

ENVIRONMENTAL REGULATION OF REPRODUCTIVE RHYTHMS IN TELEOSTS

KATSUMI AIDA

*Department of Fisheries, Faculty of Agriculture,
The University of Tokyo,
Bunkyo, Tokyo, Japan*

K. Aida (1991) Environmental regulation of reproductive rhythms in teleosts. *Bull. Inst. Zool., Academia Sinica, Monograph 16: 173-187*. Reproductive rhythms of fishes consist of both annual (long) and spawning (short) rhythms. Typical seasonal breeders which can easily be maintained under laboratory conditions and induced to undergo gonadal maturation and spawning were chosen as experimental fishes. Fishes were classified into 6 groups according to their timing of annual breeding: spring spawner, spring-summer spawner, summer spawner, autumn spawner, winter spawner and spring/autumn spawner. The environmental factors which initiate and terminate the breeding season were investigated in several species, and these results are summarized.

Multiple spawners possess their own spawning rhythm. Their spawning cycles range from 1 day to a few months. In the rose-bitterling, its cycle was shown to be dependent on water temperature, being long under lower temperatures, and short under higher temperatures. In each species examined in this study, spawning time was basically fixed at a specific time of day, and was regulated mainly by a photoperiodic cue under 24 hr LD cycles. Spawning time, however, was regulated by circadian rhythms when placed under continuous darkness. These cues are considered to determine the timing of the GTH surge, which results in final oocyte maturation, ovulation and oviposition. High levels of plasma steroid hormones are also considered to be an important physiological requirement for the occurrence of the GTH surge.

Key words: Environmental regulation, Annual rhythm, Spawning rhythm, Teleosts.

Reproductive rhythms of fishes consist of an annual reproductive rhythm (long rhythm) and a spawning rhythm (short rhythm). Fishes are roughly classified into 3 groups according to the patterns of their annual rhythms and spawning rhythms. Fishes which belong to the first group spawn only once a year. The annual reproductive rhythm of

this group coincides with their spawning rhythm, such as in rainbow trout. The second group consists of multiple spawners. It has been ascertained that in a number of species, spawning is repeated during the breeding season. Spawning cycles are considered to depend on genetic characteristics, the physiological conditions of the fish and environmental conditions. Many tropical species comprise the third group which do not exhibit any annual rhythm and continuously spawn throughout the year.

In aquacultural development, the manipulation of a fish's reproductive activity through the control of environmental factors is one of many important objectives. To achieve the goal of controlling reproduction, we must first understand the annual reproductive rhythms of the targeted fish species in the wild, and then identify the environmental cues which regulate their annual reproductive rhythm. It is, however, often difficult to obtain sufficient information on such environmental factors in the wild for those species which are desirable for culture. Therefore, we employed as a model, freshwater and seawater species which are easily maintained and can be induced to spawn under laboratory

conditions. The initiating and terminating factors of their breeding seasons were examined. Knowledge obtained from model fishes will be applicable in the control of reproduction in species targeted for aquaculture.

ANNUAL REPRODUCTIVE RHYTHMS

Fishes inhabiting the temperate zone can be tentatively classified into six groups according to their annual breeding seasons. Here, I shall describe how annual reproductive rhythms are regulated by environmental factors, based on our data obtained from cyprinid and gobiid species and several other seawater species which are easily maintained in the aquarium.

Spring spawner

A number of species are included among the spring spawners. We employed the akahiretabira-bitterling *Acheilognathus tabira* (Shimizu and Hanyu, 1981, 1982, 1991), honmoroko *Gnathopogon caerulescence* (Okuzawa *et al.*, 1986, 1990) and goldfish *Carassius auratus* (Razani and Hanyu, 1986, 1987a, 1987b; Razani *et al.*, 1988a, 1988b) as model experimental fishes, to examine the precise effects

of water temperature and photoperiod on the initiation and termination of their breeding season. Using akahiretabira-bitterling as an example, I will describe how its' annual reproductive rhythm is regulated.

Judging from the elongation of its' ovipositor, occurrence of ovulation and GSI values in the akahiretabira-bitterling, breeding occurs for about two months between late April to late June. In late February, the bitterlings were acclimated to experimental conditions under several combinations of temperature (8, 13 or 16°C) and photoperiod (11 or 15L). After 60 days, GSIs of both sexes were greatly elevated in the 16°C group, regardless of photoperiod. Some females possessed mature eggs. At 13°C, male GSI increased, whereas females did not exhibit any significant increase in their GSI changed slightly in the 8°C group. Therefore, it was concluded that increasing temperature is the only initiating factor of the breeding season in this species. The critical temperature is about 13°C, although a slight difference exists between sexes.

The terminating factors of the spawning season were investigated next. Fish that continued cyclic spawning in late May were transfer-

red to several temperature (22, 26 or 30°C) and photoperiod (8, 12, or 15L) combination. After 3 weeks of exposure, both female and male GSIs decreased greatly in the 30°C group, whereas conditions of maturity changed little in the 22°C group. The decrease in female GSI was less at 26°C than at 30°C. These results indicate that the termination of spawning period in this species is related to temperature increasing beyond the upper critical limit of about 25°C.

Photoperiodism was observed to develop at the end of the breeding season. When mature fish kept under 22°C-15L for one to two months were transferred to 22°C-11L, gonadal regression took place rapidly. This indicates that some cool habitats of the northern parts of Japan, spring spawners may terminate their breeding season by responding to shortening day-length.

Subsequently, regressed fish were transferred in September to 22°C-8L, 22°C-11L, 22°C-15L or 27°C-15L. After 6 weeks, a slight increase in GSI was observed in all the 22°C group, whereas no changes occurred in the 27°C group. The 22°C-15L and 22°C-11L groups were reared for another 4 weeks. GSI increased sharply in the

22°C-15L group, but remained unchanged in the 22°C-11L group. Therefore, it was concluded that the first stage of recrudescence is induced by the declining temperature in autumn, but the second stage of recrudescence does not readily proceed because of shortening day-length.

Environmental regulation of the annual reproductive rhythms in honmoroko and goldfish are similar to that in the akahiratabira-bitterling. Thus, the above results indicate that in spring spawners, the breeding season is initiated by rising water temperature in the spring and is terminated by high temperature in summer; gonadal maturation is inhibited by shortening day-length in autumn, although the temperature is permissive. These spring-spawners are considered to be long-day spawners, since their gonadal maturation is inducible under artificial long day-length in autumn.

Spring-summer spawner

We employed 4 species of summer spawners, the rose-bitterling (tairikubaratanago) *Rhodeus ocellatus ocellatus* (Asahina *et al.*, 1980; Asahina and Hanyu 1983, 1985, 1991), the medaka *Orhyzias latipes* (Awaji and Hanyu, 1987, 1988, 1989a, 1989b), chichibu-goby *Tridentiger obscurus*

(Kaneko, 1986; Kaneko and Hanyu, 1985), small filefish *Rudarius ercodes* (Lee and Hanyu, 1984; Lee *et al.*, 1984), as model experimental fishes. The details of how the rose-bitterling's annual reproductive rhythm is regulated is presented in the following.

During the breeding season, the rose-bitterling continues cyclic oviposition for almost 6 months from late March to mid-September. The female GSI shows a sudden rise in March followed by a plateau, and then an abrupt fall in September.

Initiating factors of the spawning period were investigated using adult fish collected from natural habitats in January. Fish were acclimated to several regimes of temperature (10, 16 or 22°C) combined with photoperiod (7, 9, 12 or 16L): Within 4 weeks, GSIs increased rapidly at 16 and 22°C regardless of the photoperiod, and some females started cyclic oviposition. There was no significant change in GSI at 10°C. However, by raising the temperature to 13°C, a rapid increase in GSI was observed in all photoperiodic groups. These results demonstrate that gonadal maturation in the pre-spawning period depends on temperature rising above 10°C. Increasing

day-length seemed to have little or no effect.

The terminating factor of the spawning period was investigated using bitterlings collected in late August. Fishes were transferred to 25°C-11, 12, 13, 14, or 15L. Within 5 weeks, spawning rhythm resumed in both the 14 and 15L groups, whereas no oviposition occurred in the remaining groups. However, by increasing day-length from 11 or 12L to 14 or 15L, respectively, ovarian rematuration was quickly induced and followed by the eventual commencement of oviposition. These results indicate that during the course of the breeding period, photoperiodism becomes involved in the control of gonadal activity, and this involvement manifests itself when natural day-length decreases to the critical level between 13 and 14L. This is in agreement with the fact that the natural spawning period of this species terminates abruptly in September, when the water is still warm.

Initiating factors of the breeding season in the medaka and chichibugoby are also related to the increase in water temperature experienced in the spring. In the small filefish, however, initiation in the spring was inhibited under increasing tempera-

ture combined with short day-length, indicating the existence of photoperiodism, and that long day-length is necessary for initiation. Under natural conditions, however, day-length in the spring is already sufficiently long for initiation to occur. The limiting factor in the small filefish does not appear to be long day-length, but the increase in temperature above a critical level. In contrast, the terminating factor is shortening day-length in September in all of the species examined. Overall, these results indicate that there is no difference in photoperiodism both in spring spawners and spring-summer spawners.

Autumn spawner

The number of autumn spawning fishes is rather limited. Fortunately, three species of bitterlings, the zenitanago-bitterling *Pseudoperilampus typus*, the kanehira-bitterling *Acheilognathus rhombea* and the itasenpara-bitterling *A. longipinnis* belong to this group. We have precisely investigated the initiating and terminating factors of the breeding period in zenitanago-(Shimizu and Hanyu, 1983) and kanehira-bitterlings (Shimizu *et al.*, 1987 and unpublished data).

Larvae of the zenitanago-bitterling were collected in May, maintained in an outdoor pond and reared to the adult size by mid-summer. In late August, fishes were exposed to experimental temperatures (18, 25 or 28°C) combined with photoperiods (12, 13, 14 or 15L). GSIs markedly increased in the 12L groups after one month regardless of the temperature, whereas no increase in GSI was observed in the 14 and 15L groups. Under 13L, only some females showed an increase in GSI. This species is therefore referred to as a "short-day" spawner, which matures in response to decreasing day-length below 13L.

To investigate the initiating factor of the spawning season in the kanehira-bitterling, underyearlings were maintained at 28°C and 20°C in combination with 15L and 12L from August 31 for 20 days. At the end of the experiment, gonads matured under 12L conditions, whereas they did not change under 15L conditions. This indicates that the initiating factor in this species is also the decreasing day-length during the autumn months. To clarify the terminating factor of the breeding season, matured fishes were maintained under 10L-20°C and 10L-13°C for 50 days in autumn. GSI decreased under 13°C,

but was maintained at high levels under 20°C, indicating that the decreasing temperature in the winter terminates the autumn spawning. From April 18, yearling fishes were reared under 9L-24°C and 15L-24°C for 45 days to investigate the initiating factor. Under such conditions, no increase in GSI was observed in either group. GSI, however, increased in fish reared under the same conditions from May 26 for 30 days. Since yearling fishes mature 2 months earlier under natural conditions, both results suggest that the breeding season in yearling fish is induced by an endogenous rhythm (circannual rhythm).

Our results indicated that autumn spawners are short-day spawners and that their spawning period is initiated by the shortening day-length in the autumn and is terminated by the decreasing temperature in the winter.

Summer spawner

We do not have enough data on the environmental regulation of gonadal maturation in summer spawners. Two seawater species, urohazegoby *Glossogobius olivaceus* and kisu *Silago japonica* have been reared in the laboratory, but we have observed spawning for several months. Both

species, the initiating and terminating temperatures of the breeding season are similar (Kaneko, 1986; Furukawa *et al.*, unpublished data). Therefore, it is speculated that temperature is the main factor which controls the annual reproductive rhythm. However, further studies on photoperiodism in summer spawners are necessary.

Winter spawner

In winter spawners, we employed two species of gobies, the agohaze-goby *Chasmichtys dolicognathus* (Kaneko *et al.*, 1984) and the ukigori-goby *Chaenogobius macrognathus* (Hibino *et al.*, 1984).

The agohaze-goby, which is quite common in tide pools, spawns from February through May. Female GSI begins to increase in November accompanying the accumulation of yolk in the ovary. In June, GSI drops suddenly with the appearance of regressed oocytes. The initiation of ovarian development of this species starts with a decrease in temperature below 18°C in November: final maturation occurred at the lowest temperatures. The breeding period was terminated when temperatures reached around 18°C in June. Similar results have been obtained in the ukigori-goby. Water tempera-

ture below 16°C was necessary for the initiation and maintenance of gonadal maturation, and increasing temperature above the critical level was necessary for the termination of maturation. Although decreasing temperature in the autumn was the main factor for initiation, short photoperiods accelerated recrudescence. In the long-jaw goby *Gillichys mirabilis*, gonadal activity was inhibited by temperature rising above 24°C and gonadal recrudescence was induced by temperature declining below 20°C (de Valming, 1972). In addition, short photoperiods accelerated recrudescence, which is however, not the case with the agohaze-goby. There have been no indications, thus far, that photoperiodism is involved in influencing the reproductive cycle of this species.

Spring/autumn spawner

Annual breeders usually exhibit a single breeding season in a year. However, we have recently found that the tobinumeri-dragonet *Repomucenus beniteguri* has two breeding seasons, occurring separately in the spring and the autumn (Zhu *et al.*, 1989). The tobinumeri-dragonet is a small seawater fish, which spawns daily during both breeding seasons. Under natural conditions, spawning was

initiated when the temperature increased above 18°C in the spring and was terminated in July under temperature near 30°C. After about a one-month pause, spawning resumed in late August at about 28°C and terminated below 14°C in November (Zhu *et al.*, 1991a). At first, it was thought that temperature is a limiting factor which controls the breeding season in this species. Under artificial conditions, however, the initiation of autumn spawning was delayed under 15L conditions in comparison with the 12L condition at the same temperature. Furthermore, daily spawning stopped after the fish were transferred from 9L to 15L conditions (Zhu *et al.*, 1991b). Although there is no information on the initiating factors of spawning in spring, long day-length and high temperatures during June to July suppressed spawning. Therefore, it is suggested that the tobinumeri-dragonet is a short-day spawner and its photoperiodism probably declines in the spring as in spring spawners.

SPAWNING RHYTHM

We now know that many teleost species repeat spawning throughout their spawning seasons. These fishes are called multiple spawners, and

show regular or irregular spawning cycles ranging from 1 day to a few months. Intervals of spawning are considered to be regulated by genetic factors, physiological conditions of fishes and environmental conditions.

Daily spawning

Several teleost species, such as the medaka, kisu (Furukawa *et al.*, 1991), tobinumeri-dragonet (Zhu *et al.*, 1991a), and red seabream *Pagrus major* (Matsuyama *et al.*, 1988), are considered to be daily spawners. In these species the spawning time is usually fixed at a certain time of the day—morning in the medaka, evening in the tobinumeri-dragonet and early night in the kisu. We reared one female kisu or tobinumeri-dragonet with 2-3 males in experimental tanks and investigated how their spawning time is determined (Furukawa *et al.*, 1991; Zhu *et al.*, 1991c). In both species, the spawning time followed the shift of photoperiod after a few days' delay, indicating that spawning time is determined by the photoperiod. Spawning time in the tobinumeri-dragonet was changed when lights-off time was shifted, whereas no shift occurred when lights-on time was changed. Spawning time, however, was changed by the shift of lights-on time in the kisu. Therefore, we

may conclude that spawning time is determined by photoperiod, but the synchronizer of spawning time differs according to species. When kisu were transferred to continuous darkness from 24h LD cycles, spawning time was delayed 15 min per day on the average, and was returned to its original pattern upon transferring the fish to the former photoperiod. This indicates that kisu possesses a circadian rhythm, and under continuous darkness, spawning time was determined by this circadian rhythm. Small fluctuations were also observed in spawning time in kisu even under a fixed photoperiod. Thus, we investigated the effects of water temperature on spawning time, and found that the spawning occurs earlier under low temperatures and is delayed under high temperatures, indicating that changes in water temperature cause minor fluctuations in daily spawning time. However, water temperature levels did not influence spawning time in the tobnumeridragonet.

Recently, it was reported that plasma GTH levels show a clear daily rhythm in a daily spawner, the red seabream (Tanaka *et al.*, 1991). Oocytes of daily spawners underwent oocyte maturation and ovulation in response to GTH or

maturation inducing steroid, (MIS). Daily rhythm in responsiveness to these hormones was also observed in the kisu and the tobnumeridragonet (Kobayashi *et al.*, 1988; Zhu *et al.*, 1989). Responsiveness to GTH appears earlier than that to MIS. Responsiveness to MIS was induced by a short period treatment with HCG (Aida, in preparation).

Spawning rhythm in bitterlings

Bitterlings possess an ovipositor with which they can lay eggs in the gill cavity of freshwater bivalves. During the spawning season the ovipositor goes through alternate elongation and shortening according to the fish's reproductive conditions. In the kanehira-bitterling (an autumn spawner), regular spawning was observed at intervals of 5 days under 25°C and 10L 14D conditions (Shimizu *et al.*, 1985). The spawning cycle consists of three physiologically distinct phases: vitellogenic (3 days), maturational (1 day) and spawning (1 day) phases. Ovipositor elongation occurred in the afternoon of the maturational phase, and ovulation occurred at midnight. The GTH surge commenced in the afternoon and peaked at mid-night. $17\alpha, 20\beta$ -dihydroxy-4-pregnen-3-one exhibited similar changes as GTH. Estradiol

culminated during the vitellogenic phase. Testosterone rapidly decreased during the maturational phase and gradually increased in the vitellogenic phase. Spawning cycles depended on water temperature; *i. e.*, long under low temperatures and short under high temperatures (Asahina, 1981).

Spawning time in carp and goldfish

In Japan, the common carp, *Cyprinus carpio*, spawns in the spring under natural conditions. After the completion of vitellogenesis, yolk-laden oocytes could be maintained under 16°C without ovulation and degeneration taking place. Ovulation and spawning were inducible by increasing temperature from 16 to 24°C (Santos *et al.*, 1986a). These events occurred between the midnight and in the early morning of the second night, when water temperature started to elevate in the evening. Plasma GTH started to elevate in the afternoon and attained a peak by midnight. Plasma 17 α , 20 β -dihydroxy-4-pregnen-3-one showed changes similar to those of GTH. Some females which failed to ovulate during the second night after exposure to the temperature increase ovulated during the third night, with the GTH surge occurring within the

same time frame.

Experiments were also conducted to clarify the effects of photoperiod on the determination of the pre-ovulatory GTH surge in female carp (Santos *et al.*, 1986b). After a 2-week acclimation under different photoperiodic regimes at 16°C, the temperature was raised to 24°C for all groups. A pre-ovulatory GTH surge was initiated during the latter part of the light phase in each photoperiodic group after water temperature was increased, irrespective of the starting time of temperature elevation. These results clearly indicate that the onset time of the pre-ovulatory GTH surge is determined by a photoperiodic cue.

Goldfish matures in the spring and spawns several times under natural conditions. Ovulation could be easily induced by raising the water temperature from 12°C to 20°C in matured females (Kobayashi *et al.*, 1985, 1987, 1988). Similar changes in plasma GTH and 17 α , 20 β -dihydroxy-4-pregnen-3-one were observed following oocyte maturation and ovulation.

Under normal and reversed photoperiod, ovulation and spawning were observed during each dark phase (Kezuka *et al.*, 1989). In fish transferred from 15L 9D to continuous

dark and light conditions, ovulation and spawning were induced at a time corresponding to the dark phase of the acclimation photoperiod. A pre-ovulatory GTH surge was observed with ovulation, suggesting that the time of GTH surge is determined by photoperiod as in the common carp.

Mature female goldfishes were reared under 14L 10D and either pinealectomized or sham operated (Kezuka *et al.*, 1989). Water temperature was raised one week following the operation. Sham operated fish ovulated during dark phase, whereas pinealectomized fish ovulated randomly coincided with the GTH surge. The pineal gland is somehow involved in the mechanism regulating this photoperiodic response.

Temperature increase induces the pre-ovulatory GTH surge in matured females kept under 12°C. However, the GTH surge does not occur in immature and maturing females following a temperature increase. This indicates that matured females acquire a responsiveness to a temperature increase. High plasma testosterone levels were consistently observed before ovulation in the goldfish (Kobayashi *et al.*, 1988). These results suggest that a high testosterone level is necessary in

order for fish to respond to the temperature elevation. We have recently tested this hypothesis (Kobayashi *et al.*, 1989). In the winter, female goldfishes were ovariectomized, implanted with Silastic capsules containing either testosterone or E₂, and were maintained at temperatures below 12°C. Three months after implantation, water temperature was increased from 12 to 20°C using the same method as for induction of spontaneous ovulation. Some of the steroid-implanted females showed a GTH surge which was similar to that observed during normal spontaneous ovulation. Females with regressed ovaries implanted with capsules containing testosterone or E₂ exhibited a GTH surge without ovulation when water temperature was increased to 20°C. No surge occurred in the fish which did not receive a steroid implant. These results clearly indicate that the plasma steroid hormone levels before ovulation are an important physiological requirement in the occurrence of the GTH surge.

CONCLUSION

As mentioned above, photoperiod and water temperature are mainly involved in the regulation of annual

reproductive rhythms in fishes. Responsiveness to photoperiod is different in each species. Most fish exhibited photoperiodism, whereas others do not respond to changes in photoperiod. These differences in response to photoperiod are probably due to the differences in their genetic background. It is suggested that autumn spawning bitterlings were derived from spring spawning bitterlings as the result of the reversal in photoperiodism during their process of evolution. A reversal in photoperiodism is also observed in the group of black porgy (*Acanthopagrus*, Sparidae) which inhabits Japan. The reversal in photoperiodism is a phenomenon which appears to have commonly occurred in fishes during their evolution. The reversal may be based on common genetic changes. One more interesting phenomenon is seasonal changes in photoperiodism in the spring and spring-summer spawners: photoperiodism develops in the early summer and declines in the winter. The regulatory mechanism of photoperiodism is an important one for future investigations.

Photoperiod is also an important factor for the regulation of spawning time in fishes. We have ascertained that photoperiod determines spawn-

ing time by controlling the onset time of the pre-ovulatory GTH surge (Santos *et al.*, 1986). Timing signals probably trigger the release of GnRH in the hypophysis. The pineal gland may be involved in the control of timing of the GTH surge as represented previously. In addition, it has been shown that the pineal gland in goldfish possesses a circadian oscillator (Iigo *et al.*, 1991). Little, however, is known on the nature of the timing signals and the route where signals are transported. Further investigation is also necessary on these points.

Water temperature which enables gonadal maturation and oviposition to occur is also species specific. Genetic factors are considered to be involved in the determination of the range of temperature. Little, however, is also known on the genetic and physiological backgrounds of the determination mechanism. Photoperiodism becomes distinct under high temperatures, but weak under low temperatures, indicating that photoperiodism is modified by the level of water temperature. The mechanism of how temperature influences the reproduction of fish is also an important point for future investigations.

REFERENCES

- Asahina, K., I. Iwashita, I. Hanyu and T. Hibiya (1980) Annual reproductive cycle of bitterling, *Rhodeus ocellatus*. *Bull. Japan. Soc. Sci. Fish.* **46**: 299-305.
- Asahina, K. (1981) Studies on reproductive rhythms in the rose-bitterling. Doctoral thesis, The University of Tokyo.
- Asahina, K. and I. Hanyu (1983) Role of temperature and photoperiod in annual reproductive cycle of the rose bitterling *Rhodeus ocellatus ocellatus*. *Bull. Japan. Soc. Sci. Fish.* **49**: 61-67.
- Asahina, K. and I. Hanyu (1985) Development of photoperiodism involved in the gonadal activity of the rose bitterling *Rhodeus ocellatus ocellatus*. *Bull. Japan. Soc. Sci. Fish.* **51**: 1655-1670.
- Asahina, K. and I. Hanyu (1991) Effects of temperature during winter on gonadal recrudescence in spring in the rose bitterling *Rhodeus ocellatus ocellatus*. *Nippon Suisan Gakkaishi* **57**: 255-260.
- Awaji, M. and I. Hanyu (1987) Annual reproductive cycle of wild type of medaka. *Nippon Suisan Gakkaishi* **53**: 959-965.
- Awaji, M. and I. Hanyu (1988) Effects of water temperature and photoperiod on the beginning of spawning season in the orange-red type medaka. *Zool. Sci.* **5**: 1059-1064.
- Awaji, M. and I. Hanyu (1989a) Temperature-photoperiod conditions necessary to begin the spawning season in wild type medaka. *Nippon Suisan Gakkaishi* **55**: 747.
- Awaji, M. and I. Hanyu (1989b) Seasonal changes in ovarian response to photoperiods in orange-red type medaka. *Zool. Sci.* **6**: 943-950.
- De Valming, V.L. (1972) The effects of temperature and photoperiod on reproductive cycling in the estuarine gobiid fish *Gillichthys mirabilis*. *Fish. Bull.* **70**: 1137-1152.
- Furukawa, K., K. Aida, M. Yoshioka, H. Satoh and I. Hanyu (1991) Effects of photoperiod and water temperature on the spawning rhythm of the kisu *Sillago japonica*. *Nippon Suisan Gakkaishi* (in press).
- Hibino, H. (1984) Studies on reproductive rhythms in the ukigori-goby. Master thesis. The University of Tokyo.
- Iigo, M., K. Hiriaki, K. Aida and I. Hanyu (1991) Circadian rhythms of melatonin secretion from superfused goldfish (*Carassius auratus*) pineal gland *in vitro*. *Gen. Comp. Endocrinol.* **83**: 152-158.
- Kaneko, T., I. Hanyu and K. Hirose (1984) Annual reproductive cycle of a tide pool-living goby *Chasmichthys dolichognathus*. *Bull. Japan. Soc. Sci. Fish.* **50**: 1535-1540.
- Kaneko, T. and I. Hanyu (1985) Annual reproductive cycle of the chichibugoby *Tridentiger obscurus*. *Bull. Japan. Soc. Sci. Fish.* **51**: 1645-1650.
- Kaneko, T. (1986) Studies on reproductive rhythms in gobiid fishes. Doctoral thesis, The University of Tokyo.
- Kezuka, H., M. Kobayashi, K. Aida and I. Hanyu (1989) Effects of photoperiods and pinealectomy on the gonadotropin surge and ovulation in goldfish *Carassius auratus*. *Nippon Suisan Gakkaishi* **55**: 2099-2103.
- Kobayashi, M., K. Aida, I. Hanyu and S. Ishii (1985) Application of radio-receptor assay to the purification of silver carp gonadotropin. *Bull. Japan. Soc. Sci. Fish.* **51**: 405-411.
- Kobayashi, M., K. Aida and I. Hanyu (1986) Annual changes in plasma levels of gonadotropin and steroid hormones in male goldfish. *Bull. Japan. Soc. Sci. Fish.* **52**: 1152-1158.

- Kobayashi, M., K. Aida and I. Hanyu (1987) Hormone changes during ovulation and effects of steroid hormones on plasma gonadotropin levels and ovulation in goldfish. *Gen. Comp. Endocrinol.* 67: 24-32.
- Kobayashi, M., K. Aida and I. Hanyu (1988) Hormone changes during the ovulatory cycle in goldfish. *Gen. Comp. Endocrinol.* 69: 301-307.
- Kobayashi, M., K. Aida, K. Furukawa, K. K. Law, T. Morowaki and I. Hanyu (1988) Development of sensitivity to maturation-inducing steroids in the oocytes of the daily spawning teleost, the kisu *Sillago japonica*. *Gen. Comp. Endocrinol.* 72: 264-271.
- Kobayashi, M., K. Aida and I. Hanyu (1989) Induction of gonadotropin surge by steroid hormone implantation in ovariectomized and sexually regressed female goldfish. *Gen. Comp. Endocrinol.* 73: 469-476.
- Lee, T. Y. and I. Hanyu (1984) Reproductive cycle of small filefish *Rudarius ercodes*. *Bull. Korean Fish. Soc.* 17: 423-435.
- Lee, T. Y., I. Hanyu and K. Furukawa (1984) Effects of photoperiod and temperature on the gonadal activity in small filefish *Rudarius ercodes*. *Bull. Korean Fish. Soc.* 17: 523-528.
- Matsuyama, M., S. Adachi, Y. Nagahama and S. Matsuura (1988) Diurnal rhythm of oocyte development and plasma steroid hormone levels in the female red seabream *Pagrus major* during the spawning season. *Aquaculture* 73: 357-372.
- Okuzawa, K., K. Furukawa, K. Aida and I. Hanyu (1986) Annual reproductive cycle of the honmoroko *Gnathopogon elongatus*. *Bull. Japan. Soc. Sci. Fish.* 52: 1957-1960.
- Okuzawa, K., K. Furukawa, K. Aida and I. Hanyu (1989) Effects of photoperiod and temperature on gonadal maturation, and plasma steroids and gonadotropin levels in a cyprinid fish, the honmoroko *Gnathopogon caerulescens*. *Gen. Comp. Endocrinol.* 75: 139-147.
- Razani, H. and I. Hanyu (1986) Annual reproductive cycle of 2-3 year-old female goldfish and its artificial modification by manipulation of water temperature and photoperiod. *Bull. Japan. Soc. Sci. Fish.* 52: 965-969.
- Razani, H. and I. Hanyu (1987a) Effect of continuous short photoperiod-cool temperature on gonadal maturation of goldfish with reference to photoperiod elongation. *Adv. Aqua. Biol. Fish.* 1987: 181-189.
- Razani, H., I. Hanyu and K. Aida (1987b) Critical daylength and temperature level for photoperiodism in gonadal maturation of goldfish. *Exp. Biol.* 47: 89-94.
- Razani, H., I. Hanyu and K. Aida (1988a) Environmental influences on ovarian activity and related hormones in yearling goldfish. *Nippon Suisan Gakkaishi* 54: 1505-1511.
- Razani, H., I. Hanyu and K. Aida (1988b) Environmental influences on testicular activity and related hormones in yearling goldfish. *Nippon Suisan Gakkaishi* 54: 1513-1520.
- Santos, A. J. G., K. Furukawa, M. Kobayashi, K. Bando, K. Aida and I. Hanyu (1986) Plasma gonadotropin and steroid hormone profiles during ovulation in the carp *Cyprinus carpio*. *Bull. Japan. Soc. Sci. Fish.* 52: 1159-1166.
- Santos, A. J. G., K. Furukawa, M. Kobayashi, K. Bando, K. Aida and I. Hanyu (1986) Photoperiodic determination of preovulatory gonadotropin surge time in the carp *Cyprinus carpio*. *Bull. Japan. Soc. Sci. Fish.* 52: 1167-1172.

- Shimizu, A. and I. Hanyu (1981) Annual reproductive cycle of a spring spawning bitterling *Acheilognathus tabira*. *Bull. Japan. Soc. Sci. Fish.* 47: 333-339.
- Shimizu, A. and I. Hanyu (1982) Environmental regulation of annual reproductive cycle in a spring-spawning bitterling *Acheilognathus tabira*. *Bull. Japan. Soc. Sci. Fish.* 48: 1563-1568.
- Shimizu, A. and I. Hanyu (1983) Environmental regulation of spawning-period in an autumn spawning bitterling *Pseudoperilampus typus*. *Bull. Japan. Soc. Sci. Fish.* 49: 895-900.
- Shimizu, A., K. Aida and I. Hanyu (1985) Endocrine profiles during the short reproductive cycle of an autumn-spawning bitterling *Acheilognathus rhombea*. *Gen. Comp. Endocrinol.* 60: 361-371.
- Shimizu, A., K. Aida and I. Hanyu (1987) Annual reproductive cycle in an autumn-spawning bitterling *Acheilognathus rhombea*. *Nippon Suisan Gakkaishi* 54: 529-536.
- Shimizu, A. and I. Hanyu (1991) Changes in photoperiodism involved in the gonadal development of a spring-spawning bitterling *Acheilognathus tabira*. *Nippon Suisan Gakkaishi* 57: 177.
- Tanaka, H., H. Kagawa, K. Okuzawa and K. Hirose (1991) Two gonadotropic glycoproteins in the red seabream (*Pagrus major*). *Proceedings of the 4th international symposium on reproductive physiology of fish.* (submitted)
- Zhu, Y., K. Furukawa, K. Aida and I. Hanyu (1989) Annual reproductive rhythm of the tobnumeridragonet *Repomucenus beniteguri* (Callionymidae) in Lake Hamana. *Nippon Suisan Gakkaishi* 55: 591-599.
- Zhu, Y., K. Aida, K. Furukawa and I. Hanyu (1990) Development of sensitivity to maturation-inducing steroids and gonadotropins in the tobnumeridragonet, *Repomucenus beniteguri*, Callionymidae (Teleostei). *Gen. Comp. Endocrinol.* 76: 250-260.
- Zhu, Y., K. Aida, K. Furukawa and I. Hanyu (1991a) Daily spawning rhythm during spring and autumn spawning seasons in the tobnumeridragonet *Repomucenus beniteguri*. *Nippon Suisan Gakkaishi* (in press).
- Zhu, Y., K. Furukawa, K. Aida and I. Hanyu (1991b) Effects of water temperature and photoperiod on the initiation and termination of the autumn spawning season in the tobnumeridragonet *Repomucenus beniteguri*. *Nippon Suisan Gakkaishi* (in press).
- Zhu, Y., K. Furukawa and K. Aida (1991c) Effects of photoperiod on spawning rhythm in the tobnumeridragonet *Repomucenus beniteguri*. *Nippon Suisan Gakkaishi* (in press).

