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Short Paper

Relationship between temperature and embryonic period of Arabesque greenling *Pleurogrammus azonus*

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Abstract: To clarify the relationship between temperature and embryonic period of Arabesque greenling *Pleurogrammus azonus*, we incubated eggs at the water-temperature conditions which approximate the range during the spawning season. The relationship between the mean rearing temperature (*T;* °C) and the mean embryonic period (*D;* days) was as follows: $D = 170.63 - 20.66T + 0.85T^2$.

Key words: Arabesque greenling; Embryonic period; *Pleurogrammus azonus*; Temperature

Natural mortality rates of fish are highest in early life (Houde 1997). In general, newly hatched larvae have a low swimming ability and are unable to avoid drifting to unfavorable areas. Consequently, one of the critical factors for survival is when and where they hatch (i.e. the match-mismatch hypothesis: Cushing 1972). Embryonic period has been related to temperature, except under conditions of hypoxia and extreme pollution (Alderdice and Forrester 1971; Greig et al. 2007). For species with demersal egg clusters that have a long embryonic period, little temporal difference is found within the spawning ground, however inter-annual variations in the timing of spawning have been observed (i.e. Gunnarsson et al. 2016). Therefore, the timing of spawning and thereafter hatching and also first feeding may be crucial for recruitment success. However, information of the relationship between temperature and the egg incubation period has not yet been applied to recruitment analysis in the field.

Arabesque greenling *Pleurogrammus azonus* is one of the most important fishery resources in Northern Japan, and its population size has decreased around Hokkaido since 2010 (Takashima et al. 2016). For coastal areas of Hokkaido, distribution and migration of *P. azonus* in the early life stage have been studied (Arabesque Greenling Research Group 1983) and the spawning grounds have been found to be located in coastal waters with rocky substrata in the Sea of Japan around Hokkaido and the Tsugaru Strait (Hoshino et al. 2009). Previous studies have estimated the spawning period using observations of ovarian maturation (Kambara et al. 1953; Kambara 1957; Takashima et al. 2016). However, female *P. azonus* spawn multiple times during a single spawning season (Takemura 1952; Kambara 1957; Suzuki and Hioki 1975; Yusa 1967) making it difficult to determine when is the spawning date of individuals which successfully recruit to the fishery. Marannu et al. (2017) have already validated the daily otolith increment, so we will be able to estimate hatch dates enable a better understanding factors related to successful recruitment. Furthermore as their main spawning ground has been revealed (Hoshino et al. 2009), we can use data of daily sea-surface (Japan Meteorological Agency 2017a) and at a depth of 50 m water temperature (Japan Meteorological Agency 2017b) near the spawning ground formed in coastal reefs in the Sea of Japan around Hokkaido (Irie 1983; Hoshino et al. 2009). If we can establish the relationship between temperature and the embryonic period for this species, we will be able to estimate the spawning date for recruited individuals. In a previous study, the incubation period was estimated to be about two months at a water temperature of approximately 10°C without aeration (Yusa 1957), but little is yet known about the relationship between temperature and the embryonic period of this species. This study investigated the relationship between water temperature and the embryonic period of *P. azonus* to enable prediction of the spawn date of successfully recruited individuals.

Broodstock were obtained from Matsumae, Setana, and Shimamaki, coastal areas of the Sea of Japan in

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Table 1. Broodstock sampling and body size, date of artificial fertilization, egg diameter (mean ± SD), percentage of fertilization (%) at 12 hours after fertilization (HAF), percentage of normal development (%) at 2-3 days after fertilization (DAF), water temperature (mean ± SD), and number of egg of *P. azonus* reared in the laboratory

Broodstock sampling			TL(mm)		Date	Egg size (mm)	12HAF	2-3DAF	Water temperature (°C)		Egg mass
Site	Year	Date	Female	Male	Fertilization	Diameter (mean ± SD)	Fertilized (%)	Normal (%)	Target	Real (mean ± SD)	Number of eggs
Setana	2013	Dec. 4	363	342	Dec. 6	2.3 ± 0.13	99	100	10	10.2 ± 0.35	238
									12	12.0 ± 0.21	318
Matsumae	2014	Dec. 4	418	356	Dec. 4	2.3 ± 0.11	100	84	8	8.0 ± 0.20	172
									10	10.0 ± 0.20	428
			350	358	Dec. 8	2.4 ± 0.06	99	94	8	7.9 ± 0.38	57
									10	10.0 ± 0.20	276
Shimamaki	2015	Nov. 15	320	372	Nov. 24	2.4 ± 0.04	100	92	8	8.0 ± 0.10	159
Matsumae		Dec. 11	403	406	Dec. 11	2.4 ± 0.05	86	88	10	9.6 ± 0.32	997
			383	385	Dec. 23	2.4 ± 0.06	100	100	8	7.9 ± 0.28	182

Hokkaido, Japan (Table 1). The broodstock were reared in two circular shaped tanks (1 k*l* seawater volume) at the temperature range of 11–12°C and then ovigerous females were used for artificial fertilization. Artificial fertilized eggs of *P. azonus* (egg mass) were placed in a 100 *l* circular shaped tank at a temperature range of 11–12°C. Thereafter, the egg clusters were incubated using hatching jar sets at three temperatures (controlled at 8, 10 and 12°C using a 200 W heater) in 30 *l* tanks. Sand filtered seawater was supplied continuously into each tanks and water exchange rate was 120%/day. Every 15 : 00–16 : 00, we counted the number of hatched individuals, and measured the water temperature (Table 1). Salinity (using EC meter, Model EC300, YSI-Nanotech Ltd., Japan), dissolved oxygen (using DO meter, Model 55A-12, YSI/Nanotech Ltd., Japan), and pH (using pH meter, HM14P, DKK-TOA Co., Japan) were within the range 33–34 ppt, and 98.3–98.6%, and 8.1–8.2, respectively. Four types of models (Power law model: $D = \mathrm{a}T^{\mathrm{b}}$; Polynomial model: $D = \mathrm{c} + \mathrm{d}T + \mathrm{f}T^2$; Exponential model: $D = \mathrm{g}{\cdot}\exp^{\mathrm{h}T}$; Réaumur's law model: $D = \mathrm{i} / (T - \mathrm{j})$, where D is the mean embryonic period in days, T is the mean incubation temperature, and a–j are constants) that have often applied to the relationship between temperature and embryonic period of fish (e.g. Brittain 1977; Hamel et al. 1997; Mihelakakis and Yoshimatsu 1998; Yang and Chen 2005; Hu et al. 2017) were calculated using the non-linear least squares regression procedure (MS-Excel solver routine). The model that had the minimum Akaike Information Criterion value (AIC) was judged to be the best data model (Akaike 1973).

Though the egg diameter of each batch was significantly different (one-way ANOVA, $p < 0.001$), no significant difference was found at the embryonic period among different egg diameter (one-way ANOVA, 8 and 10°C: $p = 0.10$, 0.14, respectively). Therefore, all replicate within each temperature setting were pooled. The mean embryonic period at 8, 10, and 12°C were 60 ± 1.0, 49 ± 1.3, and 44 ± 1.9 days (± standard deviation), respectively (Fig. 1). The AIC value of the Polynomial model was the lowest (33.8) among the four models (Power law model: 37.5; Exponential model: 41.0; Réaumur's law model: 38.1). The relationship between the mean rearing temperature (T; °C) and the mean embryonic period (D; days) was as follows: $D = 170.63 - 20.66T + 0.85T^2$ ($r^2 = 0.98$, $P < 0.001$, $n = 9$; Fig. 2). The range of embryonic period was estimated to be approximately 40–60 days in the field. The cumulative temperature unit (CTU = mean embryonic period × mean water temperature) was calculated to be 488 ± 21.0 day°C (mean ± standard deviation, $n = 9$). In this study, the mean embryonic period of 10°C was 49 days, though the embryonic period is about 60 days at a water temperature of approximately 10°C without aeration (Yusa 1957). Incubation of eggs at low dissolved oxygen concentrations resulted in an increase in the duration of embryonic development (Alderdice and Forrester 1971; Hamor and Garside 1976; Greig et al. 2007). All of the experiment, dissolved oxygen concentrations were retained over 98% with aeration, and there was no effect for shortage of oxygen. Differences in the results will be due to differences of dissolved oxygen. This study allows the estimation of the spawning date of fish to be estimated based on daily otolith rings count which enables the determination of their hatch date.

Not only the estimation of the date of hatching using otolith microstructure analysis, but also the estimation of the spawning date using the data of the relationship between temperature and the egg incubation period will enable estimation of the recruitment success for the fish that have long and multiple times spawn during a single spawning season. Data of the relationship between water temperature and the egg incubation period of fish have been useful to improve seed production of various fishes (e.g. Fukuhara 1990; Yang and Chen 2005), and in addition, the results will be useful to improve methods for the fisheries resource management in the field.

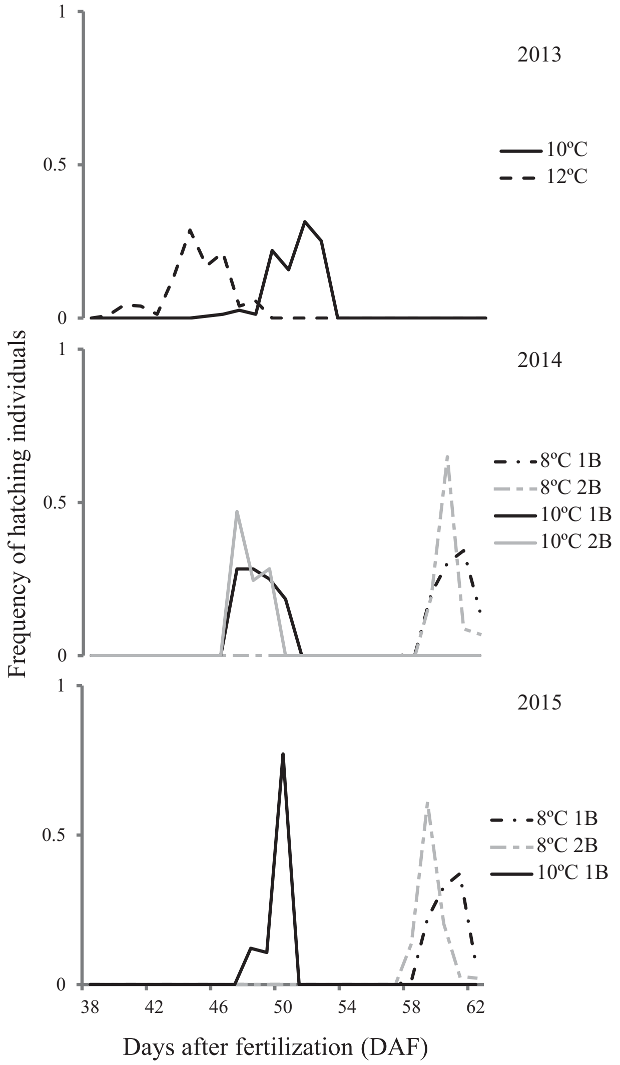

Fig. 1. Frequency of the hatching individuals of Arabesque greenling *Pleurogrammus azonus* under the different water temperature conditions (8, 10 and 12°C).

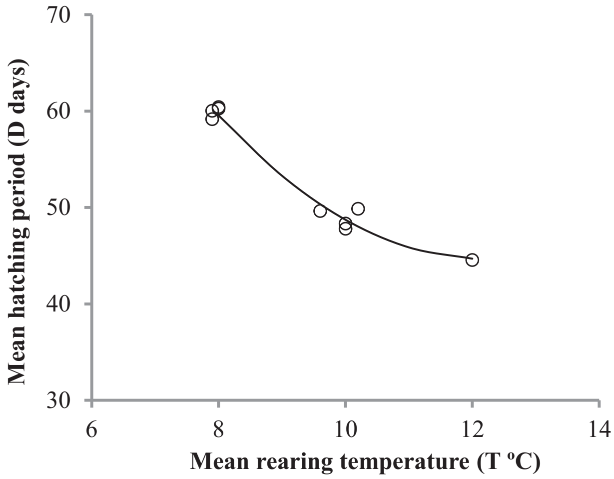

Fig. 2. Relationship between mean rearing temperature and the mean embryonic period. Regression formula is given adjacent to the respective mean regression line for the embryonic period model under different rearing temperature conditions.

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