ZOOLOGICAL SCIENCE 5: 1059–1064 (1988)

Effects of Water Temperature and Photoperiod on the Beginning of Spawning Season in the Orange-red Type Medaka[1]

MASAHIKO AWAJI[2] and ISAO HANYU

Laboratory of Fish Physiology, Faculty of Agriculture, University of Tokyo, Bunkyo, Tokyo 113, Japan

ABSTRACT—Rearing experiments were conducted to study effects of photoperiod and water temperature on the beginning of spawning season in the orange-red type medaka, *Oryzias latipes*. Fish, cultured in Tokyo and ready for rapid gonadal growth in spring, was transferred to 7 conditions (16°C 14L, 14°C 14L, 14°C 12L, 14°C 10L, 12°C 14L, 10°C 14L, 8°C 14L), and reared for 4 to 8 weeks. Changes in gonadosomatic index (GSI) and gonadal histology were examined. Groups at 14 and 16 °C showed rapid GSI increase irrespective of photoperiod. Active yolk globule accumulation and spermatogenesis began, and spawning was observed in these groups. After 8 weeks, many regressive oocytes were noticeable in 14°C groups. In groups at temperature below 14°C, GSIs of both sexes remained low and spawning was not observable. But yolk globule accumulation proceeded slowly in 10 and 12°C groups. When medaka in early spring were transferred to 4 conditions (16°C 10L, 8L, 6L, 4L), and reared for 4 weeks, they could mature and spawn in all experimental regimes, which eliminates possibility for photoperiodic response in spring. From these results, it is clear that temperature rise in spring only is responsible for the beginning of spawning season in medaka cultured around Tokyo.

INTRODUCTION

Most teleost fishes have their own annual reproductive cycles. Though some fishes seem to have endogenous reproductive rhythm [1], the annual reproductive cycle is generally believed to depend upon seasonal changes in environmental factors: photoperiod and water temperature in temperate zone, and rainfall in tropical zone [1–6].

Medaka, *Oryzias latipes*, is a small freshwater teleost native to Japan and her adjacent areas [7]. Two types of medaka, namely the wild and the orange-red types [8], are well known. Their annual reproductive cycles have already been investigated [9–12], and the spawning season is understood to extend usually from mid-April to early September.

There have been several works concerning environmental effects on the beginning of spawning season in medaka, with attention engaged by effects of photoperiod and/or water temperature [10, 13–19]. But obtained results did not always coincide with one another. As for the orange-red type medaka, in additions, effects of photoperiod in combination with water temparature have not been clearly shown yet, warranting further investigations.

This study was conducted to clarify environmental factors responsible for the beginning of spawning season in the orange-red type medaka. Rapid and remarkable gonadal growth, i.e. commencement of yolk globule accumulation in ovaries and retrieval of meiosis in testes, is leading and inevitable process for the beginning of spawning [9, 10]. Efforts were made to reveal environmental factors which cause this rapid gonadal growth. Thus medaka in early spring, which had been reared under natural condition and was ready for the rapid gonadal growth, was used as material. Photoperiod and temperature regimes were selected to be comparable to, or not too far from, natural environmental changes in spring.

Accepted January 29, 1988
Received October 23, 1987

[1] Studies on the Reproductive Rhythms of Fishes-XXIV.

[2] To whom reprints should be requested. Present address: National Research Institute of Aquaculture, Nansei, Mie 516-01, Japan.

MATERIALS AND METHODS

Two experiments were conducted in 1980 (Experiment 1) and 1981 (Experiment 2). The orange-red type medaka *Oryzias latipes*, born in the preceding breeding season, were obtained from a hatchery in Edogawa-ku, Tokyo, kept in a pen (area 14 m^2, depth 40 cm) placed in a circulating outdoor tank (area 14 m^2, maximum depth 50 cm) at least for 4 months till experiments, and fed tubifex (freshwater oligochaetes) to satiation.

During experiments, water temperature in the outdoor tank ranged from 6 to 16°C, and the daylength [20] increased from 11.3 to 13.3L.

Experiment 1 was started on February 27 and ended on April 24. Fish ranging from 21 to 31 mm in standard length were transferred to following 7 conditions: 8°C 14L (14 hours of light/day), 10°C 14L, 12°C 14L, 14°C 14L, 14°C 12L, 14°C 10L, 16°C 14L. The three 14°C groups were reared for 8 weeks, and the others for 4 weeks. Twenty and 30 fish of each sex were used for 8°C, 10°C, 12°C or 16°C regime and three 14°C regimes, respectively. Part of fish in each group was taken on March 13 and 27, and the rest at the end.

Experiment 2 was started on March 6 and ended on April 7. Thirty fish of each sex, with standard length of 20 to 30 mm, were reared under 16°C 4L, 16°C 6L, 16°C 8L or 16°C 10L. Part of the fish were sampled halfway also in this experiment.

Each experimental group was kept in a 50 to 60 liter, light proof aquarium with circulation and some water plants. Photoperiod was controlled with a 10 W fluorescent lamp connected to a timer, and water temperature with a thermostat and heater. Fish were fed with tubifex *ad lib.*

After taken up, fish were anesthetized with tricaine methane sulfonate. The whole body with opened abdominal cavity was fixed in Holland's solution-sublimate in Experiment 1 and in Bouin's solution in Experiment 2. Standard length, body weight and gonad weight were measured while in 70% alcohol. GSI (gonad weight$\times$100/body weight) was calculated and gonads were embedded in paraffin by ordinary method. Ovarian sections in 10 μm were stained with AZAN and testicular sections in 5 μm with Mayer's hematoxylin and eosin.

RESULTS

Experiment 1

Figure 1 shows the changes in GSI under experimental regimes. Average GSI of initial controls was around 2.5% in female and 0.5% in male. The ovary contained oocytes up to yolk vesicle stage. The testis had spermatogonia, primary spermatocytes and residual sperm.

Females exposed to higher temperature (14 or 16°C) had rapid increases of GSI, reaching 6 to 9% in the fourth week, irrespective of photoperiod. The beginning of yolk globule accumulation was observed in the second week, and fully matured oocytes were noticeable in the fourth week. First spawning was observed in 14°C 12L group 25 days after the commencement, followed by spawning in the other groups at higher temperatures. GSIs of three groups at 14°C kept high till the eighth week, but ovaries of these groups contained many regressive oocytes at the end of the experiment.

Females exposed to lower temperatures (12, 10 or 8°C) had little increase of GSI in spite of long daylength. GSIs of 10°C 14L and 12°C 14L groups were 3 to 4% in the fourth week. Their ovarian histology in the second week was unchanged, but in the fourth week early yolk globule stage oocytes were observed. GSI and ovarian histology of 8°C 14L group in the fourth week were equal to those of initial control. No spawning was observed in these groups.

GSIs of males exposed to higher temperatures showed rapid increase, reaching 1.0 to 1.2% in the fourth week, and three groups at 14°C kept that value till the eighth week. Increase of spermatogonia and primary spermatocytes was observed in the second week, leading to active spermatogenesis shown by meioses of spermatocytes in the fourth and eighth weeks.

Males exposed to lower temperature showed a little increase of GSI, reaching 0.6 to 0.8% in the fourth week. But active spermatogenesis was not observed.

Experiment 2

Results of Experiment 1 indicated importance of rising temperature in the beginning of spawning

season in medaka, daylength changes having little effect on gonads. Experiment 2 was conducted to examine the possibility of photoperiodic response in gonadal development.

Figure 2 shows the changes of GSI under experimental regimes. Average GSI of initial controls was around 2.0% in female and 0.5% in male. Gonadal histology of initial controls was much the

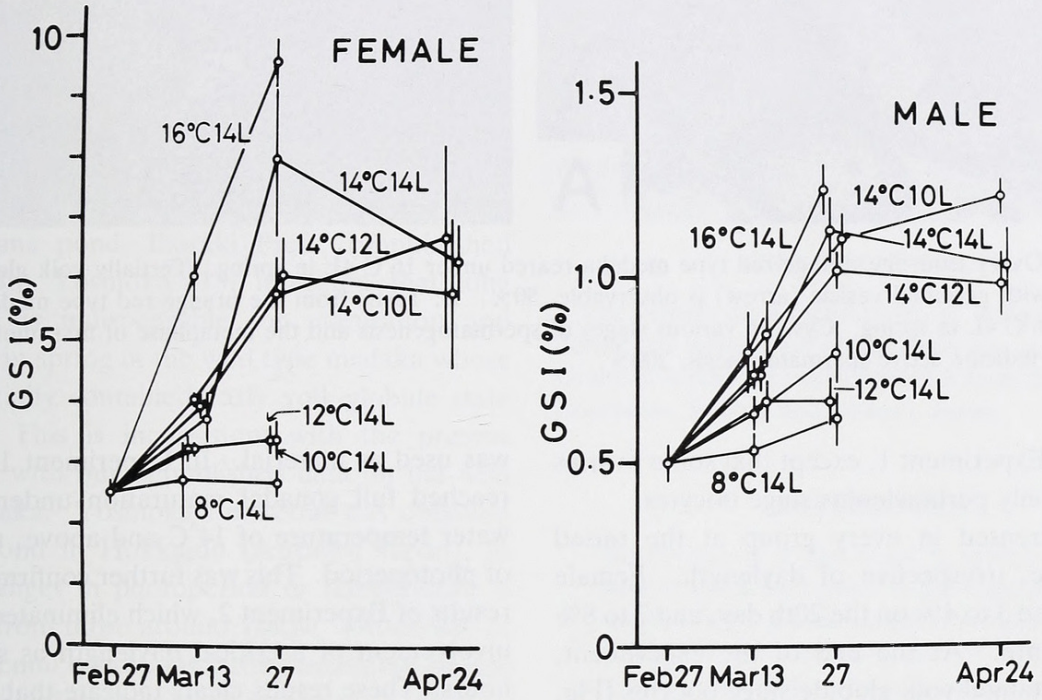

FIG. 1. Changes in the gonadosomatic index (GSI) of the orange-red type medaka reared under various temperature-photoperiod regimes in Exp. 1.

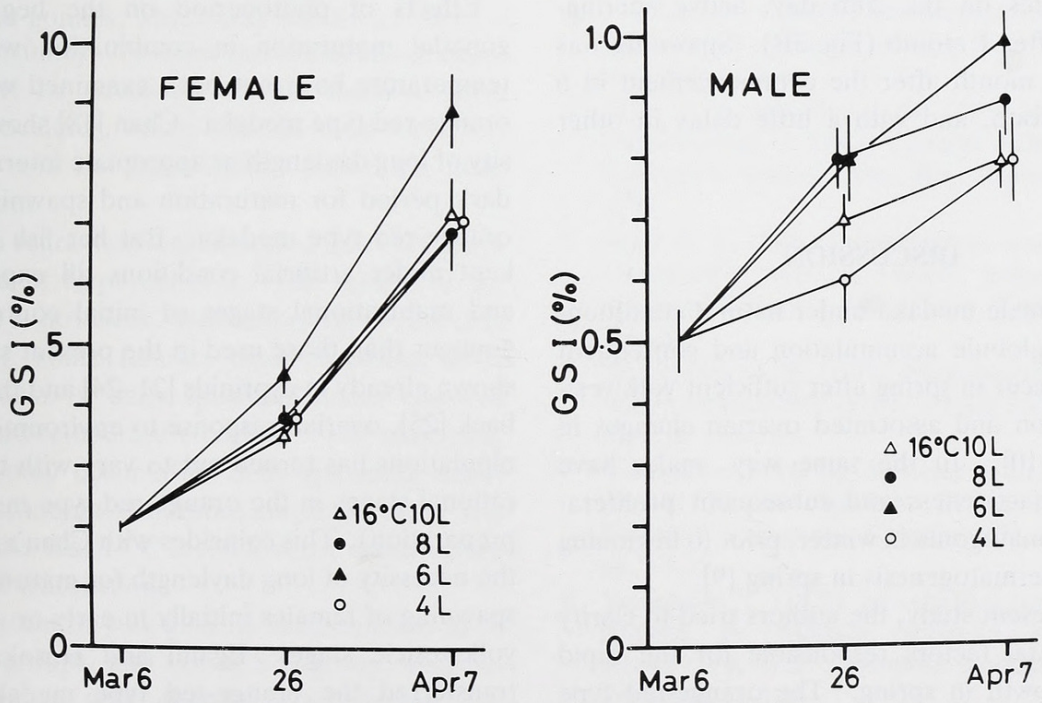

FIG. 2. Changes in the gonadosomatic index (GSI) of the orange-red type medaka reared under short day regimes in Exp. 2.

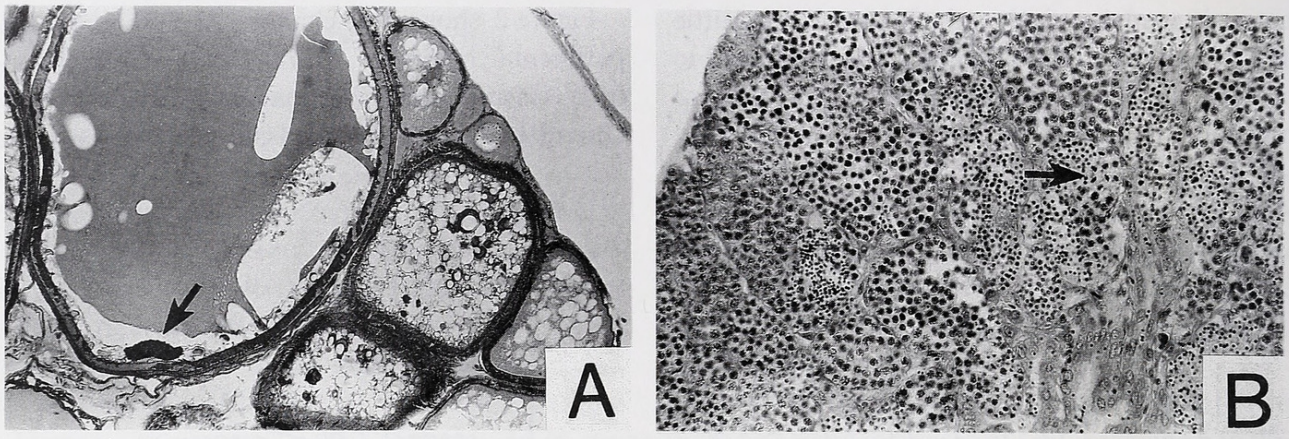

FIG. 3. A: Ovary from the orange-red type medaka reared under 16°C 4L in spring. Tertially yolk globule stage oocyte with germinal vesicle (arrow) is observable. 50×. B: Testis from the orange-red type medaka reared under 16°C 4L in spring. Cysts in various stages of spermatogenesis and the metaphase of first miotic division (arrow) indicate active spermatogenesis. 200×.

same as in Experiment 1, except that some ovaries contained only perinucleolus stage oocytes.

GSIs increased in every group at the raised temperature, irrespective of daylength. Female GSIs reached 3 to 4% on the 20th day, and 7 to 8% after 1 month. At the end of the experiment, ovaries contained yolk globule stage oocytes (Fig. 3A) and some ovulatory follicles. Male GSIs were 0.6 to 0.8% on the 20th day, and 0.8 to 1.0% after 1 month. Testes showed some increase in primary spermatocytes on the 20th day, active spermatogenesis after 1 month (Fig. 3B). Spawning was observed 1 month after the commencement in 6 and 10L group, and with a little delay in other groups.

DISCUSSION

In the female medaka under natural condition, rapid yolk-globule accumulation and consequent spawning occur in spring after sufficient yolk vesicle formation and associated ovarian changes in winter [9, 10]. In the same way, males have testicular inactiveness and subsequent proliferation of spermatogonia in winter, prior to beginning of active spermatogenesis in spring [9].

In the present study, the authors tried to clarify environmental factors responsible for the rapid gonadal growth in spring. The orange-red type medaka, ready for the rapid gonadal growth and therefore fully responsive to environmental cues, was used as material. In Experiment 1, medaka reached full gonadal maturation under constant water temperature of 14°C and above, regardless of photoperiod. This was further confirmed by the results of Experiment 2, which eliminated possible involvement of a critical daylength as short as 4 hours. These results clealy indicate that the rising temperature only is responsible for the rapid gonadal growth and subsequent beginning of spawning season in spring.

Effects of photoperiod on the beginning of gonadal maturation in combination with water temperature have not been examined well in the orange-red type medaka. Chan [18] showed necesity of long daylength or appropiate interruption of dark period for maturation and spawning of the orange-red type medaka. But her fish had been kept under artificial conditions till experiments, and maturational stages of initial controls were younger than those used in the present study. As shown already in cyprinids [21–24] and the stickleback [25], ovarian response to environmental manipulations has turned out to vary with the maturational stages in the orange-red type medaka (in preparation). This coincides with Chan's results in the necessity of long daylength for maturation and spawning of females initially in early or middle of yolk-vesicle stage. Egami and Hosokawa [10] transferred the orange-red type medaka maintained under natural temperature and photoperiod to aquaria at 23 to 26°C in various seasons. From

October to February, the gonads developed by the treatment without photoperiod change, and the females began to lay eggs earlier than those under natural condition. Their results agree with ours in the importance of rising temperature in spring, but not for the other seasons due to unidentifed reason.

In the wild type medaka, we found that the rising temperature only is responsible for the beginning of spawning season by the same methods as ones in this study using the fish obtained from Ushikunuma pond, Ibaraki Pref. (unpublished data). But Yoshioka [15] indicated that long daylength is indispensable for maturation and spawning in spring in the wild type medaka whose ovary initially contained early yolk-globule state oocytes. This is inconsistent with the present results or with our unpublished data on the wild type medaka. Yoshioka's material was obtained from a pond in Hokkaido (northern Japan), in which changes in photoperiod or temperature is different from those around Tokyo. Difference in environmental conditions during immature period may cause different gonadal response to environmental manipulations in advanced stages. On the other hand, Sawara and Egami [19] reported possible existence of racial difference in photoperiodic response of gonads, which may also be a reason of the inconsistency. To show racial difference clearly, it must be necessary to use material from different localities, which is in the same reproductive stage and has been reared under the same environment.

Ovarian regression was observed in 14°C groups at the end of Experiment 1. The reason of the regression is not clear. But according to Shiraishi *et al.* [26], some females cease spawning after several weeks of daily spawning under constant photoperiod-temperature regimes. The same phenomenon may occur after daily spawning under some experimental conditions employed here. It is also possible that 14°C is too low for maintenance of gonadal maturation.

Observation of yolk globule accumulation in 10 and 12°C groups, and not in 8°C group, suggests two phases of yolk globule accumulation: fast accumulation at above 14°C, slow accumulation at 10 to 14°C. In goldfish Yamazaki [27] obtained similar results by observing slow yolk globule accumulation at 15°C, and fast accumulation at 20 and 25°C in early spring. It is also known that female goldfish can mature at 13 to 14°C, but can not ovulate without temperature rise to 20°C [28]. It will be necessary to ascertain the slow yolk globule accumulation of medaka at 10 and 12°C with longer rearing for understanding relationship between water temperature and maturation or ovulation.

ACKNOWLEDGMENT

The authors are grateful to Dr. K. Aida for his advice during this work. Part of this study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan

REFERENCES

1 Sundararaj, B. J. and Vasal, S. (1976) Photoperiod and temperature control in the regulation of reproduction in the female catfish *Heteropneustes fossilis*. J. Fish. Res. Board Can., **33**: 959–973.

2 Crim, L. W. (1982) Environmental modulation of annual and daily rhythms associated with reproduction in teleost fishes. Can. J. Fish. Aquat. Sci., **39**: 17–21.

3 Hanyu, I., Asahina, K., Shimizu, A., Razani, H. and Kaneko, T. (1983) Environmental regulation of reproductive cycles in teleosts. Proc. 2nd N. Pac. Aquaculture Symp., 173–188.

4 Peter, R. E. and Crim, L. W. (1979) Reproductive endocrinology of fishes: Gonadal cycles and gonadotropin in teleosts. Ann. Rev. Physiol., **41**: 323–335.

5 Peter, R. E. (1981) Gonadotropin secretion during reproductive cycles in teleosts: Influences of environmental factors. Gen. Comp. Endocrinol., **45**: 294–305.

6 De Vlaming, V. L. (1972) Environmental control of teleost reproductive cycles: a brief review. J. Fish. Biol., **4**: 131–140.

7 Yamamoto, T. (1975) Systematics and zoogeography. In "Medaka (Killifish), Biology and Strains". Ed. by T. Yamamoto, Keigaku Publ. Co., Tokyo, pp. 17–29.

8 Yamamoto, T. (1975) Introductory remarks on the medaka. In "Medaka (Killifish), Biology and Strains". Ed. by T. Yamamoto, Keigaku Publ. Co., Tokyo, pp. 1–16.

9 Awaji, M. and Hanyu, I. (1987) Annual reproductive cycle of the wild type medaka. Nippon Suisan Gakkaishi, **53**: 959–965.

10 Egami, N. and Hosokawa, K. (1973) Responses of the gonads to environmental changes in the fish, *Oryzias latipes*. In "Response of Fish to Environmental Changes". Ed. by W. Chavin, Charles C. Thomas, Springfield, pp. 279–301.

11 Nishikawa, S. (1956) Histological observations on the seasonal spermatogenetic cycle of a medaka, *Oryzias latipes* (T & S). Zool. Mag., **65**: 203–206.

12 Yamamoto, K. and Yoshioka, H. (1964) Rhythm of development in the oocyte of the medaka, *Oryzias latipes*. Bull. Fac. Fish. Hokkaido Univ., **XV**: 5–24.

13 Yoshioka, H. (1962) On the effects of environmental factors upon the reproduction of fishes. I. the effects of day-length on the reproduction of the Japanese killifish, *Oryzias latipes*. Bull. Fac. Fish. Hokkaido Univ., **XIII**: 123–136.

14 Yoshioka, H. (1963) On the effects of environmental factors upon the reproduction of fishes. 2. Effects of short and long day-length on *Oryzias latipes* during spawning season. Bull. Fac. Fish. Hokkaido Univ., **XIV**: 137–151.

15 Yoshioka, H. (1966) On the effects of environmental factors upon the reproduction of fishes. 3. The occurrence and regulation of refractory period in the photoperiodic response of medaka, *Oryzias latipes*. J. Hokkaido Univ. Education, Sec. II (B), **17**: 23–33.

16 Yoshioka, H. (1970) On the effects of environmental factors upon the reproduction of fishes. IV. Effects of long photoperiod on the development of ovaries of adult medaka, *Oryzias latipes*, at low temperatures. J. Hokkado Univ. Education, Sec. II (B), **21**: 14–20.

17 Yoshioka, H. (1971) On the effects of environmental factors upon the reproduction of fishes. The significance of combinations of light and dark periods in photoperiodic response of the ovaries of medaka, *Oryzias latipes*, in out-of-breeding seasons. Seibutu kyozai, **8**: 76–82.

18 Chan, K. K.-S. (1976) A photosensitive daily rhythm in the female medaka, *Oryzias latipes*. Can. J. Zool., **54**: 852–856.

19 Sawara, Y. and Egami, N. (1977) Note on the differences in the response of the gonad to the photoperiod among populations of *Oryzias latipes* collected in different localities. Annot. Zool. Japon., **50**: 147–150.

20 Rika nenpyo (Chronological Scientific Tables) (1984), ed by Tokyo Astronomical Observatory, Maruzen Co., Ltd., Tokyo, pp. 12–16.

21 Asahina, K. and Hanyu, I. (1983) Role of temperature and photoperiod in annual reproductive cycle of the rose bitterling *Rhodeus ocellatus ocellatus*. Nippon Suisan Gakkaishi, **49**: 61–67.

22 Shimizu, A. and Hanyu, I. (1982) Environmental regulation of annual reproductive cycle in a spring-spawning bitterling *Acheilognathus tabira*. Nippon Suisan Gakkaishi, **48**: 1563–1568.

23 Razani, H. and Hanyu, I. (1986) Annual reproductive cycle of 2–3 years old female goldfish and its artificial modification by manipulations of water temperature and photoperiod. Nippon Suisan Gakkaishi, **52**: 965–969.

24 Razani, H. and Hanyu, I. (1986) Effects of continued short photoperiod at warm temperature and following changes of regimes on gonadal maturation of goldfish. Nippon Suisan Gakkaishi, **52**: 2061–2068.

25 Baggerman, B. (1972) Photoperiodic responses in the stickleback and their control by a daily rhythm of photosensitivity. Gen. Comp. Endocrinol. Suppl., **3**: 466–476.

26 Shiraishi, M., Tanaka, S. and Deguchi, Y. (1977) The number of eggs spawned by himedaka, *Oryzias latipes*, under artificial photoperiod. Bull. Coll. Agr. Vet. Med., Nihon Univ., **35**: 315–320.

27 Yamazaki, F. (1965) Endocrinological studies on the reproduction of the female goldfish, *Carassius auratus* L., with special reference to the function of the pituitary gland. Mem. Fac. Fish. Hokkaido Univ., **XIII**: 1–64.

28 Yamamoto, K., Nagahama, Y. and Yamazaki, F. (1966) A method to induce artificial spawning of goldfish all through the year. Nippon Suisan Gakkaishi, **32**: 977–983.