ANNOTATIONES ZOOLOGICAE JAPONENSES
Volume 50, No. 4—December 1977

Published by the Zoological Society of Japan

Studies on Oocyte Maturation of the Medaka, *Oryzias latipes*
IV. Effect of Temperature on Progesterone- and Gonadotropin-Induced Maturation

With 6 Text-figures

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ABSTRACT The effect of temperature on the ability of isolated oocytes of the medaka, *Oryzias latipes*, both to respond to maturational stimulation by progesterone and gonadotropin and to mature following stimulation at normal temperature was examined. Maturation was induced in part of the oocytes exposed to progesterone or pituitary hormones at low temperature. The rate of germinal vesicle breakdown (GVBD) is increased with increasing temperature in oocytes exposed to progesterone at temperatures below 40°C, but decreased at higher temperatures and fell to 0 at 44°C. The enlargement of the oocytes and the rate and initiation of GVBD *in vitro* were dependent on the temperature during incubation after exposure of the oocytes to progesterone. The optimum temperature for post-treatment incubation was found to be 30°C.

These experiments indicate that in this fish, oocyte maturation can take place within 24 hours only with temperatures between 20° and 39°C, and explain that the daily cycle of oviposition can be maintained only in this range of temperatures.

Under natural conditions, the fresh-water fish, *Oryzias latipes* has an annual reproductive cycle in which the female usually breeds for about 6 months during the spring and summer (cf. Briggs and Egami, 1959; Yamamoto, 1975). During reproductive cycle, the ovaries enlarge and large yolky oocytes that are at growing and maturing stages of oogenesis appear. In the winter, the ovaries are small and have only small oocytes that have not reached the yolk formation stage. Small winter ovaries can be induced to develop by keeping the fish at the temperature characteristic of the breeding season (Egami, 1954 a, b; Iwamatsu, 1973). This indicates that the water temperature is an important environmental factor in determining the annual reproductive cycle of this fish (Rugh, 1941; Yamamoto, 1949; Yamao *et al.*, 1950; Egami, 1954 a). According to Egami (1959), if fish that had spawned every morning were cooled to a lower temperature, a delay in the subsequent oviposition was observed which increased with the length of the period of cooling. This delay was due to the inhibitory effect of low temperature on the action of the pituitary

substance that stimulates ovarian growth (Egami, 1954 a). However, the effect of temperature on the oocyte maturation process that is induced by steroid and pituitary hormones had not yet been investigated. We have therefore studied the effects of temperature on the actions of pituitary hormone and progesterone in the process of *in vitro* oocyte maturation.

Material and Methods

Mature medaka, *Oryzias latipes* were purchased from a local fish farmer (Yatomi, Aichi Prefecture). Females were bred by keeping them in an aquarium (26± 0.5°C) with males for more than two weeks before use. The aquarium was illuminated (more than 1,000 lux) from 15: 00 to 5: 00 in a dark room. Most of the females spawned from 14: 00 to 16: 00 every day. Twenty-40 oocytes (800–950 μ) were dissected out of an ovary of each female 22–23 hours before spawning. These oocytes were not capable of initiating maturation *in vitro* with culture in Earle's Medium 199 (EM 199, Dainipponseiyaku, Osaka; pH adjusted with M/2 $NaHCO_3$ to 7.3) containing no exogeneous hormone (Iwamatsu, 1974; Yamauchi, 1974). To induce maturation, the oocytes were pre-incubated in the presence of 10 μg/ml of progesterone for 30 minutes or 200 IU/ml of pregnant mare serum gonadotropin (PMS; Serotropin, Teikoku-zoki Co.) for 10 hours. They were then rinsed twice with EM 199 supplemented with 50 μg/ml of penicilline K and 50 μg/ml of streptomycin sulfate and cultured for intervals varying from 18 hours to 160 hours.

First, to test the effect of temperature during the hormone-treatment period on oocyte maturation, oocytes were incubated in fresh medium (25°C) after they were pre-incubated at various temperatures in medium containing the hormone. Second, the effect of incubation temperature on oocyte maturation was examined by incubating oocytes at various temperatures after exposure to the hormone at 25°C. In order to minimize the variation among oocytes, oocytes obtained from an ovary were divided into experimental groups (5–10 oocytes in a culture dish) as evenly as possible. The outer envelope of the germinal vesicle (GV) disappears at the initial step of GVBD of *Oryzias* oocytes (Iwamatsu, 1965). The mean value of GVBD at given temperature was obtained from experiments repeated more than 3 times. The time of GVBD was determined as the time when 50% of the oocytes used showed complete disappearance of the nuclear membrane ($GVBD_{50}$). During incubation, the diameter of each oocyte with its covering of follicle cells was measured.

Results

1) *The effect of temperature during hormone-treatment period on oocyte maturation*

a. *Progesterone*

When 73 oocytes were treated with hormone at temperature above 43°C for 30 minutes, few survived for 12 hours. When oocytes were incubated at 25°C for 18

hours after progesterone-treatment at various temperatures ranging from 0 to 43°C, GVBD was induced. The frequency of GVBD in these oocytes increased with increasing temperatures up to 40°C (Fig. 1). There was little or no difference in the size of these mature oocytes. The most striking incidences of oocyte enlargement and GVBD were observed between 6 and 12 hours after hormone treatment.

b. *Gonadotropin*

Oocytes (560) were pre-incubated in EM 199 (25°C) containing 200 IU/ml of PMS for various intervals, and then after rinsed were incubated in fresh EM 199 until the presumed ovulation time. Oocytes that were incubated in the presence of the hormone for more than 5 hours underwent maturation with further incubation in the absence of the hormone. Of 35 oocytes pre-incubated for 10 hours, about 50% showed GVBD by the presumed ovulation time (Fig. 2).

In the next experiment, 370 oocytes were incubated for 10 hours in the presence of the hormone at various temperatures and then rinsed and incubated in EM 199 (25°C) for 14 hours. Maturation was induced in oocytes that were treated with the hormone at temperatures in the range of 15° to 38°C. At temperature below 10°C, oocytes responded poorly to the hormone showing little maturational reaction (Fig. 3).

2) *The effect of temperature during incubation after progesterone-treatment on oocyte maturation*

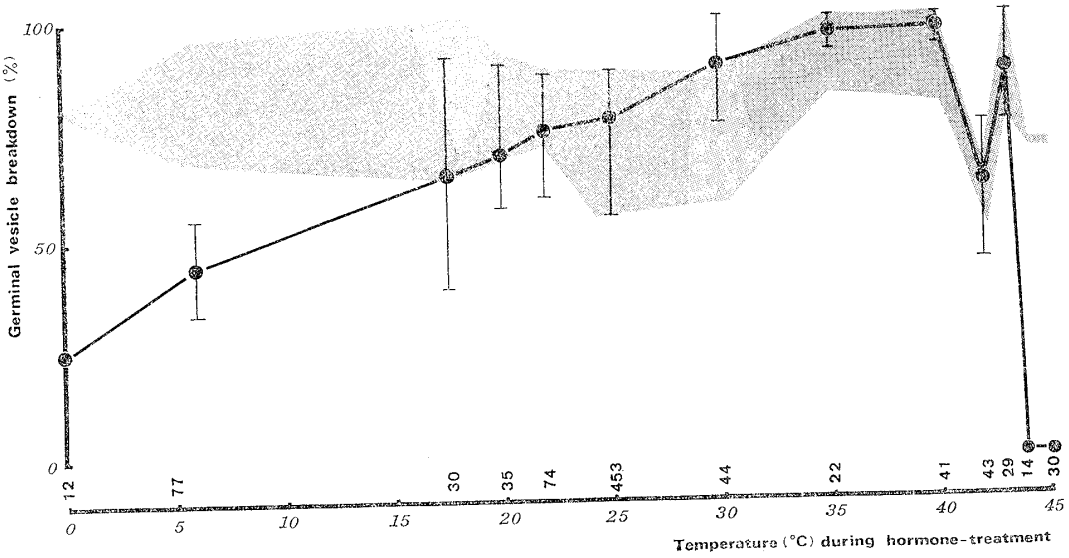

Fig. 1. Relationship between temperature during hormone-treatment period and rate of GVBD *in vitro* of *Oryzias* oocytes. GVBD was examined after 18 hours-incubation. Each point represents the mean and the range (vertical line) of the percentage of GVBD. Shaded portion indicates the range of the percentage of GVBD in the control which was exposed to hormone at 25°C. Numbers below each data point indicate number of oocytes used.

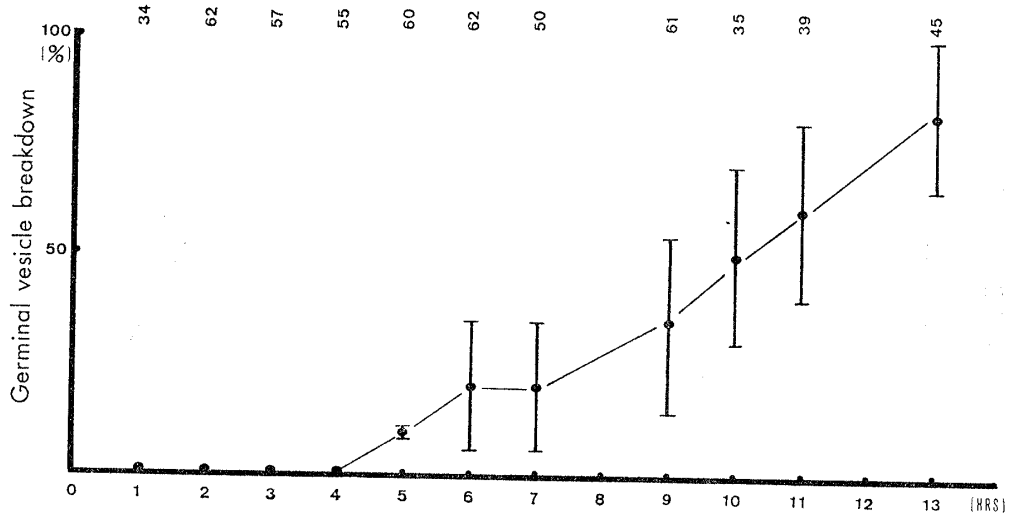

Fig. 2. Duration of exposure to gonadotropin and percentage of GVBD at 25°C. Each point represents the mean±S.E. (vertical line) of the percentage of GVBD. Numbers above each data point indicate number of oocyte used.

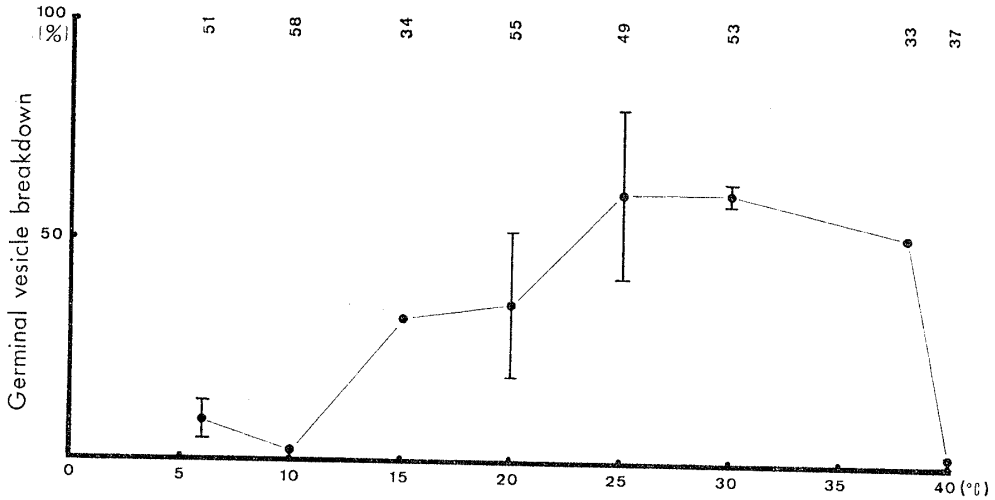

Fig. 3. Relationship of temperature during incubation after exposure to gonadotropin to GVBD. Each point represents the mean±S.E. (vertical line) of the percentage of GVBD. Numbers above each point indicate number of oocytes used.

When 1913 oocytes were incubated at temperatures ranging from 6° to 42°C, most of them survived at 39°C or less but not at 40°C or more. The size of the oocytes at the end of 12 hours-incubation varied depending on the temperature during incubation (Fig. 4). At low temperatures (6–8°C), enlargement of the oocytes was noticeably delayed.

The frequency of GVBD at various temperatures during incubation is summarized in Fig. 5. At temperatures above 40°C and below 18°C, no oocyte showed GVBD within 18 hours from the start of incubation. However, when the incubation

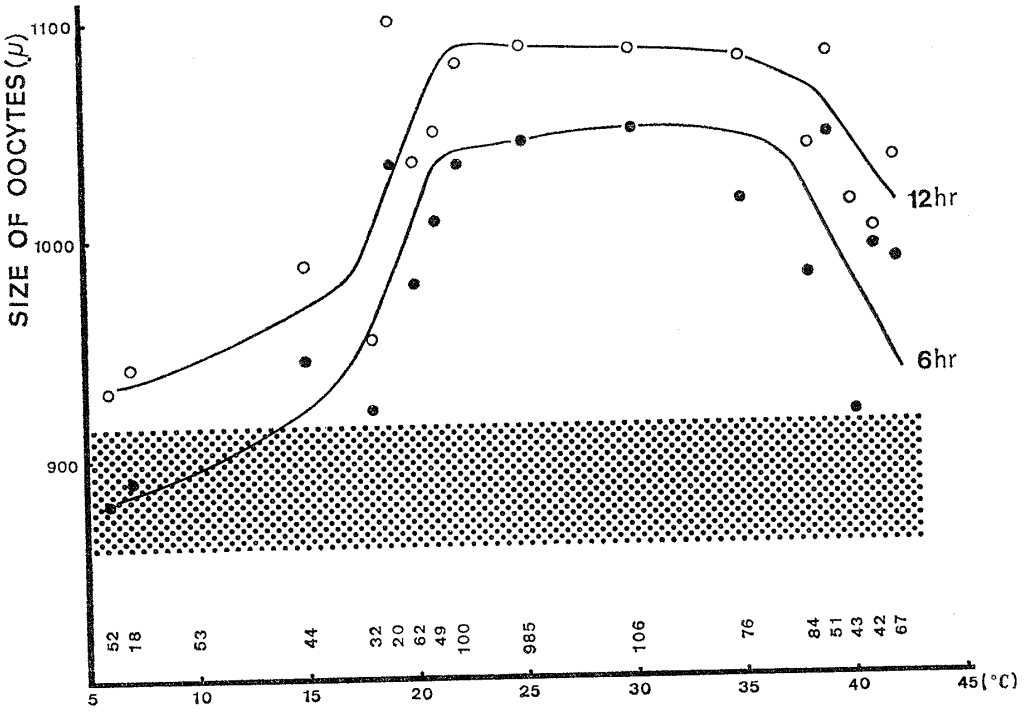

Fig. 4. Relationship between temperature and size obtained by oocytes during incubation. Shaded portion is the standard deviation from the mean size of the oocytes before incubation. Numbers below each data point indicate number of oocytes used.

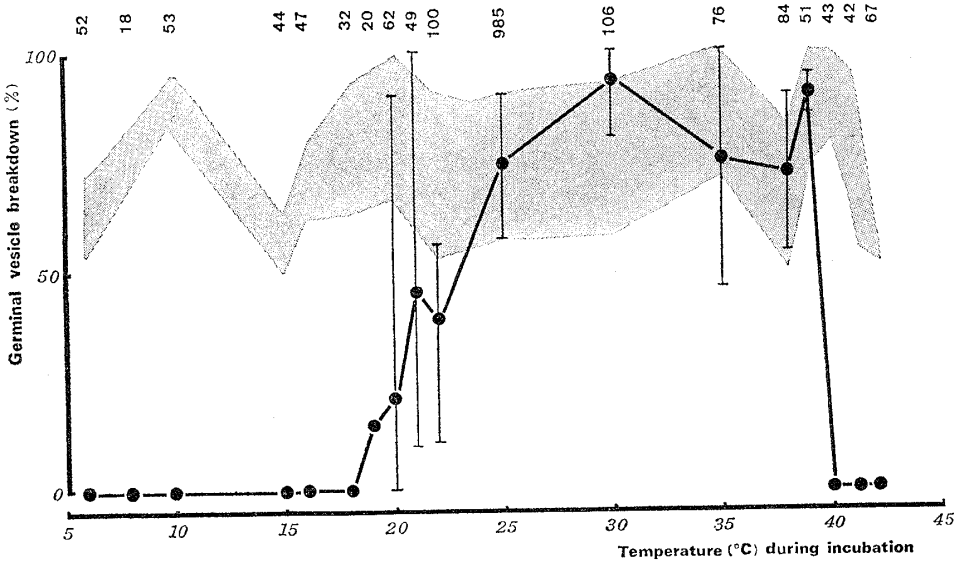

Fig. 5. Relationship between temperature during incubation and rate GVBD of *Oryzias* oocytes. GVBD was examined after 18 hours-incubation. Each point represents the mean and the range (vertical line) of the percentage of GVBD. Shaded portion indicates the range of the percentage of GVBD in the control which was incubated after hormone-treatment. Numbers above each data point indicate number of oocytes used.

time was increased to 114 hours (about 5 days), GVBD was observed in some oocytes (about 27% at 10°C). The frequency of GVBD increased as the temperature increased from 10° to 30°C. No GVBD could recognize at 6° and 8°C.

The time required for $GVBD_{50}$ was shortest in the temperature range between 30° and 38°C. It was slightly greater at 39°C and increased rapidly at temperatures below 25°C. Also, the initiation of GVBD was earlier as the temperature increased from 10° to 30°C. GVBD at the low temperatures of 19°C, 18°C, 16°C and 10°C began at 18 hours (15%), 24 hours (3%), 36 hours (10%) and 96 hours (3%) respectively (Fig. 6).

Discussion

The medaka under a long photoperiod can lay eggs until the water temperature declines to about 13–14°C (Yoshioka, 1962). According to Egami (1954 a), the stimulative effect of the pituitary substance on ovarian growth was hardly observable at temperatures below 13°C. At such a low temperature, oocyte maturation is not induced *in vitro* in the presence of gonadotropin, as shown in the present experiments. In these cases, the ineffectiveness of gonadotropin in inducing ovarian growth and oocyte maturation is probably due to poor responsiveness of the oocytes to the gonadotropin at the low temperatures. Oocytes stimulated by progesterone and gonadotropin are able to mature *in vitro* when the temperature is more than 10°C. From these observations, it is inferred that the threshold of water temperature for the induction of *in vivo* oocyte maturation in this fish is within the limits of 8–13°C.

Temperature has an influence on the daily cycle of oviposition (Egami, 1959). The interval between ovipositions shortens during the reproductive season as the water temperature rises (Yoshioka, 1962). The interval between ovipositions reveals the period required for the maturation of the oocytes, which shortens with

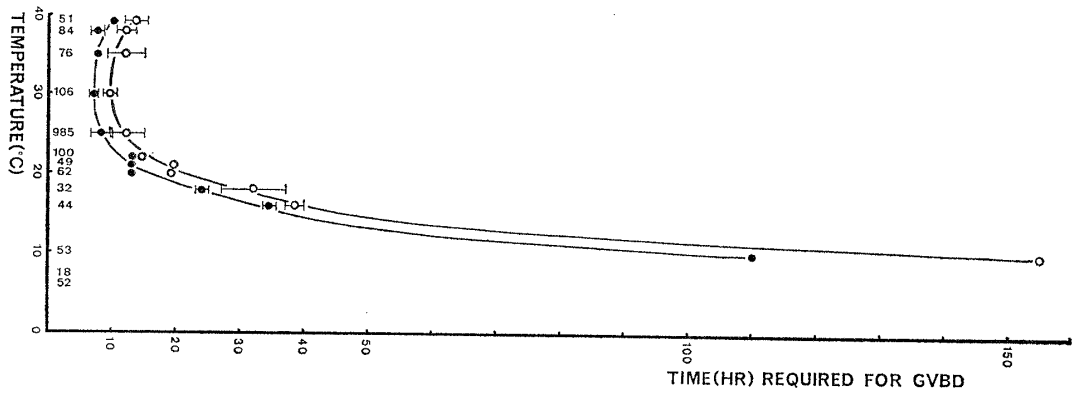

Fig. 6. Effect of temperature during incubation on time required for GVBD in *Oryzias* oocytes. Oocytes were treated with 10 μg/ml progesterone at 30°C for 30 minutes. —○—, Time required for $GVBD_{50}$. —●—, Time required for initiation of GVBD.

an increase in temperature. Below 19°C, oocytes require considerably more than 24 hours for maturation, as shown by the present observation *in vitro*. Fish at such low temperatures should not exhibit the daily cycle of oviposition. Actually, although oocytes develop to the maturation stage at temperatures from 11.5° to 21°C, all fish lay eggs at intervals of 48 or 72 hours (Yoshioka, 1962, 1963). This failure in the daily cycle of oviposition seems to cause a delay in oocyte maturation at temperatures below 19°C.

An effect of temperature during the maturation process after exposure to progesterone has been ascertained in the present experiment. Temperature may affect several steps of oocyte maturation after hormonal stimulation: a change in ATPase activity (Morrill *et al.*, 1971), the uptake and intracellular binding of calcium (Merriam, 1971 a, b), the synthesis of a polypeptide or protein (see Smith, 1972; Baltus *et al.*, 1973; Brachet *et al.*, 1974), the formation and conformational change of an inactive maturation-promoting factor (MPF) to its active form (Wasserman & Masui, 1975), and dissolution of the nuclear membrane. The uptake and intracellular binding of calcium are inhibited by a reduction in temperature from 30° to 10°C (Hurwitz *et al.*, 1975) but the activation of (Na^+-K^+)-ATPase by cation is less affected (Charnock *et al.*, 1975). These results suggest an inhibitory effect of low temperature on calcium transport. At present, we have little information on the temperature-dependent steps of the maturation process after stimulation by progesterone.

REFERENCES

Baltus, E., J. Brachet, J. Hanocq-Quertier and E. Hubert, 1973. *Differentiation*, **1**: 127–143.
Brachet, J., E. Baltus, A. De Schutter, E. Hanocq, J. Quertier, E. Hubert, S. Iacobelli and G. Steinert, 1974. *Molec. Cell. Biochem.*, **3**: 189–205.
Briggs, J. C., and N. Egami, 1959. The medaka (*Oryzias latipes*). A commentary and a bibliography. *J. Fish. Res. Bd. Canada*, **16**: 363–380.
Charnock, J. S., A. F. Almeida and Rebecca To, 1975. *Arch. Biochem. Biophys.*, **167**: 480–487.
Egami, N., 1954 a. *Annot. zool. Japon.*, **27**: 13–18.
———— 1954 b. *Endocrinol. Jap.*, **1**: 75–79.
———— 1959. *J. Fac. Sci. Tokyo Univ.*, (IV), **8**: 539–548.
Hurwitz, L., G. Debbas and S. Little, 1975. *Molec. Cell. Biochem.*, **8**: 31–41.
Iwamatsu, T., 1965. *Embryologia*, 327–336.
———— 1973. *Bull. Aichi Univ. Educ.*, **22**: 73–88.
———— 1974. *Annot. zool. Japon.*, **47**: 30–42.
Merriam, R. W., 1971 a. *Exptl. Cell Res.*, **68**: 75–80.
———— 1971 b. *Ibid.*, **68**: 81–87.
Morrill, G. A., A. B. Kostellow and J. B. Murphy, 1971. *Exptl. Cell Res.*, **66**: 289–298.
Rugh, R., 1941. Experimental Embryology. New York University Press.
Smith, L. D., 1972. Oogenesis (Biggers J. D., and W. Schuetz, eds.), 227–239. University Park Press, Baltimore.
Wasserman, W. J., and Y. Masui, 1975. *Exptl. Cell Res.*, **91**: 381–388.
Yamamoto, T., 1949. Experiments in Animal Physiology. Kawade-shobô, Tokyo. (In Japanese.)

Yamamoto, T., 1975. Series of Stock Culture in Biological Field. Medaka (Killifish) Biology and Strains. Keigaku Publishing Company, Tokyo.
Yamao, Y., N. Egami and A. Saito, 1950. *Collect. & Breed., Tokyo,* **12**: 108–110. (In Japanese.)
Yoshioka, H., 1962. *Bull. Fac. Fish. Hokkaido Univ.*, **13**: 123–136.
———— 1963. *Ibid.*, **14**: 137–171.