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Seasonal Development of Gonads of the Hagfish, *Eptatretus burgeri*, Correlated with Their Seasonal Migration

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ABSTRACT—Seasonal development of gonads was studied in the hagfish, *Eptatretus burgeri*, caught near the Misaki Marine Biological Station facing the Sagami Bay of the Pacific coast of Japan, during periods from October 1971 to March 1973 and from November 1977 to December 1978. The hagfish were collected at water depths of 6 to 10 m (shallow water) in Koajiro Bay close to the Station or water depths of 50 and 100 m (deep water), about 1,600 and 4.800 m west to the Station. They were collected monthly from both shallow and deep waters, but they could not be collected from shallow water during warmer summer months (July-September) due to seasonal migration. The mean total lengths of the females collected in both shallow and deep waters during January to April were 40.7±0.4 cm (±SEM; n=317) and 34.5±0.3 cm (±SEM; n=556), respectively. In both locations, developing eggs longer than 5 mm were found in females larger than 39 cm in total length. There was no difference in the annual growth curves of developing eggs between shallow and deep water: the sizes of the developing eggs were the smallest in October, and was the largest in September. There was no apparent difference in testicular development between two locations, so these data were combined. Testicular development occurs in males larger than 38 cm in total length. The testis weight was the heaviest in July, and was the lightest in September. In autumn and winter, most testicular follicles contained only spermatogonia. In spring, follicles containing spermatocytes increased. Follicles with spermatids or maturing sperms were relatively abundant in summer. Although egg deposition is supposed to occur in September and/or early October somewhere in deep water, both testis weight and spermatogenesis were minimal at this time. Why testis development is lowest at the time when females presumably deposit eggs is discussed in relation to the question of the location of the spawning ground.

INTRODUCTION

Previously Kobayashi *et al*. (1972) and Ichikawa *et al*. (2000) have reported that there is a seasonal migration of the hagfish, *Eptatretus burgeri*, in the vicinity of the Misaki Marine Biological Station of the University of Tokyo, in Sagami Bay off the Pacific coast of Japan. Further, our previous studies showed for the first time that *E. burgeri* migrates to water of more than 100 m depth presumably for egg deposition (Kobayashi *et al*., 1972; Ichikawa *et al*., 2000), although the spawning ground has not yet been located. Seasonal migration is an unusual phenomenon seemingly limited to *E. burgeri*, because until now it has been concluded that hagfishes usually live in deep water with no apparent limited spawning time (Conel, 1931; Walvig, 1963; Gorbman and Dickhoff, 1978; Gorbman, 1983). This special situation of *E. burgeri* has prompted further studies of this animal at the Misaki Marine Biological Station and at another locality. Thus, Fernholm (1974) confirmed seasonal migration by direct underwater observations. Patzner (1977, 1978) examined annual histological changes in the gonads. Tsuneki *et al*. (1983) observed seasonal migration and gonadal changes in this species in another locality near the Oki islands in the Japan Sea. From these studies, annual changes in the ovary became clear (Fernholm, 1975; Patzner, 1978; Tsuneki *et al*., 1983). However, annual changes in the testis are relatively unknown, since previous studies were based on a small numbers of individuals (Patzner, 1977; Tsuneki *et al*., 1983). Moreover, although subpopulation of the hagfish is also found in water of greater than 50 m depth throughout the year (Ichikawa *et al*., 2000), anuual changes in gonads of those living in deep water have not yet been studied. Here, we report annual changes in gonadal development of the hagfish, *E. burgeri*, living in both shallow and deep waters using a larger number of individuals.

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Dedicaed to the memory of late Dr. Tomoyuki Ichikawa.

MATERIALS AND METHODS

Collection of the hagfish

The hagfish, *Eptatretus burgeri* Girard, were caught in baited traps in water of 6 to 10 m depths (shallow water) in Koajiro Bay near the Misaki Marine Biological Station of the University of Tokyo, and in water of 50 and 100 m depths (deep water) in Sagami Bay, 1,600 and 4,800 m west from the Station, respectively. See previous papers (Kobayashi *et al.*, 1972; Ichikawa *et al.*, 2000), for the exact localities of these collection sites and collection methods. Collections were performed during periods from October 1971 to March 1973 and from November 1977 to December 1978.

Observations of gonads

Hagfish were anesthetized with ethyl m-aminobenzoate methane-sulfonate (MS222) and their total lengths were measured. They were dissected and the gonads were examined. In females, the length of the long axis of the largest egg was measured; in males, the body and testicular weights were measured only in those caught during November 1977 to December 1978, and the gonadosomatic index (GSI = testicular weight / body weight $\times 10^5$) was calculated. The presence of post-ovulatory follicular capsules was also noted in females.

For histological study of the testis, several males of medium size (40 to 50 cm in total length) were randomly chosen in each month (see Fig. 8 for numbers of specimens examined). The testes of the selected animals were fixed in Bouin's solution for more than 24 hr, and were dehydrated through a series of increasing concentrations of ethanol. Tissues were embedded in Paraplast, and serial sections of 7 μm were mounted on glass slides.

The testis of the hagfish consists of follicles filled with spermatogenetic cells. Percentages of follicles containing spermatogonia, spermatocytes, spermatids, or maturing sperm were calculated in a few randomly chosen sections and their averages were determined. Follicles containing both spermatogonia and spermatocytes were included with follicles containing only spermatocytes. Follicles containing both spermatocytes and spermatids were included with follicles having only spermatids. Any follicles that contained spermatozoa were treated as follicles containing spermatozoa irrespective of the stages of maturity of remaining cells in the follicle.

Statistics

Kolmogorov - Smilnov two-sample tests were used to compare the frequency distribution histograms of the body length between the hagfish caught in Koajiro Bay and in water of 50 m depth, and the hagfish with or without developing eggs. χ^2-test was used for analyses of the ratio of number of the hagfish bearing developing eggs to that without them, the ratio of numbers of hagfish bearing mature testes to those not mature, and to sex ratio. Student's t-test was used to compare the body lengths between males and females.

RESULTS

1. Ovarian development

Eggs were visible to the naked eye in the hagfish larger than 20 cm in total length. During January through September, sexually mature females collected in any locations contained 20 to 40 (29.3±1.4; mean±SEM, n=24) large developing eggs as well as many small pre-vitellogenic eggs (oocytes) less than 2 mm in diameter. Pre-vitellogenic eggs were round, whereas more advanced eggs were ovoid. The sizes of developing eggs were uniform in individual animals.

(1) Relation between total length and development of eggs (Fig. 1)

To examine the relationship between the stage of ovarian development and total length, fish caught in water of 6 to 10 m depths and in water of 50 m depth during January through April were analyzed. The mean total lengths of the females collected in water of 6 to 10 m depths and water of 50 m depth during that period were 40.7±0.39 cm (±SEM, n=317) and 34.5±0.28 cm (±SEM, n=556), respectively, and thus hagfish in shallow water were significantly larger than those in deep water. In both shallow and deep waters, most females larger than 39 cm in total length contained developing eggs more than 5 min long. Accordingly, in shallow water 64.4% of females contained developing eggs (≥5 mm in length), whereas only 25.4% of females were those with developing eggs in deep water.

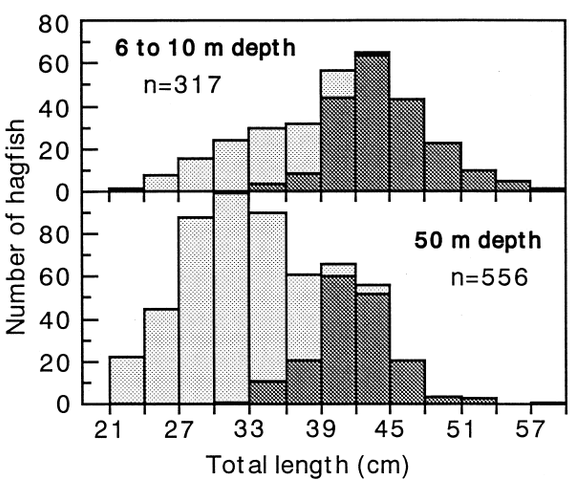

Fig. 1. Frequency distribution histograms of the total length of the female hagfish with (darker columns) and without (lighter columns) developing eggs caught in shallow water of 6 to 10 m depths (upper panel) and water of 50 m depth (lower panel). Fishes caught during months from January to April, 1971–1973, were combined. Eggs more than 5 mm are considered to be "developing". Total body lengths are expressed in 3 cm intervals.

(2) Monthly distribution of egg size (long axis) in the females caught in water of 6 to 10 m depths (Fig. 2)

In October, small numbers of hagfish (1 male and 18 females) were caught in shallow water. All females contained pre-vitellogenic eggs less than 1 mm in diameter, in addition to the post-ovulated follicular capsules (see below). Females caught in November contained developing eggs less than 7 mm in length. Thereafter, the size of the developing eggs increased linearly until the disappearance of the hagfish from shallow water around June. A clear bimodal distribution was noted in the size of eggs after January, indicating that there were two groups of the females, one with developing eggs and other with pre- or early vitellogenic eggs, in the shallow water. The appearance of females, which contained small eggs after January, possibly can be explained by the migration of

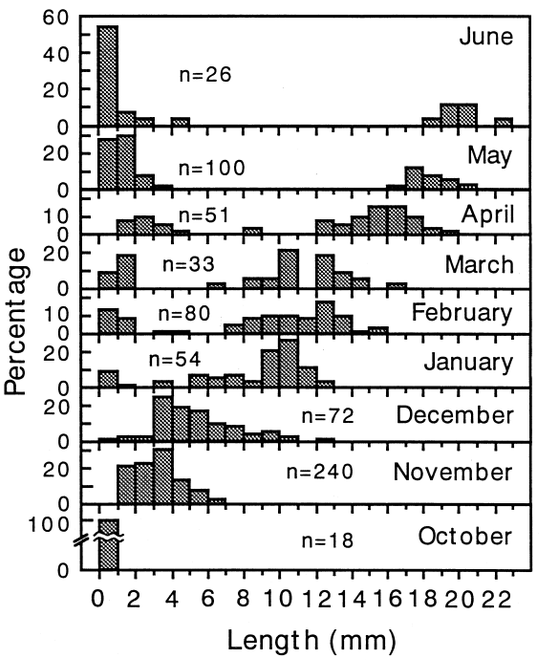

Fig. 2. Distribution of egg size (long axis) in each month in the female hagfish caught in shallow water of 6 to 10 m depths. Fishes caught in the same months during November 1971 to June 1972 and October 1972 to March 1973 were combined. Egg lengths (long axis) are expressed in 1 mm intervals.

smaller hagfish with pre-vitellogenic eggs from deep water into shallow water. An abrupt increase in females with pre-vitellogenic eggs less than 2 mm after May may be attributed to two possibilities: out migration of larger females with developing eggs from shallow to deep water and of the shoreward migration of smaller females with pre-vitellogenic eggs from deep to shallow water.

(3) Monthly distribution of egg size (long axis) in the females caught in water of 50 m depth (Fig. 3)

A bimodal distribution was noted in the size of eggs during the year, although the bimodality was less clear during the October to December period. After January, females were clearly divided into two groups, one with developing eggs more than 5 mm in length, and other with small eggs less than 5 mm in length. The size of developing larger eggs increased linearly until September, when it reached the maximum. Although females with maturing larger eggs were relatively abundant until August, they were few in number in September and October.

(4) Annual changes in the size (long axis) of developing eggs (Fig. 4)

There was no difference in the annual growth curves of developing larger eggs between water of 6 to 10 m and water of 50 m depths. A very similar growth curve was obtained in the hagfish collected in water of 100 m depth. In both shallow water and water of 50 m depth, the size of the developing

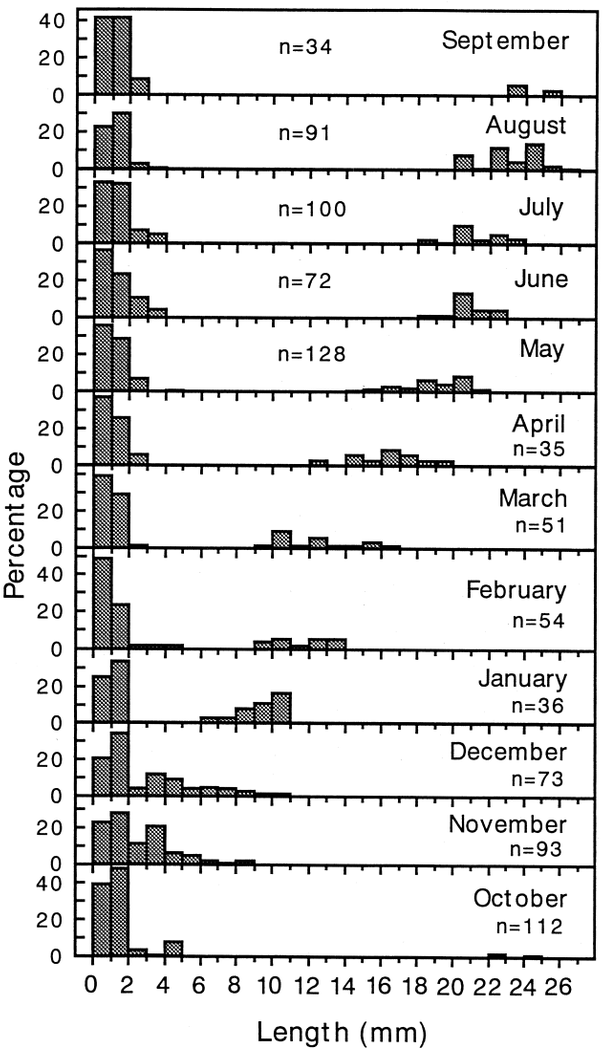

Fig. 3. Distribution of egg size (long axis) in each month in the female hagfish caught in water of 50 m depth. Fishes caught in the same months during November 1971 to March 1973 were combined. Egg lengths are expressed in 1 mm intervals.

eggs was the smallest in October, and thereafter it increased linearly until the disappearance of the hagfish from shallow water around June or until September in water of 50 m depth.

(5) Post-ovulatory follicular capsules

Post-ovulatory follicular capsules were found in the hagfish caught in both shallow water and water of 50 m depth in October through December, but they became less recognizable after January due to tissue involution. In shallow water, the percentages of females which contained post-ovulatory follicular capsules were 100% (n=18/18) in October, 83.5% (n=203/243) in November, and 68.5% (n=50/73) in December, respectively. On the other hand, in water of 50 m deep, they were 9.5% (n=6/63) in October, 39.8% (n=37/93) in November, and 20.2% (n=18/89) in December, respectively.

The high percentages of females which contained post-ovulatory follicular capsules in shallow water can be attrib-

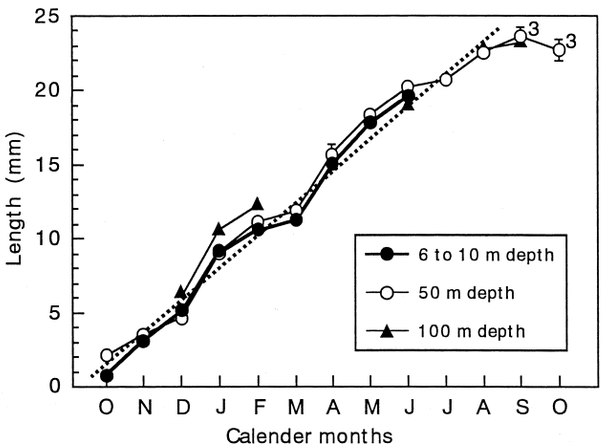

Fig. 4. Annual growth curve of developing eggs of the female hagfish caught in three different locations: water of 6 to 10 m depths (closed circles), water of 50 m depth (open circles), and water of 100 m depth (triangles). Fishes caught during November 1971 to March 1973 were studied. Numbers of animals studied were mostly 20 to 40 otherwise indicated in the figure. In most cases, ranges of the standard errors are within the size of the mean data points. Broken line indicates a regression line, which is made by the mean data points of all three locations.

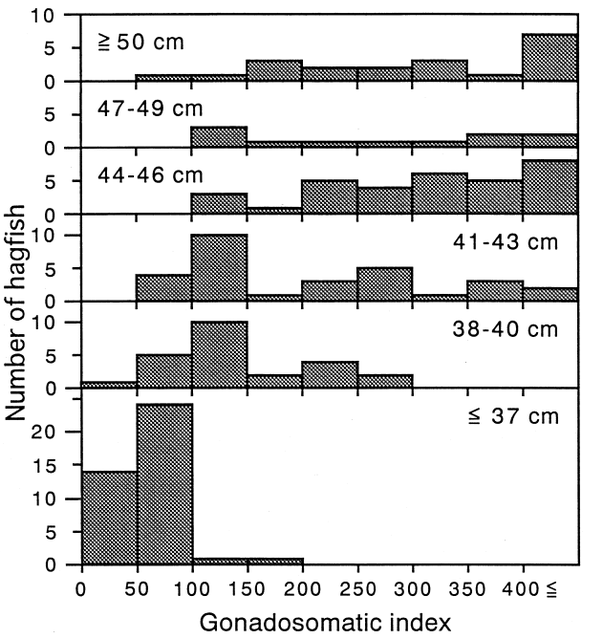

Fig. 5. Frequency distribution histograms of gonadosomatic index (GSI = testicular weight / body weight $\times 10^5$). Male fish caught in both water of 6 to 10 m depths and water of 50 m depth during May through July 1978, were studied. Numbers with centimeter (cm) in each panel represent the ranges of total length of the hagfish. GSI are expressed in 50 intervals.

uted to the possibility that post-spawning larger hagfish (≥39 cm) first migrate to shallow water, whereas the low percentages in water of 50 m depth are apparently due to the fact that more than half of the females are smaller ones with immature ovaries.

2. Testicular development

Because of the limited number of the males and no apparent difference in the testicular development between shallow water and water of 50 m depth, data of the hagfish caught in both locations were combined.

(1) Relation between total length and development of testis (Fig. 5)

Since testes were most active from May through July in terms of both testicular weight and spermatogenesis (see below), data collected only during those months were further analyzed. Gonadosomatic index (GSI) was less than 200 in all males smaller than 37 cm, whereas GSI greater than 200 was found in males larger than 38 cm. Thus, it is apparent that testicular development occurs in the hagfish larger than 38 cm in total length.

(2) Monthly distribution of the frequency of GSI (Fig. 6)

Next, monthly changes in GSI values were studied in the male hagfish larger than 38 cm. There was no significant difference in the mean total length of males during these months (mean±SEM; 44.4±1.0 cm). Males with a GSI greater than 200 were most abundant only during the period from March through July. A clear bimodality was noted only between May and August. Most adult males had inactive testes in terms of GSI during the months from August through February of the following year. Most males caught in August had immature testes; no maturing males with higher GSI were collected in September.

(3) Annual changes in GSI (Fig. 7)

Annual changes in GSI were also studied in the males larger than 38 cm collected in both shallow and deep waters. GSI was the lowest in September, followed by a gradual increase during autumn through early summer. It reached a peak in July, and then decreased to a nadir in September. A slight decrease in GSI was observed in February, but the reason for this was not clear.

(4) Annual changes in spermatogenesis (Figs. 8 and 9a–h)

Follicles containing spermatogonia predominated during September through February (Figs. 8 and 9a), whereas follicles containing spermatocytes were abundant during March through May (Figs. 8 and 9b). Follicles containing spermatids and maturing sperm were relatively abundant in June through August, and July and August, respectively (Figs. 8, 9c and 9d), but they were few in number in September (Fig. 8). Individual variations were small in autumn and winter, but were large in spring and summer. It is noteworthy that spermatids were few during the months from September thorough May, but some spermatozoa were found throughout the year, although the frequency was consistently low, except for July and August.

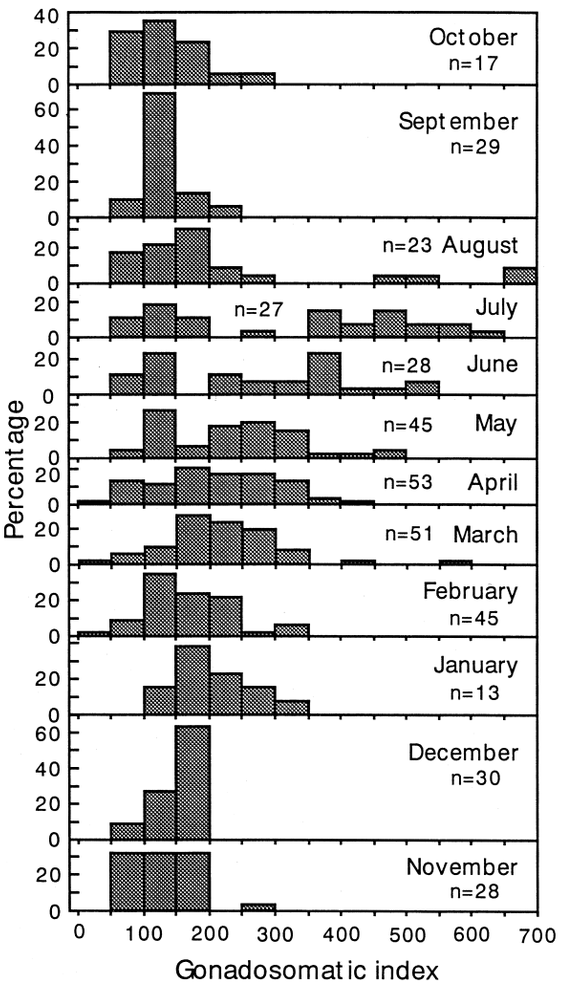

Fig. 6. Frequency distribution histograms of gonadosomatic index (GSI) in the male hagfish larger than 38 cm in total length. Fishes caught in both shallow water of 6 to 10 m depths and water of 50 m depth during November 1977 to December 1978, were studied. GSI are expressed in 50 intervals.

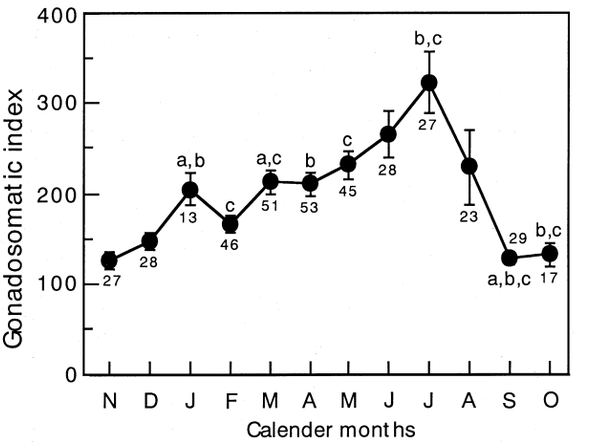

Fig. 7. Annual changes in gonadosomatic index (GSI) of the male hagfish. Only males larger than 38 cm in total length were considered. They were collected in shallow water of 6 to 10 m depths and/or water of 50 m depth during November 1977 to December 1978. Bars indicate standard errors. Numbers indicate number of individuals studied. a to c by the mean data points represent significant differences ($P < 0.05$) compared to the previous first, second and third months, respectively.

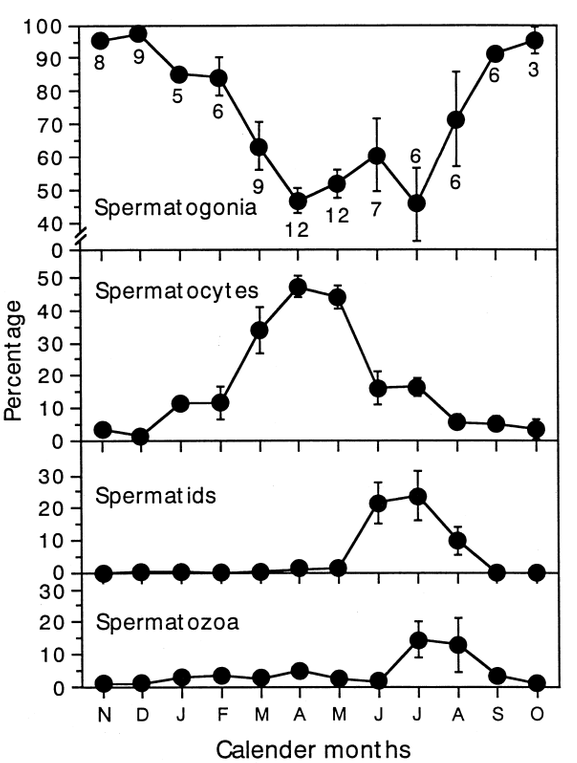

Fig. 8. Annual changes in percentage of follicles containing each developmental stage of spermatogenetic cells in the testis of hagfish. Solid circles and bars indicate the mean and standard errors, respectively. Numbers in the upper-most panel indicate number of individuals studied.

DISCUSSION

The present study has demonstrated that there is a clear seasonality in ovarian development in *E. burgeri*. Females with maximal development of eggs were found in September (October as well in some specimens) in water of 50 m depth, whereas those with post-ovulatory follicular capsules were found after October in both shallow water and water of 50 m depth. Thus, it is clear that egg deposition must occur in September and/or early October. These observations on ovarian development are in good accordance with those of Dean (see Conel, 1931) and Patzner (1978) at the same locality and Tsuneki *et al.* (1983) near the Oki Marine Biological Station of Shimane University in Oki Islands in the Japan Sea. Post-ovulatory follicular capsules have also been described in this species by Dean (see Conel, 1931) in October and November in shallow water adjacent to the Station in 1900, and by Tsuneki *et al.* (1983) in Oki populations.

Annual changes were also observed in testicular development, but a significant apparent discordance was found in

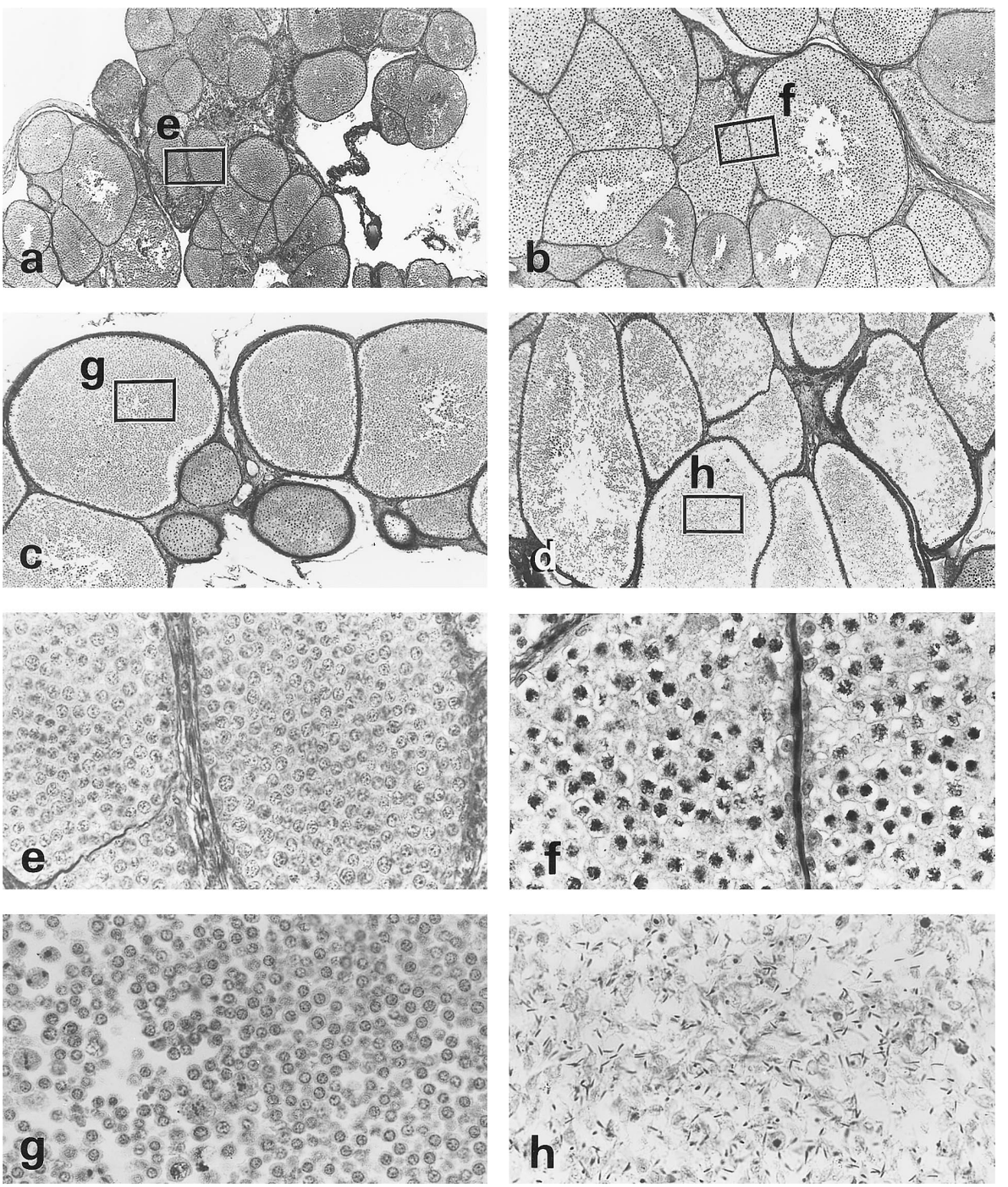

Fig. 9. a–d, Representative sections through the testicular follicles of the hagfish collected in November (a), April (b), June (c) and July (d). The areas outlined by rectangles in a-d are enlarged and shown in e–h, respectively. Note follicles full of spermatogonia in e, spermatocytes in f, spermatids in g, and spermatozoa in h, respectively. a–d, ×40; e–h, ×320.

regard to the time of maximum development of gonads between males and females. The testes were the most active in June and July in terms of both GSI and spermatogenesis, whereas they were the least active in September, at the time of presumable egg deposition. Patzner (1977) believed the peak of male GSI to be immediately before egg deposition apparently without actual observation. His figure (Fig. 6) may thus be misleading. Tsuneki *et al.* (1983) also reported annual changes in male GSI of *E. burgeri* near the Oki Islands in the Japan Sea. They did not find a clear seasonal change in GSI of males. Such discrepancies between our studies and theirs may be partly attributable to different methods

of sampling. In our study, males smaller than 38 cm were not considered, whereas in their study all available males were included.

In agreement with the present study, Dean (1904, also cited by Conel, 1931) reported that (1) *E. burgeri* was found in relatively shallow water near the Misaki Marine Biological Station of University of Tokyo, during the year except September and October, (2) in females, growth of eggs (in length) was completed by the middle of August, and (3) all males collected at the end of October were completely spent, whereas those collected during July showed clearly late stages in spermatogenesis, and ripe specimens were not uncommon. He also supposed that the period during which hagfish were not collected was the spawning season, but he was not aware of the possibililty of migration. More recently, Tsuneki *et al*. (1983) also reported the seasonal migration accompanying spawning of this species in the Japan Sea: hagfish were abundant in water of about 20 m depth of Kamo Bay in the Oki Islands during late October to July in the following year, but they were absent from Kamo Bay in August and September, during the spawning season. Although the hagfish were found outside Kamo Bay at a depth of about 50 m in August and early October, no mature males were collected there. Thus, *E. burgeri* living in both the Pacific and the Japan Sea coasts exhibit very similar features in seasonality accompanying reproduction.

It is puzzling why the testis is relatively immature at the time of egg deposition. A similar situation is also observed in females: females with fully grown eggs measuring more than 20 mm were easily collected in August, but were few in number in September. In a previous paper (Ichikawa *et al*., 2000), we reported attempts to locate the spawning ground, but all searches, which were carried out in water of 40 to 110 m depths by net sweeping, failed to collect either fertilized eggs or juveniles. The investigated areas included the collection sites of the hagfish used in the present study. Incidentally, hagfish smaller than 20 cm in total length were not collected in any collection sites at water of less than100 m depth (Ichikawa *et al*., 2000). Thus, we (Ichikawa *et al*., 2000) suggested that the spawning ground seems to be water of more than 100 m depth, and that mature males move to there from August while mature females move to there in September. Another possibility for the difficulty to collect peri-spawning hagfish in September is that hagfish with mature gonads do not eat during the spawning period, through they are present in the same place. Therefore, they are not attracted to baits. However, the latter possibility seems unlikely, since neither fertilized eggs nor juveniles were collected by net sweeping there (Ichikawa *et al*., 2000).

It is puzzling also why not all fully-grown males contain a maturing testis, although almost all fully-grown females had maturing ovaries. In our study only males more than 38 cm in total length were used for the analysis of the GSI, and only medium-sized adult males were further selected for the analysis of spermatogenesis. Indeed, there was no significant difference in total length of male specimens over a period of months. Thus, it seems that there might be two kinds of males, those with and those without maturing testes, of which total length are not different, in the same population of hagfish in water of 50 m depth. Clearly, further studies are needed to solve these questions. Comparative studies may also be relevant, because in the Atlantic hagfish, *Myxine glutinosa*, mature males are fewer than mature females (Walvig, 1963).

The present study has shown that the selection of maturing ovarian follicles occurs during December in females living in both shallow water and 50 m in depth, and the boundary between developing and atretic eggs was about 5 mm in length. Our report supports the finding by Gorbman and Dickhoff (1978) and Gorbman (1983) who also reported in *E. stouti* that eggs develop in sequence up to a length of 4.5 mm: at 4.5 mm length a selection of about 20–30 follicles occurs forming a clutch. They further reported in *E. stouti* that during growth of the larger selected follicles, others continue to reach the 4.5 mm threshold length, but these become atretic. The development of smaller eggs after January in maturing females is the object of a future study in *E. burgeri*.

Finally, the present study reports the clear seasonality in the development of both ovaries and testes in *E. burgeri*. Although several questions, such as the locus of the spawning ground, and the discordance of the timing of gonadal maturity between males and females, remain unresolved, the present study may provides basis for study of the enigmatic features in the hagfish reproduction.

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