short and similar duration. The duration of alpha and beta atresia probably is <2 wk, since the incidence of these two stages dropped to very low levels 2 wk after the onset of feeding; a lag of about 1 wk existed between the first high incidence of females with beta atresia (9 d) and that for gamma+delta (16 d), indicating that the duration of the beta stage may be about 1 wk. The continued high incidence of gamma+delta stages of atretic follicles long after the onset of feeding indicates that these late atretic stages must persist in the ovary for much longer periods than alpha or beta stages. Although gamma+delta stages were present in all ovaries on the last day of the experiment their abundance within an ovary had decreased indicating that even the delta stage would eventually disappear, eliminating the last histological sign of past reproductive activity. We conclude from these inferences that the alpha and beta stages persist in the ovary for 1 wk or less whereas gamma+delta stages persist for over a month, but eventually all signs of past reproductive activity are lost.

The occurrence of alpha stage atresia of yolked oocytes is the best characteristic to use to back-calculate the time of past reproductive activity in field-caught specimens because the stage is of relatively short duration and the time required to resorb all yolked oocytes is relatively short. On the other hand, alpha stage atresia of unyolked oocytes, and beta and gamma+delta stages are less useful for back-calculations because these stages may occur in an ovary for extended periods while atretic oocytes are recruited from the large reservoir of unyolked oocytes in the ovary. In addition, estimates of the time since the onset of atresia in ovaries without yolked oocytes (using the incidence of beta or gamma+delta atretic stages) will always be uncertain because atresia of unyolked oocytes may occur at low levels in immature or developing ovaries as well as in regressing ovaries.

For the laboratory specimens, we calculated the average elapsed time from the onset of ovary resorption using various classes of alpha stage atresia of yolked oocytes and beta atresia in ovaries without yolked oocytes (Table 3). We prefer the criteria of 50% or more of the yolked oocytes with alpha stage atresia because it is likely that no spawning will occur in such females. The average duration of this stage (alpha, yolked, ≥50%) in the starving laboratory females was about 9 d and ranged from <3 to 20 d from the onset of starvation.

Starvation may have induced a higher rate of

TABLE 3.—Mean and maximum duration of various atretic characteristics of the ovaries of starved northern anchovy.

Atretic characteristics	Mean duration (d)	Maximum duration (d)
Alpha atresia of yolked oocytes present	8.0	20
Alpha stage atresia in:		
<50% of yolked oocytes	4.5	9
50-90% of yolked oocytes	8.1	13
91% or more of yolked oocytes	9.3	20
50% or more of yolked oocytes	9.0	20
No yolked oocytes present and beta atresia present	>16	>27

oocyte resorption than usually occurs under natural conditions. Variation in the female nutritional state, food ration, water temperature, day length, and a host of other variables may affect rates of atresia. In addition, field data indicate (see next section) that some spawning may occur in females with low to moderate levels of alpha (yolked) atresia, indicating that such stages may persist under natural conditions for extended periods. Despite these uncertainties we believe that our laboratory estimates of atretic rates are useful for making a rough estimate of the minimum time elapsed since the end of the spawning season in sea-caught females.

Natural Rates of Atresia

In this section we analyze sea data taken since 1977 for the occurrence of four ovarian atretic states in a northern anchovy population:

Atretic state 0—no alpha atresia of yolked oocytes (yolked oocytes present).

Atretic state 1—alpha atresia of yolked oocytes where <50% of the yolked oocytes are affected.

Atretic state 2—alpha atresia of yolked oocytes where 50% or more of the yolked ooctyes are affected (Fig. 5a, b).

Atretic state 3—ovaries with no yolked oocytes present and beta stage atresia present (Fig. 5c).

In addition to the atretic condition of the ovary, we also include histological evidence of recent or imminent spawning using the system of Hunter

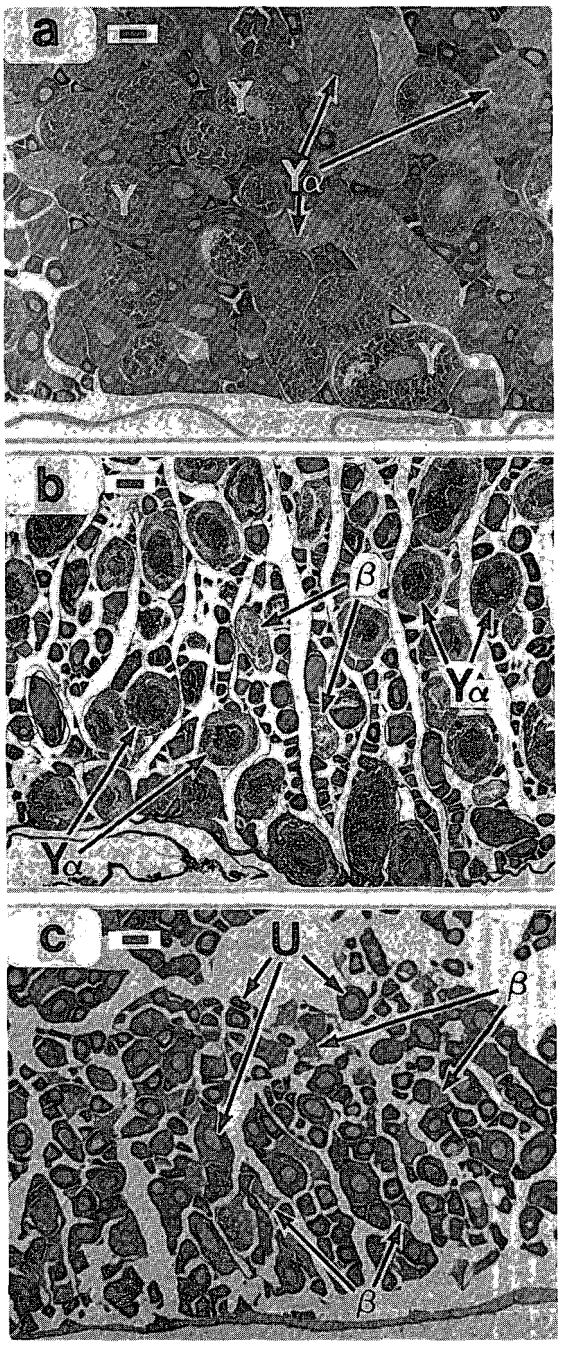

FIGURE 5.—Northern anchovy ovaries with increasing atresia states (bar = 0.1 mm). a) 50% of all yolked oocytes (Y) are in an alpha (α) stage of atresia (both early and late are counted). This is the division point between atretic state 1 and atretic state 2. b) 100% (all) yolked oocytes are in an alpha stage of atresia (Yα). Also present are a few unyolked alpha atretic oocytes and several beta (β) stage atretic follicles. This is still in atretic state 2. c) All yolk has been resorbed leaving only unyolked oocytes (U) and many beta (β) stage atretic follicles. This is atretic state 3.

and Goldberg (1980), i.e., presence of hydrated eggs (imminent spawning), day 0, or new postovulatory follicles (spawning on the night of capture), and 1-d-old postovulatory follicles (spawning on the night before capture). We also include the number of females judged to have inactive or immature ovaries with no evidence of atresia. All data on the incidence of reproductive states are given in Table 4. In the discussion that follows we select and regroup these data in various fashions to test hypotheses and document trends.

Incidence of Spawning in Atretic Females

An important assumption underlying interpretation of ovarian atresia is that the spawning season has or is going to cease, in other words, the probability of spawning in females with atretic ovaries would be expected to be low. To test this assumption we selected from Table 4 the females which had alpha stage atresia of yolked oocytes (atretic states 1 and 2) or yolked oocytes without alpha atresia (atresia state 0) and calculated the percentage of these females that had hydrated oocytes, new (day 0) postovulatory follicles, and 1-d-old postovulatory follicles. Of the females classed in atretic state 1 (females with <50% of the yolked oocytes in alpha stage of atresia), 14% showed evidence of recent or imminent spawning (postovulatory follicles or hydrated oocytes); 29% of the females without atresia showed evidence of spawning (Table 5). Only 1% of those in atretic state 2 (females 50% or more atretic yolked oocytes) had recently been reproductively active. That 1.8% of females in atretic state 1 had hydrated eggs and 3.7% had age 0 d postovulatory

TABLE 4.—Numbers of female northern anchovy in various atretic and reproductive states northern California

Collection dates number of mature females/collection	Atretic state[1]	Hydrated oocytes	Postovulatory follicles		Yolked oocytes	No yolked oocytes	Immature no histology[4]	Total
			0 day[2]	1 day[3]				
1977	0	1	2		13	4		20
09/09-09/10	1							0
	2							0
(10)	3					3		3
	x	1	2	0	13	7	0	23
1978	0				1			1
05/07-05/11	1			1	5			6
	2				4			4
(10)	3					6		6
	x	0	0	1	10	6	0	17
1979	0	39	44	52	279	89	110	613
01/26-02/16	1			1				1
	2				1			1
(10)	3					2		2
	x	39	44	53	280	91	110	617
1979	0	16	51	45	284	27	18	441
03/22-04/14	1		1	5	36			42
	2				6			6
(10)	3					16		16
	x	16	52	50	326	43	18	505
1979	0			1	8	4		13
06/09-06/19	1				3			3
	2				1			1
(12)	3					16		16
	x	0	0	1	12	20	0	33
1979	0				9	31		40
09/19-09/23	1							0
	2				5			5
(10)	3					25		25
	x	0	0	0	14	56	0	70
1980	0	25	72	52	241			390
03/20-04/10	1				9			9
	2				1			1
(20)	3					2		2
	x	25	72	52	251	2	0	402
1980	0		4	9	63	8	6	90
04/24-04/27	1				11			11
	2				14			14
(20)	3					63		63
	x	0	4	9	88	71	6	178

[1]Atretic state 0 = no alpha stage atresia of yolked oocytes.
state 1 = alpha stage atresia of yolked oocytes present but <50% oocytes affected.
state 2 = alpha stage atresia of yolked oocytes present, 50% or more oocytes affected.
state 3 = no yolked oocytes present and beta stage atresia present.

follicles indicate that some of the females in atretic state 1 spawn despite the atretic condition of their ovaries. On the other hand, only two females classed in atretic state 2 had a recent history of spawning. These two females had few yolked oocytes remaining, all but one was in alpha atresia. This atresia may have increased or even started during the time elapsed between hydration and capture. In short, the females in atretic state 2 probably did not spawn in the highly atretic state in which they were captured. No doubt exists that females with moderate levels of ovarian atresia are capable of spawning because they often are hormonally induced to do so in the laboratory. In summary these data indicate that significant numbers of females in atretic state 1 may continue to spawn under natural conditions, although the probability of spawning appears to be about half that of fish without ovarian atresia. Few or none of the females in atretic state 2 continue to spawn indicating that this stage is probably the best one to use to calculate cessation of spawning in the population.

Forcasting the end of Spawning Using Ovarian Atretic States

In our laboratory study atretic state 1 had an average duration of about 5 d and atretic state 2, 9 d; state 3 was in excess of 16 d and probably persists for 30 or more days (Table 3). In the sea, linear projections of the end of the spawning season from early incidence of atresia is not realistic since the numbers of females with regressing ovaries would be expected to increase sharply at the end of the season.

listed in order of collection dates for southern and Baja California (1977-82) and for (1979, 1982).

Collection dates number of mature females/collection	Atretic state[1]	Hydrated oocytes	Postovulatory follicles		Yolked oocytes	No yolked oocytes	Immature no histology[4]	Total
			0 day[2]	1 day[3]				
1980	0			3	32	11		46
05/15-05/28	1		1		5			6
	2				2			2
(20)	3					16		16
	x	0	1	3	39	27	0	70
1981	0	119	122	148	862	58		1,309
02/05-03/06	1		2	1	19			22
	2				3			3
(15)	3					10		10
	x	119	124	149	884	68	0	1,344
1981	0	77	96	113	559	7		852
04/01-04/19	1		3	2	57			62
	2		1		45			46
(15)	3					19		19
	x	77	100	115	661	26	0	979
1981	0		2	1	7			10
04/15-04/30	1				5			5
	2				8			8
(15)	3					7		7
	x	0	2	1	20	7	0	30
1982	0	104	101	189	1,172	52	8	1,626
01/28-03/08	1	2	2	10	32			46
	2				2			2
(15)	3					6		6
	x	106	103	199	1,206	58	8	1,680
1979[5]	0		1		42			43
03/20-03/22	1		1		40			41
	2				41			41
(30)	3					25		25
	x	0	2	0	123	25	0	150
1982[5]	0	2			27	2	11	42
01/22-01/25	1	3		2	14			19
	2			1	5			6
(15)	3					13		13
	x	5	0	3	45	16	11	80

[2]New postovulatory follicles <24 h old.
[3]Postovulatory follicles about 24 h old.
[4]Female not examined histologically, ovary ≤1% of body weight.
[5]Female northern anchovy from northern California; rest of females were from southern and Baja California.

TABLE 5.—Percentage of northern anchovy females taken from 1977 to 1982[1] that were classed in three atretic states that occurred in each of five reproductive classes.

	Reproductive state						
			Postovulatory follicles				
Atretic state	Percent yolked oocytes with alpha stage atresia	Hydrated oocytes (%)	0 day (%)	1 day (%)	Spawning recent or imminent[2] (%)	No evidence of recent spawning[3] (%)	Total number of females
0	0	7.5	9.7	12.0	29.3	70.7	5,090
1	<50	1.8	3.7	8.1	13.6	86.4	273
2	≥50	0	0.7	0.7	1.4	98.6	140

[1]Calculated from data given in Table 4; only females with yolked oocytes are considered.
[2]Females with either hydrated oocytes or postovulatory follicles ages 0 or 1 d (the sum of the first three columns).
[3]Females with yolked oocytes but without hydrated oocytes or postovulatory follicles.

This nonlinearity becomes obvious when the end of the spawning season is extrapolated from numbers of females classed in atretic state 2. For example, of the 1,620 mature females taken during the peak of spawning (28 January-18 March 1982) in southern California (Table 4), only two were in atretic state 2 and 1,612 had yet to pass through state 2. Since laboratory data indicate that about 9 d are required to pass through atretic state 2, it would require $(1{,}612/2) \times 9$, or over 7,000 d for the entire population to become atretic at the rates of atresia observed in February, which, of course, is nonsense. Projections of the end of the spawning season using higher rates of atresia taken in April in southern California (24-27 April 1980) give a more realistic projection ($(87/14) \times 9 = 56$ d). Such an arithmetic projection may be inappropriate for collections which have a very high rate of atresia such as those taken in Monterey in March 1979 ($(84/41) \times 9 = 18$ d), and a geometric model might be preferable. The point we wish to emphasize is that atretic rates are nonlinear over the season with the rate increasing markedly as the season closes. Thus only samples taken near the close of the spawning season are of value for forecasting the end of spawning for the population.

Seasonal Changes in Atresia Among Females of Different Lengths

To evaluate how atretic rates change among females of different lengths, we segregated our data into two length classes (females ≤10 cm SL and those >10 cm SL) and calculated the percentage of mature females that had atretic ovaries (atretic states 1-3 combined). Mature is defined here as all females except those which have yet to reach first maturity (small females with small immature non-atretic ovaries). We also calculated the fraction of females in each length class with 1-d-old postovulatory follicles, a measure of the percentage of females spawning daily (Hunter and Goldberg 1980).

In every case, regardless of cruise or season, small females (≤10 cm SL) consistently had a higher rate of ovarian atresia than did larger ones (>10 cm SL) (Table 6). This is a strong trend as the probability of such an event (9 pairs of the same sign) is $(1/2)^9$. In addition, the difference between pairs was statistically significant (chi-square test) even when the levels of atresia were quite low. For example, in February-March 1981, only 4.1% of the small females and 1.9% of the large females were atretic, yet this difference was significant at $P < 0.05$ using the chi-square test. As would be expected, the percentage of females with atretic ovaries increased in both length classes as the season progressed from January through June.

The consistency of the differences in the incidence of atresia between large and small females indicates that the smaller ones must have a much shorter spawning season than larger ones. Females <10 cm long are typically about 1-yr-old and are in their first spawning season whereas those longer than 10 cm are predominantly 2-3 yr old and have spawned during the previous seasons. These data indicate that the first spawning season of females may be quite short with significant numbers of females leaving the spawning population in early April, while the older fish continue to spawn. That the rates of atresia in young fish were always higher even in the peak months of spawning such as February and March indicates that a small percentage of small females may only spawn a few times during the season in contrast to the older females which appear to be spawning at about weekly intervals for months. The fraction of small females spawning per day would be expected to be less than larger females since the small females have a higher incidence of ovarian atresia. We calculated the fraction of females spawning per

TABLE 6.—Percentage of mature northern anchovy females in two length classes with atretic ovaries. Females from north of Point Conception and groups with fewer than nine females per length class excluded.

Cruise period			Number of mature females[1]		Percent of mature females with atretic ovaries[2]		Fraction mature females spawning per day percent[3]	
From	to	year	≤10 cm	>10 cm	≤10 cm	>10 cm	≤10 cm	>10 cm
01/26	→02/16	1979	121	297	1.7	0.7	8.0	15.9
01/28	→03/18	1982	97	1,523	14.6	2.6	14.1	12.2
02/05	→03/06	1981	462	824	4.1	1.9	10.2	13.7
03/20	→04/10	1980	68	334	8.8	1.8	11.7	15.2
03/22	→04/14	1979	30	430	23.3	13.3	3.8	11.8
04/01	→04/19	1981	102	870	39.2	10.0	10.5	12.9
04/24	→04/27	1980	64	100	96.9	26.0	0	8.6
04/15	→04/30	1981	10	20	80.0	60.0	0	5.3
05/15	→05/28	1980	15	44	73.3	29.5	6.2	4.4

[1]All females given in Table 4 except those with immature ovaries and those not examined histologically.
[2]All females in atretic states, 1, 2, and 3 combined.
[3]Fraction of females spawning = F, where $F = \dfrac{m_{1i}}{2m_{1i} + m_{ni}}$

m_{ni} = mature nonspawning females, and m_{1i} = females with 1-d-old postovulatory follicles.

day for the two length classes to test this assumption. We used the Stauffer and Picquelle (footnote 3) method for estimating spawning fraction as it corrects for biases in the numbers of females with hydrated eggs, i.e.,

$$F = \frac{M_{1i}}{2M_{1i} + m_{ni}}$$

where F = fraction of females spawning per day, M_{1i} = number of females with 1-d-old postovulatory follicles, and m_{ni} = number of mature females with no recent spawning history (females with postovulatory follicles or hydrated eggs are excluded). Examination of Table 6 indicates that differences in spawning fraction between the two size classes of females were much less distinct than were the differences in ovarian atresia. Using only the 8 cruises in which the numbers of females in each of the two length classes exceeded 10, the mean difference in spawning fraction (fraction for large females − fraction for small females) for the set of 8 cruises was +3.76% with 95% C.I. ±3.50% indicating a small difference in spawning frequency between the two length classes that is just barely significant at the 5% level. We believe the reason that differences in atretic fraction between large and small females are much more consistent than those in spawning fraction is that spawning fraction has a greater variability and a much more limited dynamic range than does the atretic fraction. Spawning fraction varies from 0 to about 16% and may be affected by time of day and schooling behavior (Hunter and Goldberg 1980). Atretic fraction varies from 0 to nearly 100%, is not linked to reproductive behavior, and consequently, is probably not affected by time of day or schooling.

DISCUSSION

Evaluation of Atretic Classification

Our objective was to evaluate the use of ovarian atretic states to characterize the reproductive biology of northern anchovy populations. We included in our analysis of laboratory data many atretic characteristics not used to construct the three atretic states utilized in the analysis of sea data. These additional characters could be used to create additional states or to more precisely delimit the existing ones. Our selection of characteristics was based in part on ease of identification since for population work thousands of histological sections were examined. Other considerations include the fact that statistical analysis indicated that classifiers frequently confused beta stage atretic follicles in yolked ovaries with postovulatory follicles older than 24 h, and, as a consequence, beta atresia was not used as a diagnostic character in ovaries with yolked oocytes. Alpha stage atresia was the most useful atretic stage because the type of oocyte (yolked) undergoing atresia is still discernible. In addition, alpha stage atretic oocytes can be easily distinguished from postovulatory follicles whereas this is not the case for later atretic stages.

Three atretic states were defined and applied to sea data. The incidence of all three atretic states combined was a sensitive index of the reproductive state of the population over the spawning season.

In fact, the atretic condition of the ovary was a more sensitive index of seasonal changes in the reproductive rate among size classes of females than was the incidence of spawning based on the presence of postovulatory follicles.

Atretic state 1 (<50% of yolked oocytes in the alpha stage of atresia) was not useful for estimating atretic rates in an absolute sense since this state appeared to persist in natural populations for extended and probably variable periods. Some spawning occurred among females classed in atretic state 1, although the frequency of spawning was less than half of that of females without ovarian atresia. Batch fecundity might also be reduced in females classed in atretic state 1, a speculation worth further study. Atretic state 1 was a useful index of atretic rates during peak spawning months. At such times it was the most common atretic condition and detection of differences in atretic rates among length classes was largely a function of the number of females in this state.

Atretic state 2 (50% or more of yolked oocytes in alpha atresia) persisted for about 9 d in the laboratory, and judging by its low frequency in field collections this state may have a similarly short duration in natural populations. Females with ovaries in this state rarely or never spawn, as might be expected, since more than half of the yolked oocytes are not viable. In addition, a short duration of this state also might be expected on the grounds that it seems maladaptive to prolong such a threshold condition. For the above reasons atretic state 2 seems to be the best absolute measure of the rates of ovary resorption in the population and the only state that might provide an accurate forecast of the end decline of reproduction in a population. Unfortunately, accurate forecasts of the end of spawning for a population can be made only near the end of the spawning season.

Atretic state 3 (no yolked oocytes with beta atresia present) identifies females in late postspawning condition. Such females cannot be separated from immature females on the basis of gonad weight or using gross anatomical criteria. This state persisted for about 30 d in the laboratory, but 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 the definitions were changed to include gamma+delta stages of atresia which have a longer life in the ovary than the beta stage. The laboratory data also indicated that even gamma+delta stages of atresia would eventually disappear from the ovary so that no signs of previous spawning activity would exist in a regressed ovary. It is doubtful that the duration of atretic state 3 or any late postspawning state will ever be accurately estimated because it is dependent on too many environmental circumstances. Nevertheless, this state is very useful in separating females in postspawning condition from females with no previous reproductive history. This is an essential distinction for estimating spawning biomass (Stauffer and Picquelle footnote 3) and for determining the size or age at first reproduction (Hunter and Macewicz 1980).

Possibly the most important future application of atretic classification of ovaries is for process oriented sea work on the reproductive biology of multiple spawning fish such as the northern anchovy. Such work does not require a large sample as do estimates of reproductive characteristics for an entire population. The reproductive state of an individual female can be accurately defined by the atretic criteria we have discussed, and the spawning state criteria described by Hunter and Goldberg (1980). The reproductive characteristics of a female can be related to its physiological state (age, fat content, biochemical composition, and instantaneous growth rate from otoliths or RNA/DNA ratios) and functional relationships established between reproduction and the environment. In this way the factors controlling the duration of the spawning season, and the total fecundity during the season, can be identified under natural conditions.

Biological Implications

Several important biological conclusions can be drawn from this work. Only a few attempts have been made to estimate the time needed for a follicle to disappear by atresia in vertebrates and no information exists for fishes (Byskov 1978). Our focus was on atretic rates of all oocytes in the ovary and not on an individual follicle; nevertheless, the striking speed with which all yolked oocytes passed through the initial stages of atresia indicate that the rate for individual follicles must be high. Similar rates were observed in the guppy by Lambert (1970a). In the guppy, alpha stage atresia of yolked oocytes appears about 1 d after parturition, and beta stage atresia appeared about 2 d after the first alpha stages were detected; beta stages persisted for only 11 d. In the anchovy, the average time for all yolked oocytes in the ovary to pass through alpha atresia was 8.0 d and the

maximum time was 29 d. Thus the effect of atresia on fecundity may be underestimated since the duration of atretic stages is short and a small standing stock of atretic oocytes could be an indication of a high loss rate. On the other hand, laboratory studies seem to indicate that atretic rates are not sufficiently high to account for the differences in fecundity observed when fish are fed high and low rations (Tyler and Dunn 1976; Wootton 1979). The duration of the atretic stages in these studies was unknown, however.

Additional evidence for the volatility of the reproductive state of anchovy is an important contribution of this study. Our laboratory data indicated that given a shortage of food the ovary can be rapidly resorbed leaving no trace of former reproductive activity in a few months or less, but when given sufficient food atresia stopped, maturation and vitellogenesis resumed, and a reproductively active ovary was rapidly reformed within 35 d. Clearly, in such multiple spawning fishes as the anchovy, more than one spawning season per year is possible given the appropriate environmental conditions. This may explain the occurrence of a second annual spawning period in the Peruvian anchoveta (Santander and Castillo 1976) and the occasional heavy fall spawning of the northern anchovy (Smith 1972). That active ovaries are consistently produced from small, inactive ones in 30-60 d in the laboratory (Leong 1971; Hunter and Leong 1981) and that some reproductively active females are found the year around also supports this view.

Food shortage does not always lead to regression of the ovary in anchovy or any other multiple spawning fishes. In addition to food ration, regression of the ovary also depends upon the level of energy reserves, the timing of the reproductive cycle, and perhaps certain environmental conditions such as temperature and day length. For example, starvation of 40-80 d did not block the initial increase in the size of ovaries of the goby *Gillichthys* at the start of the reproductive cycle in July but only 23 d of starvation resulted in ovarian regression in January when active vitellogenesis was occurring (de Vlaming 1971). Similarly we noted in a preliminary experiment that starving anchovy of 25% greater wet weight than those used in this study produced a slower regression of the ovary over a 36-d period than occurred in the present study. The present study is more representative of natural conditions since the fish were taken in midspawning season when their ovaries were active whereas in the preliminary study the fish were taken out of season and fed heavily for 30 d to induce gonad maturation before the onset of the 36-d starvation period.

Another important conclusion from this study was that young female anchovy spawning for the first time probably have a much shorter reproductive season than do older females. Hunter and Leong (1981) estimated that the average female spawns about 20 times per year. Thus the older females must spawn considerably more often than 20 times per year, and probably contribute a much larger fraction of the reproductive output of the population than a proportionate share by weight. This indicates the importance of maintaining older fish in the population and that danger may exist if older fish are overharvested.

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