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Maturation and Spawning Behavior of the Puffer, *Fugu niphobles*, Occurring on the Coast of Sado Island in the Sea of Japan (A Preliminary Report)

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Abstract The spawning behavior of the puffer *Fugu niphobles* was observed at Fukiagé Beach, Aikawa Town on Sado Island in the Sea of Japan during its reproductive season in 1978 and 1979. The spawning beds, consisting of gravel, pebbles and boulders, were somewhat influenced by underground water coming from the paddy-field located on a coastal terrace just behind this beach. The spawning season lasted for more than a month from early June to late July. The spawning occurred almost every evening during a period of fifty minutes before and after sunset. It is remarkable that at Fukiagé Beach spawnings show a weak and rather vague lunar periodicity. Males outnumber females in and near the spawning beds. It is estimated, on the basis of gonadal index and histological examination of both sexes, that the peak of maturation is in early June in the male and in late June in the female, although the highest frequency of spawning behavior was not seen until early July. Furthermore, false spawnings were noticed toward the end of the spawning season.

The puffer, *Fugu niphobles* (Jordan et Snyder), is a very common species of fish along the coasts of Japan. It is well-known that its spawning behavior is linked with moon phases. This behavior was first reported by Uno (1955) from Kominato Beach, Chiba Prefecture, on the Pacific coast. Following this discovery, nearly identical behavior was observed at several places in the vicinity of Hikari City and Hofu City in the Inland Sea (Katayama et al., 1964; Katayama and Fujita, 1967). Encouraged by these findings, the staff at the Misaki Marine Biological Station of the University of Tokyo and Keikyu Aburatsubo Marine Park Aquarium conducted an extensive investigation at several places along the coast of the Miura Peninsula (Nozaki et al., 1976; Kobayashi et al., 1978; Tsutsumi, 1978; Tsutsumi et al., 1978). This spawning behavior was observed again by Hayashi (1977) and Suzuka and Isogai (1979) on beaches of Yokosuka City also located on the Miura Peninsula.

Since there is a great difference in the water level and its amplitude between the Pacific coast and the Sea of Japan coast, it is logical to ask whether or not the spawning behavior of the puffer on the Japan Sea coast is also under the influence of the lunar periodicity, which influences light intensity as well as tidal flow. In order to answer this question, the present work was designed and carried out on the coast of Sado Island in the Sea of Japan. A concurrent histological examination of the gonads of both sexes was also attempted for understanding the rate of maturation.

Material and methods

In the past several years, we have tried to locate spawning sites of *Fugu niphobles* around the coast of Sado Island. However, only a single site near the Sado Marine Biological Station of Niigata University was found to be utilized for spawning (Fig. 1). Field observations were carried out from April, 1978 to September, 1979 at this site (Fukiagé Beach of Aikawa Town) and the period and duration of spawning, and number of individuals in each spawning group were recorded. The tidal heights and lunar phases were plotted every day acccording to the data published by the Aikawa Meteorological Station.

In order to determine the gono-somatic in-

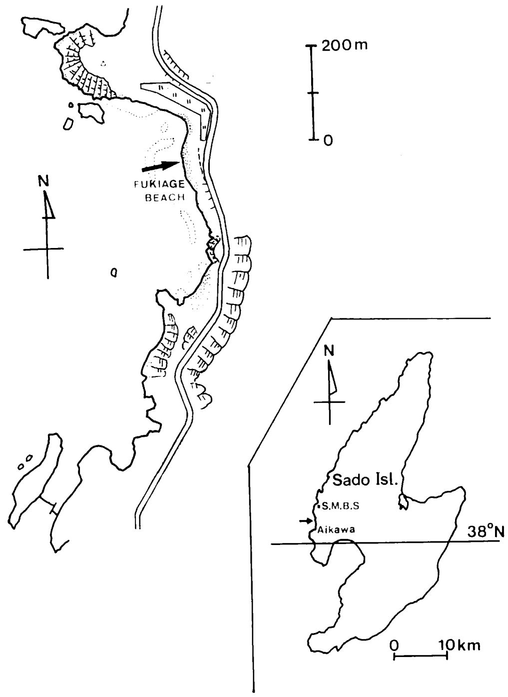


Fig. 1. Map of Fukiagé Beach, Aikawa Town, Sado Island, showing the spawning place (arrow) of the puffer, *Fugu niphobles*. Inset map indicates Sado Island in the Japan Sea to show the area concerned (arrow). S.M.B.S.: Sado Marine Biological Station, Niigata University.

Fig. 2. View of Fukiagé Beach, Aikawa Town, showing three spawning beds (A, B and C).
Fig. 3. Picture of puffers spawning on the evening of June 24, 1978, at Fukiagé Beach.

dices (GSI; (gonad weight/body weight) $\times 100$) and the rate of maturation of both sexes, a number of fish were collected from shoals of this species in and near the spawning bed, shelters, and feeding grounds for making macroscopical and histological observations. After taking the length and weight of the body, the gonads were removed and weighed. Then, several pieces of each gonad from more than 10 individuals in every week were fixed in Bouin's solution, dehydrated with alcohol or butanol series, embedded in paraplast (Sherwood), and then cut serially in 8 to 10 μm sections. These sections were stained with Delafield's hematoxylin-eosin double staining and Heidenhain's azan triple staining. The diameter of the egg was measured with an ocular micrometer under a light microscope.

Results

Spawning behavior. Fukiagé Beach is located on the northern corner of a small inlet, Aikawa Bay, extending about 1 km in a northern direction and consists mainly of gravel and pebbles mixed with boulders (Figs. 1, 2). Just in front of this beach, there is a large flat rock that protects the beach from heavy surf. Behind this beach is a constructed terrace 20 m in height. There is a paddy-field on the coastal terrace north of this beach. A part of the spawning bed is somewhat under the influence of underground water coming from this paddy-field. Three beds, A, B and C, were identified on this beach. Bed A, consisting of pebbles less than 10 cm in diameter, is the calmest place among the three. Bed B, consisting of rocks, and bed C, consisting of boulders 20 to 30 cm in diameter, are less protected from the surf.

At dusk, the mature puffers come to the

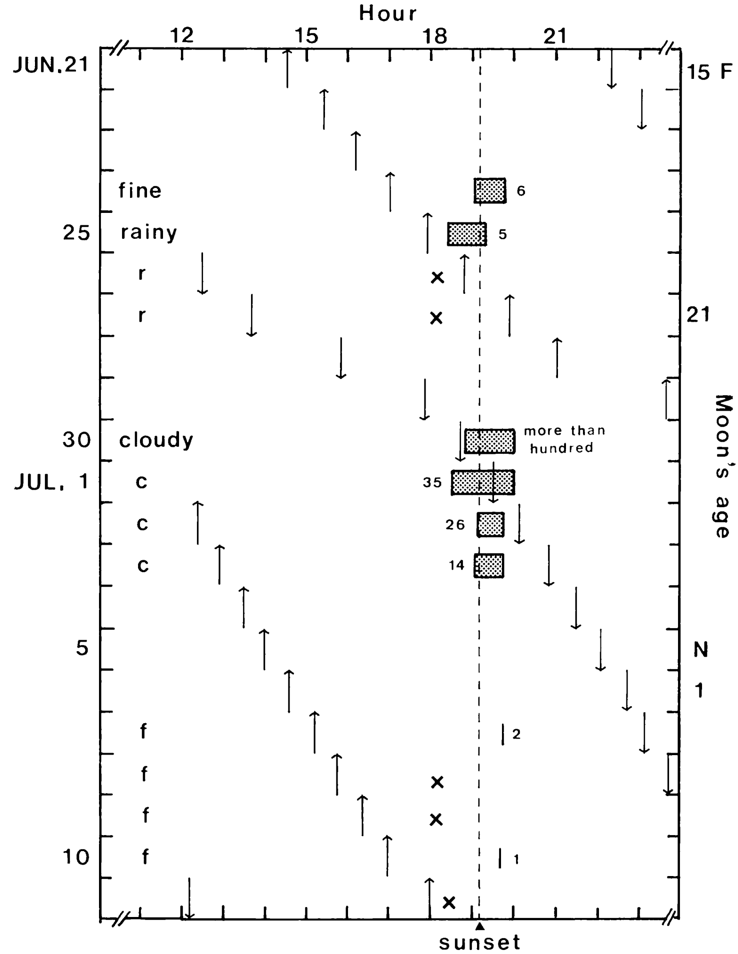


Fig. 4. Observation of puffers' spawnings at Fukiagé Beach in 1978, with weather, time of full and low tide, duration and frequency (number) of spawnings, and moon phase. ×, no spawning; ▩, duration of spawning; F, full moon; N, new moon; ↑, time of full tide; ↓, time of low tide.

beach a few at a time and then gather to form a group consisting of 10 to 30 individuals. While swimming together in a group, several males would actively pursue a female, nuzzling and biting. After sunset, courtship behavior was intensified and suddenly the puffers swam rapidly out of the water and onto the beach, where they vigorously flopped and trembled their bodies (Fig. 3). Eggs and sperm were released at this moment and fertilization took place. The fertilized eggs were left on the surface of pebbles or rocks. Ejection of eggs and sperm was noticeable because the water of outgoing waves turned milky. Most of the eggs, 0.8 to 1.0 mm in diameter, were washed away gradually by the waves, and the number of eggs left on the spot was very small. Therefore, the eggs on the beach that were exposed to the air during the low tide were hardly recognizable.

The number of fish that gathered at Fukiagé Beach to spawn was estimated at less than a thousand per evening. Small schools consisting of merely a hundred individuals were occasionally seen (Figs. 4, 5).

In and near the spawning bed, the sex ratio was highly skewed toward males. Early in July, 1978, no female fish was detected in the 55 individuals caught with a dip net. Nearly identical results were again obtained in the

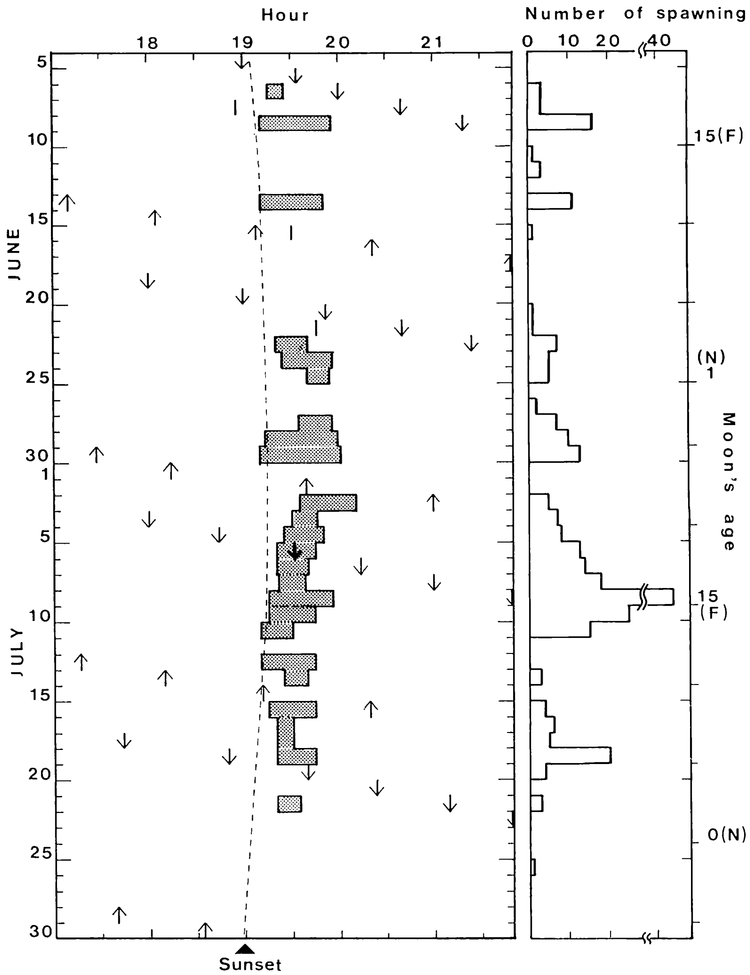
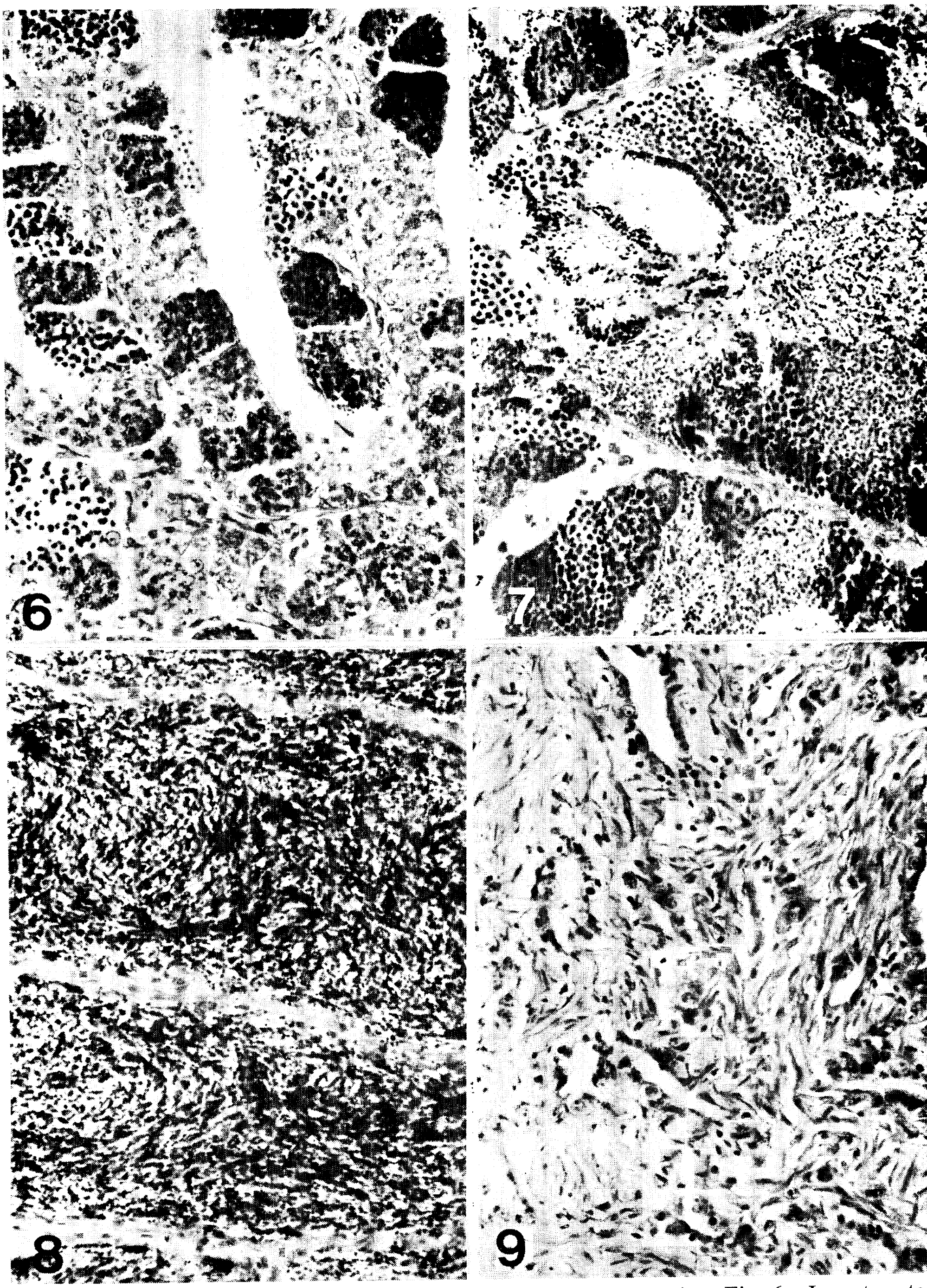


Fig. 5. Observation of puffers' spawnings at Fukiagé Beach in 1979, with the time of high and low tide, duration and frequency (number) of spawnings, and moon phase. Legend as in Fig. 4.

early phase of spawning season in 1979, in which only one female was caught in every spawning group.

Frequently, toward the end of the spawning season, neither deposited eggs nor milky turbidity were seen, even following courtship, indicating false spawnings with no gametes. These false spawnings perhaps resulted from the lack of ripe females in the spawning group. Accordingly, it is speculated that, in general, one spawning group may consist of only one ripe female and a number of males.

Observations of the puffer's spawning commenced on June 24, 1978 and June 7, 1979. The periods of spawning observed were from June 24 to July 10 in 1978, and June 7 to July 26 in 1979. As evident from the figures (Figs. 4, 5), there is a rather weak correlation between the spawning day and tidal change or moon phase. During the spawning season, spawnings took place every evening except on billowy and/or stormy days. The commencement of spawning behavior also seems to have no relation with the hours of high tide. Spawning usually took place shortly before or after sunset, i.e., between 19 : 00 and 20 : 00 hours. By a combination of meteorological data, it is seen that there is no appreciable relationship between the hours of onset of spawning and the weather of that day. Spawning was not seen if algae and discarded plastic bags had drifted to the beach.



Figs. 6~9. Section of the testis of the puffer. Hematoxylin-eosin stain. Fig. 6. Immature testis of fish caught on April 10, 1978, showing the tubules containing the spermatogonia, spermatocytes and spermatids. ×400. Fig. 7. Maturing testis of fish caught on June 5, 1978, showing the active spermatogenesis and free spermatozoa in the lumen of the tubule. ×400. Fig. 8. Ripe testis of fish caught on July 10, 1978, showing a marked dilation of tubule containing a lot of sperm. ×400. Fig. 9. Spent testis of fish caught on August 13, 1978, showing a large amount of proliferative fibrous connective tissues. ×400.

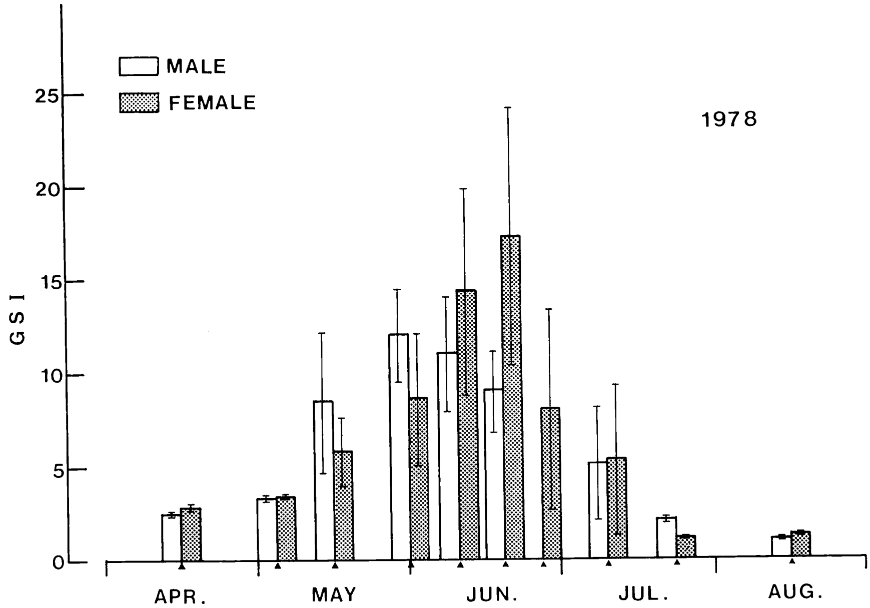


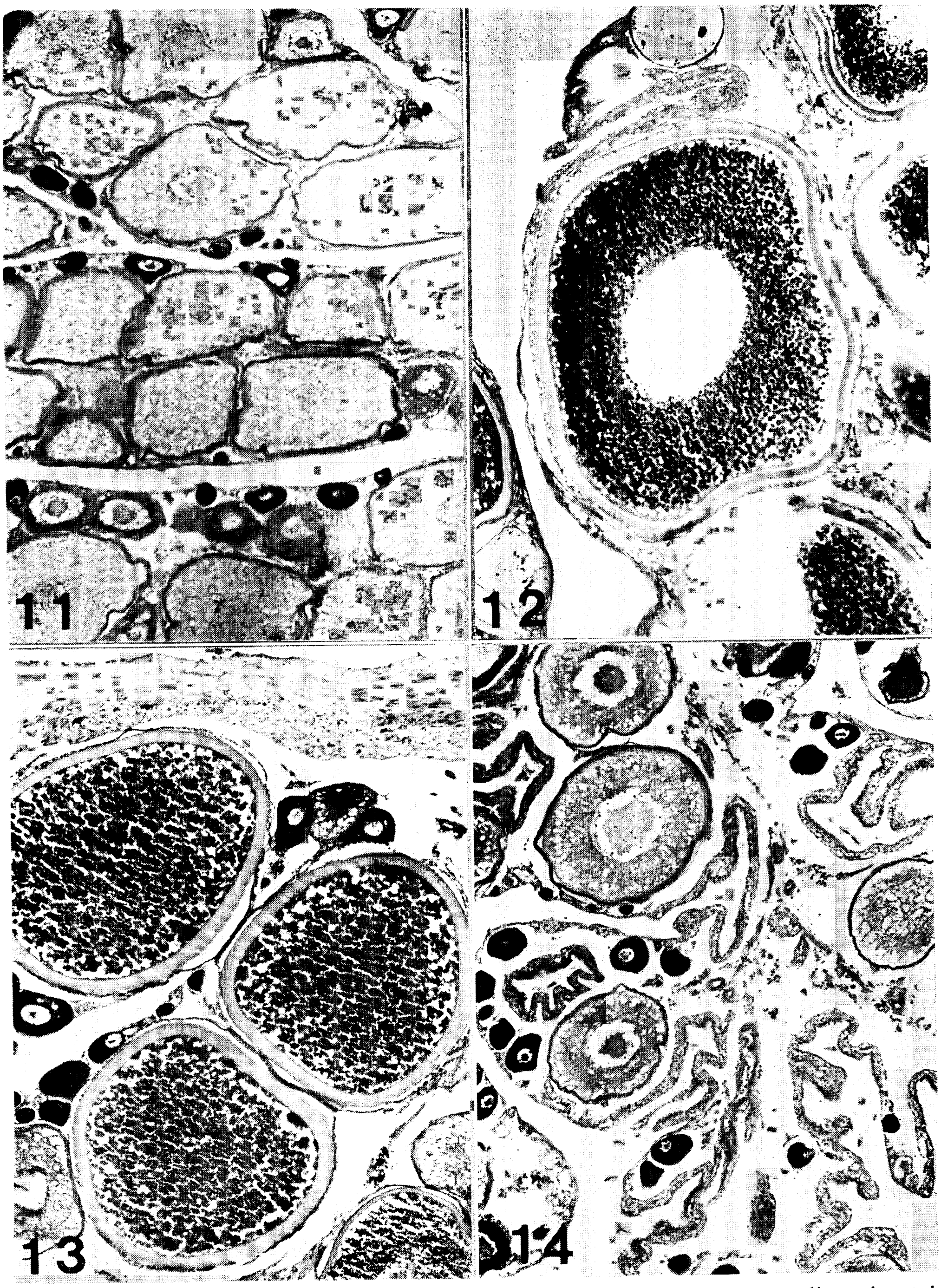
Fig. 10. Histogram showing the changes in the gono-somatic indices (GSI) of testis and ovary of the puffer.

During the spawning season observations were also conducted at daybreak for detection of morning spawning. However, no such spawning was encountered.

Frequency of spawning varied considerably from day to day, occurring from one to a hundred times. Therefore, it seems that there is little rhythmicity or periodicity in the frequency of spawning and intensified spawning behavior. However, according to our collected field data, the peak of spawning activity tends to occur between late June and early July. Observed spawnings show a weak or rather vague correlation with the lunar cycle, and at Sado Island they synchronize with the hour of sunset (Figs. 4, 5).

Maturity. **Testis:** Males collected from April to the middle of May had immature testes, with their seminiferous tubules containing a great deal of spermatogonia and spermatocytes but only a few spermatids (Fig. 6). The spermatozoa were yet to be produced. The mean GSI in this period was 2.5 ± 1.4 (Fig. 10). In the seminiferous tubule of fish collected in the middle of May, spermatids were actively undergoing spermiogenesis and, furthermore, some spermatozoa were seen free in the lumen of the tubule (Fig. 7). The mean GSI of this period increased to 8.5 ± 3.7 (Fig. 10). From late May to early July, almost all the males examined were capable of emitting sperm. Macroscopically, the sperm duct of the ripe testis appeared whitish. Histologically, a large number of sperm were seen in the lumen of the markedly dilated seminiferous tubule, while spermatogenesis was still going on in the cysts (Fig. 8). The mean GSI reached the highest ratio at 12.0 ± 2.5 in this period from late May to early June (Fig. 10). This index declined gradually to 5.1 ± 3.0 in early July (Fig. 10). According to the observed spawning behavior and histological preparation, it seems that one mature male is capable of emitting sperm several times in a spawning season. Toward July, the testis became reduced in size, even if ejection was still possible on the occasion of mating.

By the end of July, further reduction of testis size brought the GSI down to 2.4 ± 1.1 (Fig. 10). The testis became grayish-white with the deposition of pigments, and neither spermatocytes nor spermatids were recognized anywhere, although a small number of relict sperm was seen in the lumen of the tubule. By the middle of August ($GSI=1.0\pm0.6$; Fig. 10), almost no testes examined contained



Figs. 11～14. Section of the ovary of the puffer. Figs. 11, 13 and 14, hematoxylin-eosin stain; Fig. 12, sudan black B stain. Fig. 11. Immature ovary of fish caught on April 10, 1978, showing the oocytes in early yolk globule stage. ×100. Fig. 12. Maturing oocytes of fish caught on June 5, 1978, showing an enlargement of the zona radiata. ×160. Fig. 13. Mature oocytes of fish caught on June 5, 1978, showing a heavy accumulation of yolk. ×90. Fig. 14. Spent ovary of fish caught on July 10, 1978, showing the ovulation scars, corpus luteum body and abortive eggs. ×90.

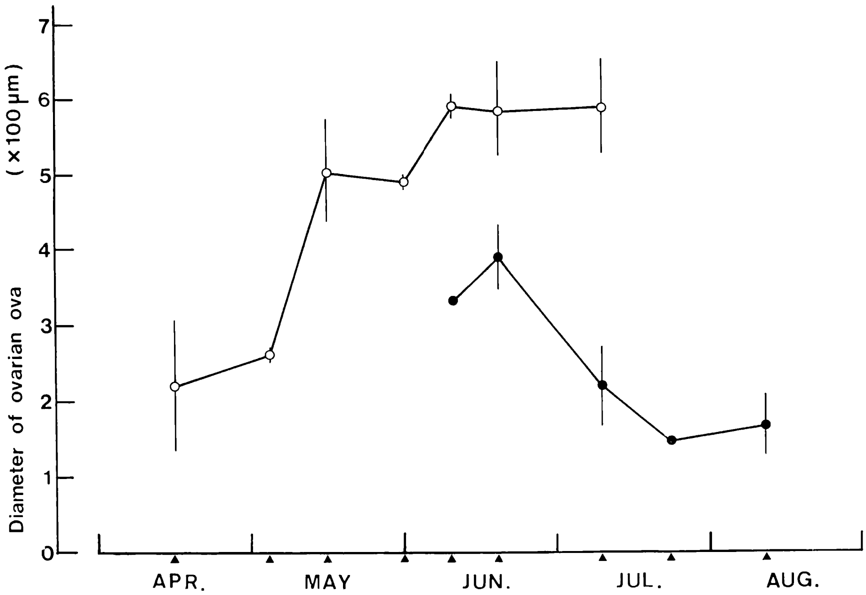


Fig. 15. Changes in the diameter of ovarian ova. ○, maturing fish. ●, spent fish.

spermatogenetic cells or sperm. Most of the testes, instead, were occupied by a massive fibrous connective tissue accompanying a heavy regression of the tubules (Fig. 9).

Ovary: Development and maturation of the ovary progressed at a slower pace when compared with those of the testis. In the middle of April the ovary was still in an immature state with its mean GSI at 2.8±0.9 (Fig. 10). The oocytes had reached the yolk vesicle to become the early yolk globule stage (Fig. 11). Their diameter was 200 to 250 μm (Fig. 15). Among these developing oocytes laden with yolk substance, a considerable number of younger oocytes in the perinucleolus stage were seen. These younger oocytes retained their developmental state throughout ovarian maturation. Increase in the volume of the ovary proceeded gradually, attaining its mean GSI only at 5.8±1.8 in the middle of May (Fig. 10). Almost all oocytes containing a great deal of yolk spherules reached the yolk globule stage, and measured 450 to 500 μm in diameter (Fig. 15). Enlargement of the zona radiata was remarkable in this stage (Fig. 12). From late May to early June, a rapid progress in maturation occurred and GSI increased to 8.6±3.6 (Fig. 10). Toward the middle of June, expansion in the abdomen of the female fish was noticeable, and the peak of GSI, 13.9±7.0, was reached (Fig. 10). Accumulation of yolk in oocytes proceeded further (Fig. 13), and the ripe eggs measured 500 to 650 μm in diameter (Fig. 15).

Spent fish with GSI at 8.4±0.2 could be collected toward the middle of June (Fig. 10). The ovary of these fish was reduced in volume, had become soft and appeared pinkish in color. A great deal of ovarian folds containing ovulation scars were found with corpora lutea-like bodies in early stages (Fig. 14). In addition, immature oocytes in the yolk globule stage, ca. 300 μm in diameter, were encountered occasionally.

About 80% of the 30 females examined, which were caught from early July to the middle of July, were spent, and showed a remarkable decrease in their GSI (5.3±4.0) (Fig. 10). As regression of the ovary proceeded further, the following changes were noticed: reduction in the volume of ovulation scars, ingestion of atretic follicles by phagocytosis of the granulosa cells, hyperplasia of the ovarian lamellae, and pigment deposition scattered in the lamellae. Females caught after late July were all spent, exhibiting rapid degeneration and atrophy of the ovary with GSI being 1.1±0.1 (Fig. 10). After spawning, all abortive eggs were regressed and absorbed. Accordingly, it is very likely that a female can spawn only once in each spawning season.

Discussion

Synchronization of the spawning cycle with the tidal cycle is a phenomenon well known in several marine and estuarine animals. This adaptive phenomenon precisely linked with lunar periodicity was fully documented in an atherinid fish, the California grunion, *Leuresthes tenuis* (Ayrs), observed on the coast of southern California (Clark, 1925; Walker, 1952, 1959). A precise lunar spawning cycle with a simultaneous ovarian maturation was also reported in a marine cyprinodontid, the mummichog, *Fundulus heteroclitus* (Linnaeus), in the creek marshes of Delaware Bay on the east coast of U.S.A. (Taylor et al., 1979; Taylor and diMichele, 1980). Similarly, a clear lunar cycle was briefly mentioned on the case of damselfish *Pomacentrus nagasakiensis* Tanaka from rocky reefs (Moyer, 1975). Although synchronization of spawning with the spring tide has also been reported for *Fugu niphobles* occurring in various places on the Pacific coast and the western part of the Inland Sea coast (Uno, 1955; Katayama et al., 1964; Katayama and Fujita, 1967; Nozaki et al., 1976; Hayashi, 1977; Kobayashi et al., 1978; Tsutsumi, 1978; Tsutsumi et al., 1978; Suzuka and Isogai, 1979), a slight difference in timing and delay in days of spawning were noted in every place observed.

Unlike on the Pacific Ocean and Inland Sea coasts where the tides fluctuate between 1.0 and 3.0 m, the tidal variation on the coast of the Sea of Japan is very low, only about 0.2 to 0.3 m in each lunar cycle. Therefore, the present study was undertaken with an assumption on the existence of a spawning behavior that is not so strongly linked to lunar periodicity. The results of our observations substantiated this assumption. As in the case of this puffer a similar adaptive feature was also observed in a species of land crab, *Sesarma haematocheir* (de Haan). On the Pacific coast, it is known that during the spawning season the egg-bearing crabs release the zoeal larvae on the beach at the full moon. However, on the coast of Sado Island the larval release occurs every night during the spawning season regardless of the moon's phase. The time of larval release extended from the sunset to midnight (Umegawa, Honma and Chiba, unpublished data). It seems, then, a diurnal rhythm, dependent probably on light intensity, should be considered for explanation of adaptation to a minor tidal fluctuation such as in the Sea of Japan. Nearly identical spawnings, i.e., crepuscular spawnings, have already been reported on the angel fish, *Centropyge interruptus* (Tanaka), and the ostraciid fishes, *Lactoria* spp. (Moyer and Nakazono, 1978; Moyer, 1979). However, more precise data of crepuscular spawnings are needed from the coast of the Sea of Japan.

The fact that the sex-ratio of the puffer in the spawning bed is considerably skewed toward males has already been noticed by several authors (Uno, 1955; Katayama, et al., 1964; Nozaki et al., 1976; Kobayashi et al., 1978; Tsutsumi et al., 1978; Suzuka and Isogai, 1979). The significance of skewed sex ratios was reviewed by Emlen and Oring (1977). However, a tentatively called "false spawning", occurring toward the end of each observed spawning season has not been documented elsewhere.

Kobayashi et al. (1978) assumed that the male released sperm every spawning act but the female spawned only once in each spawning season. Our histological observation of the gonad supports their assumption. However, significant differences in the diameter of ripe eggs were found between the materials from Misaki and those from Sado Island. The egg diameters given by Kobayashi et al. (1978) are less than half or one third of our measurements. Fertilized eggs of 0.90 to 0.95 mm were recorded by Uno (1955), Katayama and Fujita (1967), in addition to the present report.

Other spawning places on Sado Island and the Sea of Japan coast will be investigated in the near future.

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佐渡島（日本海）におけるクサフグの成熟と産卵習性

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1978 年および 1979 年の両年に，干満差がわずか 20～30 cm にすぎない佐渡島北西海岸の相川町吹上浜において，クサフグの産卵習性を観察した．産卵床は砂礫，小石ないし玉石からなり，後背地の海岸段丘ならびに田圃から流入する淡水の影響を受けている．産卵期は 6 月初旬より 7 月下旬にわたり，ほぼ毎夕日没頃より 50 分間に汀へ打ち上って放卵放精を行う．したがって，従来太平洋岸や瀬戸内海沿岸で報告されていたような，月齢ないし当日の潮位との関係は明瞭ではない．産卵場やその付近では雄魚の個体数が圧倒的に多く，産卵期の終りには，雄魚のみによる“偽産卵行動”が観察された．生殖腺指数の変化ならびに組織標本の検索により，雄魚は 6 月初旬に，雌魚は 6 月下旬に成熟のピークがみられる．しかし，産卵の盛期は 7 月初旬であることがわかった．

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