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Maturation Process of the Japanese Common Squid *Todarodes pacificus* in Captivity*

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Abstract: Adult individuals of the Japanese common squid *Todarodes pacificus* (males and females with dorsal mantle length ranging from 150–270 mm) were maintained in the tank of a raceway system using circulating seawater (12,000 liters total) at the Marine Biological Station of Hokkaido University for period ranging from 20 to 60 days in 1988, 1989 and 1990. Male squid matured earlier than females. Mating behavior of males with immature females was observed about two weeks after beginning of the breeding experiment. Male mating behavior continued to the end of the experiments. Females matured two or three weeks after male mating behavior was first observed and this phase was followed by spawning and death. Mature females spawned once and died in the 1988 and 1989 experiment. However, in 1990 one individual spawned twice in a week. Spawning occurred between midnight and early morning. Histological changes were found in the seminal receptacle and in the nidamental gland of spawning females. In the ovaries of spawning females oocytes existed at all stages of development, from yolkless to mature stages.

Introduction

Japanese common squid, *Todarodes pacificus* is one of the commercially most important species in Japanese fisheries. For this reason, many studies have focused on the fishery biology of this species (Soeda *et al.*, 1956; Araya *et al.*, 1958; Hamabe and Shimizu, 1966; Murata *et al.*, 1971, 1973). However, its reproductive features have not yet entirely been described since. *T. pacificus* shows some biological complexities in nature, related for example, to a long distance migration around the Japanese Islands and to the existence of three different populations (Araya, 1957; Kasahara, 1978). Therefore, field surveys of this species are sometimes difficult and egg masses of *T. pacificus* have not yet been found in nature. It is useful to maintain the animal in captivity to get information not obtained during field surveys. Many researchers have tried to culture squid in the laboratory, e.g., *Illex illecebrosus* (O'Dor *et al.*, 1977), *Sepia officinalis* (Richard, 1971; Boletzky, 1974), *Loligo bleekeri* (Matsumoto, 1976), *L. plei, L. pealei* and *Lolliguncula brevis* (Hanlon *et al.*, 1983) and *L. opalescens* (Yang *et al.*, 1986). Hamabe (1962) tried to keep the mature female *T. pacificus* in a barrel and he observed the females spawn. The egg mass consisted of ripe egges and a jelly coat. Since his rearing experiment lasted only a few days, information about the process of maturation was lacking. In addition, histological observations of the sexual organs have not been done. The entire maturation process of *T. pacificus* has not been yet been described. Generally, it is very difficult to rear *T. pacificus* for long periods since this species is nektonic and perhaps more nervous than coastal species such as *Sepia officinalis* or *Loligo bleekeri* in the restricted rearing environment. In the present study, we tried to get basic information about the reproduction of *T. pacificus,* especially for reproductive behaviors and histological changes of sexual organs during maturation. This was done using the method of chronic breeding experimentation; it was the first attempt to use this method for this species.

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Materials and Methods

Animal collection

Adult *T. pacificus* were obtained from local fishermen who caught the squid by set nets located in the coastal waters off southern Hokkaido in waters of about 50 m depth. Squid sampling was done two or three times in each season which ranged from July to October between 1988 and 1990. About fifty to eighty individuals were caught at each sampling. For live collection, squid were first caught by a vinyl landing net with water and transported to a circular acrylic tank aboard the tending vessel with a continuous strong flow of water for periods of about ten minutes during the 2 km return trip to port. Squid were then transferred by buckets with water to a portable tank stationed on the truck and supplied with air from a battery driven compressor during the 20 minutes ride needed to move them to the Marine Biological Station. Squid were transferred from the portable tank to the breeding tank in buckets of sea water to minimize stimuli in handling.

Maintenance system

The Marine Biological Station's raceway tank system uses circulating sea water (12,000 liters total). The tank is 5.5 m in length, 2.5 m in width and 1.3 m in depth. Three underwater windows, two of them approximately 1 m square and the third 0.5 m square are located around the perimeter of the tank to provide observation opportunities from any depth or angle. A stripe pattern was drawn with black paint on the inner tank wall to prevent the squid from shooting into the wall.

Rearing conditions

Sea water was pumped up from the coastal waters near the Marine Biological Station. Water flows

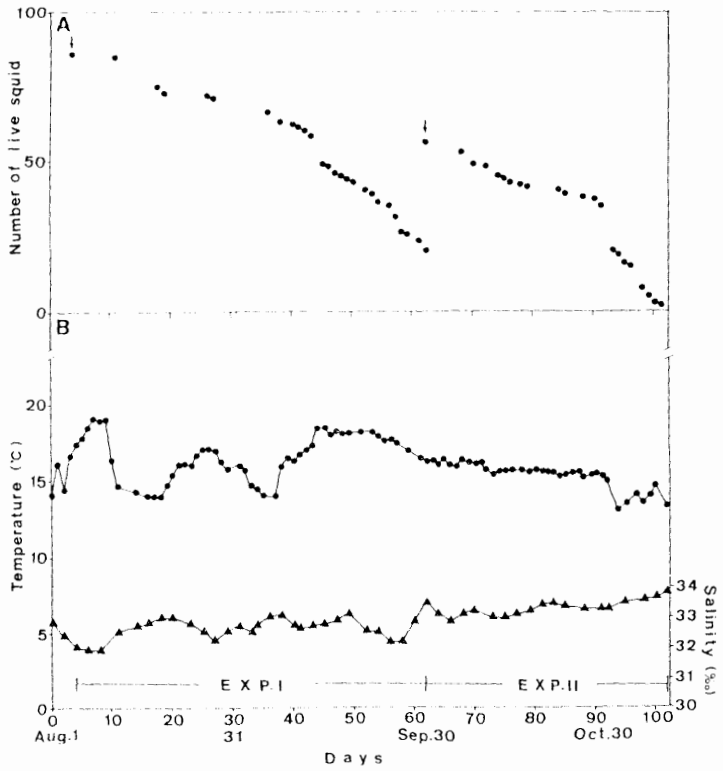

Fig. 1. Physical conditions and survival of squid during 1988 experiments. (A) Number of live squid during 1988 experiments. Arrows indicate the onset of experiment I and experiment II. (B) Water temperature (● ●) and salinity (▲ ▲) in the breeding tank.

into the tank through the bacterial sand filter at a rate of 60 liters/minute. Aeration is provided by air bubblers in the breeding tank and in the filter. Temperatures were kept between 15°C and 18°C by a thermoregulator during the entire experimental period. Salinity varied from 32‰ to 34‰, which reflected the salinity of waters around the Marine Biological Station (Fig. 1). Lighting consisted of natural light received through the window on one side of the tank and eight 130 watt White Lamps which were timer controlled. The on-off cycle of the lamps was made to coincide with the natural photoperiods (10~13L:11~14D) in each season.

Feeding

Live specimens of sardine (*Sardinops melanosticta*), anchovy (*Engraulis japonicus*) and fry walleye pollack (*Theragra chalcogramma*) were added when squid were first placed in the tank. After a few days, frozen specimens of these fish and sand lance (*Ammodytes personatus*) without head and backbone were given using a feeding bar made from a fishing rod with a bamboo tweezer at the tip for pinching the food specimens. When squid were accustomed to feeding on dead fish, about one week after the tranfer, the frozen fish were tossed directly into the tank generally in the morning.

Observation

Video camera (Victor VHS) recordings of squid behaviors in the tank were made every day, both through the underwater windows and from above the water surface.

Histological observation

Both fresh-dead and selected live squid from the tank were measured at various dimensions and sexual organs were fixed in Bouin's solution. The tissue was embeded in paraffin wax and sectioned at thicknesses 5 μm to 8 μm on a microtome. Sections were stained with Delafied' hematoxylin and counter stained with eosin. Each individual was assigned a maturity stage based on the histological observation of ovary or testis.

Results

Since the breeding experiment was carried out in 1988, 1989 and 1990 using the method mentioned above, the present paper mainly deals with the 1988 experiment which had the longest duration of all the sets of experiments done during these three years. Two sets of breeding experiments were carried out in 1988. Experiment I and experiment II lasted from August 5 to October 2 and from October 2 to November 12, respectively. The number of squid transferred to the tank at the onset of each experiment was 86 in experiment I and 56 in experiment II (Fig. 1). The dorsal mantle length of squid ranged from 150

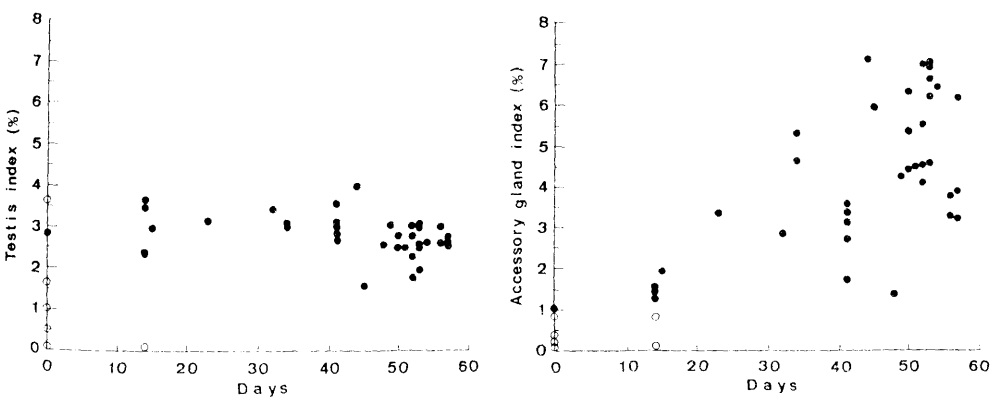

Fig. 2. The progress of male sexual maturation in the Japanese common squid, *Todarodes pacificus* held in captivity in 1988. Testis index (testis weight as a percentage of total body weight); Accessory gland index (accessory gland weight as a percentage of total body weight). ○: Immature to maturing male; ●: Mature male with spermatophores in the accessory gland.

mm to 270 mm. The results of these two experiments in 1988 which were almost identical are described below.

Maturation and spawning

At the outset of the breeding experiment most male squid were immature. They were in the spermatogonial proliferation stage. Their testes were filled with spermatogonial cells and both testis and accessory gland weight were low, 0.1% to 1.0% of body weight. However, a few squid had already become mature showing some spermatophores in the accessory gland (Fig. 2, Fig. 3A). Most males matured with many spermatophores in their accessory gland and they started to mate with immature females about two weeks after the beginning of the breeding experiment. Therefore, the onset of mating behavior coincided with production of spermatophores. The testes were filled with sperm and all stages of germ cells.

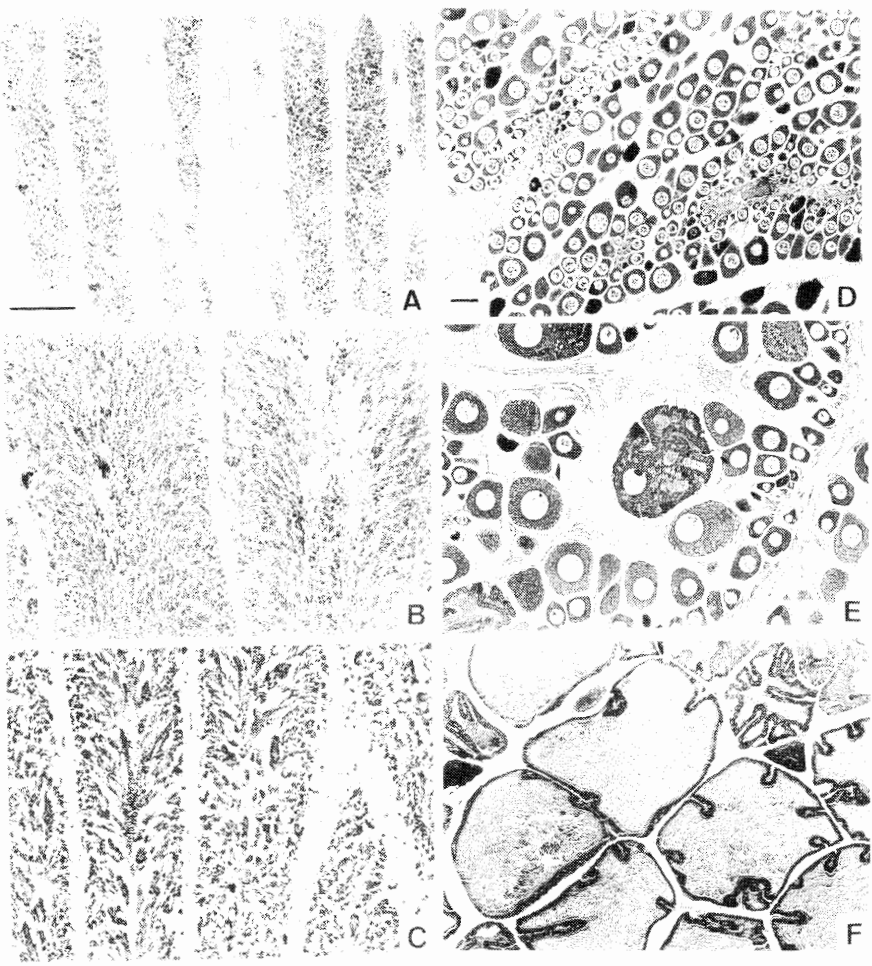

Fig. 3. Histological sections through testes and ovaries in various stages of maturation in the Japanese common squid, *Todarodes pacificus* held in captivity in 1988. Bouin-fixed and Delafield hematocylin-eosin stained. **A**. Testis in immature stage, the day at outset of the breeding experment; **B**. Testis in mature stage, two weeks after outset of the breeding experiment; **C**. Testis in regressive stage, thrity days after outset of the breeding experiment; **D**. Ovary in immature stage, the day at onset of breeding experiment; **E**. Ovary in yolk formation stage, three weeks after outset of breeding experiment; **F**. Ovary in mature stage, thirty days after outset of the breeding experiment. Bar indicates 100 μm for A to F.

Both testis and accessory gland weight increased, 3.5% to 11% of body weight (Fig. 2, Fig. 3B). Male mating behavior continued to the end of the experiment. Accessory gland weight high which signified continuous production of spermatophores in the accessory gland. However, histological regression of the testis was observed one month after the start of the experiment (Fig. 3C).

On the other hand, all females were immature at the beginning of the experiment. They were in the early yolkless stages of oogenesis. The ovaries were filled with early yolkless oocytes and both ovary and oviduct weights were low, 0.2% to 0.5% of body weight (Fig. 3D, Fig. 4). Females began to mature, *i.e.* beginning of yolk formation in the ovary, a few days after mating behavior was first observed. Ovulation to the oviduct occurred about two weeks after that and both the oviduct and nidamental gland developed very rapidly (Fig. 3E, F, Fig. 4). Ovulation was easily detected. Since the ripe eggs in the oviduct were clear amber-colored, we could observe these ripe eggs very clearly through the trans-

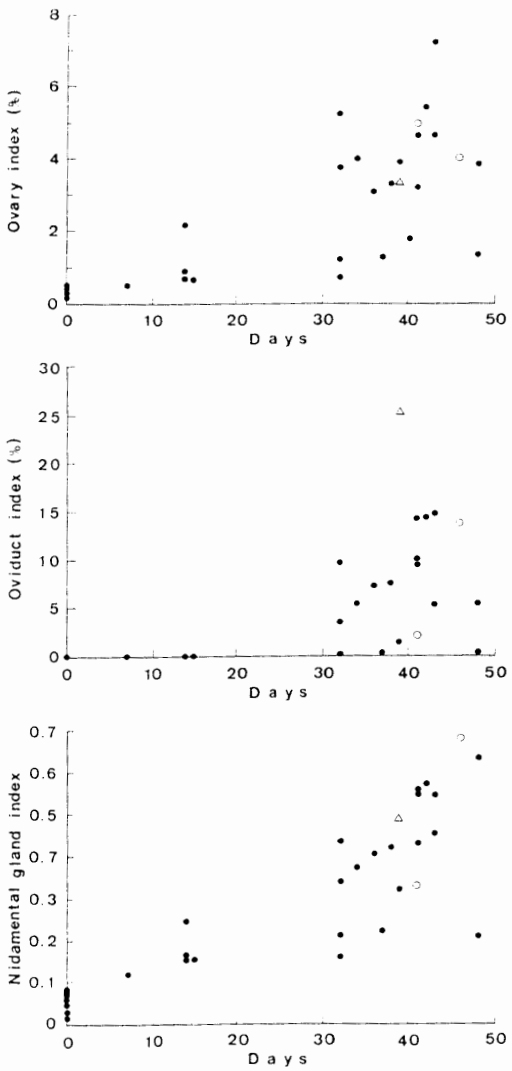

Fig. 4. The progress of female sexual maturation in the Japanese common squid, *Todarodes pacificus* held in captivity in 1988. Ovary index (ovary weight as a percentage of total body weight); Oviduct index (oviduct weight as a percentage of total body weight); Nidamental gland index (a ratio of nidamental gland length to mantle length). ●: Pre-spawning female; ○: Post-spawning female; △: Exhausted female just prior to spawning.

parent mantle. Spawning occurred a few days after ovulation. Spawning occurred soon after ovulation was first observed in experiment I (2 spawns) and experiment II (1 spawns). That occurred about one month after the outset of the experiment. Spawning behavior was not observed directly since every spawning occurred between midnight and early morning. Female squid spawned once and died soon after that in the 1988 and 1989 experiments. However, one individual spawned two times in one week, between September 5 and September 11 1990. The egg mass was approximately 1m in dimater but it was subsequently destroyed by the other swimming squid and live sardine, which were in the tank as food. Squid tended not to feed as their maturation progressed. Females preferred to stay near the bottom when the oviduct was filled with ripe eggs and they moved only when disturbed by males.

An unusual situation occurred in 1988 when two fully mature females died just prior to spawning, one in experiment I and the other in experiment II. Their mantles had become much thinner and these squid seemed to be exhausted. Total weight of ovary and oviduct was 28% of total body weight (Fig. 4). When nidamental gland weight was included in the gonadal weight of this exhausted female, the total percentage of gonadal weight was almost 50% of total body weight.

There were some histological changes in the seminal receptacle and the nidamental gland in post-spawning females. In pre-spawning individuals, the sperm mass was stored in the seminal receptacle on the outer lip of the buccal membrane. The inner walls of the seminal receptacle were observed around sperm mass (Fig. 5A). On the other hand, in post-spawning females there were a few sperm mass inside the seminal receptacle and the goblet cells of the inner walls elongated as if secretion emanated from these cells at the time of spawning (Fig. 5B). In the nidamental gland, a number of gladular cells were observed in pre-spawning females but in spawning female the mucous secretion occurred. This suggests that these glandular cells play the role of producing and releasing the jelly substance, the egg mass coat (Fig. 5C, D).

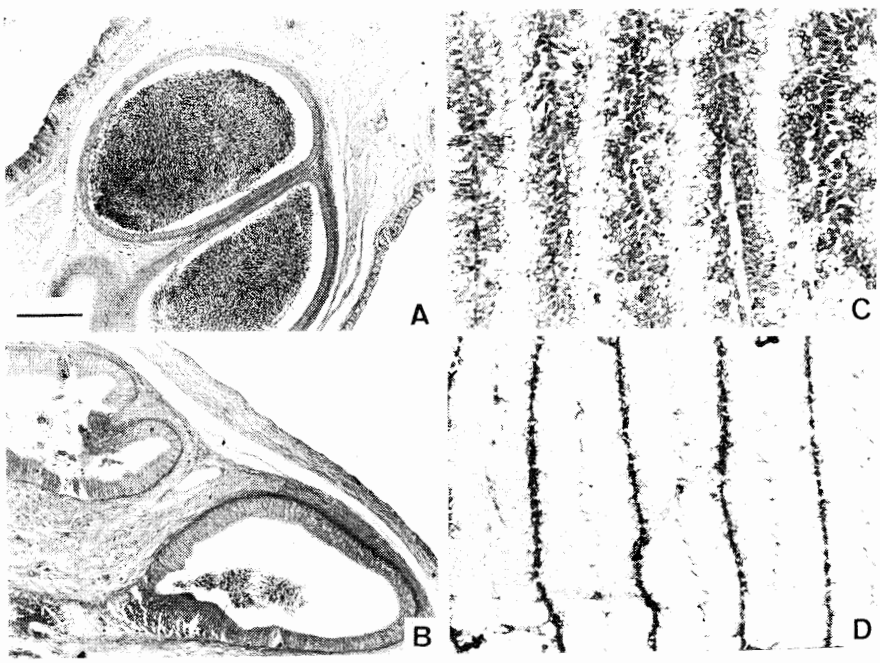

Fig. 5. Histological sections through seminal receptacles and nidamental glands of the Japanese common squid, *Todarodes pacificus* held in captivity in 1988. **A**: Seminal receptacle of pre-spawning female; **B**: Seminal receptacle of post-spawning female; **C**: Nidamental gland of pre-spawning female; **D**: Nidamental gland of post-spawning female. Bar indicates 200 μm for A to D.

Discussion

This is the first long term experiment about the maturation process of *T. pacificus*. Mortality was low during the experiment and nearly all squid remained in healthy condition in the tank. This suggests the tank is satisfactory for breeding experiments with this species.

According to Hamabe (1962) sexual maturity takes place in male three to six months earlier than females of this species. However in the present experiment, males reached sexual maturity only two to three weeks earlier than females. Because Hamabe used wild population samples which included some different populations, he might have overestimated the duration needed for sexual maturaiton in females. Sexual maturation should be a fast process in *T. pacificus*. But at this point, we must be careful in analyzing our findings, since the present results were obtained from squid maintained in the aquarium. In general, precocious maturaiton occurs in laboratory cultured squid (Durward *et al.*, 1980; Hanlon *et al.*, 1983; Yang *et al.*, 1986). Mangold *et al.* (1975) reviewed environmental factors which influence maturation in cephalopods and reported that light, temperature, and nutrition are the key stimuli. In the present experiment, these environmental factors were acting but may not have been able to mimic nature perfectly. Unfortunately, at present we do not know the major factor or combination of factors which affect sexual maturaiton in this species. During our experiments, the beginning of female sexual maturation, which means yolk formation in the ovary, occurred after male mating behavior was observed. In nature the onset of sexual maturation is reflected by an increase in the proportion of mated individuals in *T. pacificus* (Murata *et al.*, 1971). Mating behavior may be one of the triggers for sexual maturation of females. The life span of *T. pacificus* is believed to be one year or slightly longer (Soeda *et al.*, 1953; Yasui and Ishito, 1955; Araya, 1957). This species grows very fast and reaches sexual maturity at the age of one year and dies after spawning (Kawana, 1948; Katoh, 1959). From these characteristics our experiment was thought to have dealt with the terminal one to two months of the life span since almost all males and females were immature at the outset of the experiment. They then reached sexual maturity approximately in one month which was followed by spawning and death. The maturaiton process centers on the last few months of the life span. But we must keep our mind that there was a possibility of precocious maturation in these experiments. Precocious maturation of this species in captivity should be the future study. As Durward *et al.* (1980) reported from breeding experiments with *Illex illecebrosus*, a closely related form to *T. pacificus*, the processes of mating and spawning are probably similar to those of *T. pacificus*. However, there is a difference in time of mating between *I. illecebrosus* and *T. pacificus*. Male *I. illecebrosus* mates only with mature females, whereas male *T. pacificus* begins mating with immature females. Another difference between these two species is the site where spermatophores are attached during mating. In *T. pacificus* spermatophores are embedded in the buccal membrane of the female and transferred to the seminal receptacle on the outer lip whereas in *I. illecebrosus* spermatophores are embedded inside the female mantle where there is no spermreservoir like the seminal receptacle of *T. pacificus* (Hamabe *et al.*, 1974). *T. pacificus* is thought to have a higher ability in sperm reservation than *I. illecebrosus*. Mating with immature females may be needed not only for triggering female maturation but also for sperm capacitation in female seminal receptacle in *T. pacificus*. We therefore put forward the following hypothesis about sperm capacitation in *T. pacificus*. Sperm proper are already produced in the testis when the gonad is very small and sperm accumulate in the testis until they are transferred to the accessory gland to be packed in a spermatophore. Then, sperm are transferred to the female seminal receptacle and kept there for several weeks until spawning when fertilization may occur. From these processes, we consider sperm capacitation is achieved in two steps, first in the male accessory gland and second in the female seminal receptacle.

In the present study, we happened to obtain exhausted squid, their mantle became much thinner and the oviduct was filled with ripe eggs, thus suggesting the animals were close to spawning. This kind of exhausted squid were caught in nature from *T. pacificus* (Araya, 1957; Hambe, 1963) and from other species, e.g., *Loligo opalescens* (McGowan, 1954) and *L. vulgaris reynaudii* (Augustyn, 1990). In all cases, these exhausted squid seemed to be in post-spawning condition. We consider that this phenomenon is one of the reproductive strategies of *T. pacificus*. They seem to use mantle muscle as an energy source during gonad development, production of ripe eggs or sperm and spermatophores. But this strategy is not common to all *T. pacificus* since we obtained only two exhausted individuals and the catch percentage of this type of squid in nature is not high (Araya, 1957; Hambe, 1963). The mechanism of exhaustion in this species should be the subject of future study.

Histological change was observed in the seminal receptacle and nidamental gland of post-spawning

females in this experiment. In the seminal receptacle of pre-spawned females, goblet cells that form the inner walls of the seminal receptacle were clearly observed, and on it sperm masses were found. On the contrary, these goblet cells elongated and a few sperm were in the seminal receptacle of post-spawning individuals. This change suggests that the goblet cells secrete certain substances which play an important role in sperm activation. Females must again be provided with spermatophores by mature males if multiple spawning is to occur. Although mature males continued their mating behavior, one month after the outset of the experiment their testis seemed to be regressed histologically and mating behavior was not as active as before. From these observations, multiple spawning seems not occur in this species. Indeed, all spawning individuals in 1988 and 1989 spawned once and died. However, histological observation of the ovary of post-spawning individuals showed that oocytes still existed at all stages of development, which suggests that a second recruitment of egg clutches can occur for subsequent spawning. From this fact, multiple spawning is thought to occur. There was an example showing multiple spawning in 1990 experiment; one female spawned two times within a week. There thought to be two cases of spawning in this species, single spawning followed death and multiple spawning. If multiple spawning occurs, the duration between the first and second spawning would not be long, perhaps, one month or slightly more since ovulation does not take several months as we oabserved in the present experiment. We consider that *T. pacificus* is basically semelparous but sometimes spawns several times in a few days to a week or more like *I. illecebrosus* (Durward *et al.*, 1980) and the maturation process which includes mating and spawning behavior may be centered on the last few months of their life span.

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