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Short Paper

Effect of Water Temperature on Embryonic Development of Yellowfin Tuna *Thunnus albacares* Inhabiting the Eastern Pacific Ocean

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Abstract: Effect of water temperature on embryonic development of yellowfin tuna was investigated. Eggs were incubated at different water temperatures: 22, 24, 26℃ (Experiment-1) and 28, 30, 32℃ (Experiment-2). Developmental condition was observed and the elapsed time from the 2 or 4 cell stages until hatching was measured. Hatching occurred at 18.58 h for eggs incubated at 32℃. Eggs incubated at 22℃ stopped development after the morula (late cleavage) stage. Experiment-3 was designed to examine the relationship between hatching time and the incubation temperature, and was expressed by two regression lines which intersected at 26.9℃.

Key words: Yellowfin tuna; Embryonic development; Water temperature

Yellowfin tuna (YFT) *Thunnus albacares* inhabits the mixed layer of all the warm seas of the world except the Mediterranean Sea, and two stocks are recognized in the Pacific Ocean: the stock of the Eastern Pacific Ocean (EPO) and of the Western-Central Pacific Ocean (WCPO) (Lehodey and Leroy 1999). Although a commercially important species, information is limited to studies in captivity on the reproductive activities and the effects of physical variables on spawning dynamics or early life stage development in YFT (Margulies et al. 2007). Water temperature and salinity are factors which have a major effect on the development of marine teleosts (Kawahara et al. 1997). Margulies et al. (2007) estimated the egg stage duration of YFT at water temperatures between 23.3 and 30.0℃, and also reported the stage-specific egg and early larval development of YFT at 27℃, where embryonic-stage duration was 21.65 h. Wexler et al. (2011) further described the relationship of the egg stage duration of YFT and mean incubation temperatures between 20 and 33℃ as an inverse quadratic function; within this range they found the stage duration to be significantly longer at mean incubation temperatures lower than 22℃. Masuma (2013) investigated the effect of different incubation temperatures on YFT in Japan and indicated that an average evaluation index such as the temperature characteristics of Arrhenius's and Higurashi and Tauchi's formula (μ, a) and values of Q_{10} constants are much higher in YFT inhabiting the WCPO than in Pacific bluefin tuna (PBT) *T. orientalis*. However an extensive study of the temperature effect on the stage-specific embryonic development of eggs has not yet been conducted in YFT inhabiting the EPO.

In the present study, the effect of temperature on embryonic development of YFT was explored as a basis for early life history studies for resource management and for aquaculture. In the experiments, we observed several stages of embryonic development at different water temperatures and recorded the elapsed time until hatching. The results were compared with embryonic development of PBT (Miyashita et al. 2000). In addition, interspecific comparisons were made between YFT inhabiting the WCPO (Masuma 2013) and the EPO in order to describe the characteristics of both independent stocks.

The Inter-American Tropical Tuna Commission established the Achotines Laboratory in the Republic of Panama. A group of 15 wild-caught YFT broodstock were collected in coastal waters in the vicinity of the Achotines Laboratory during 2008–2010 (1.6–4.6 years old), and were reared in an indoor concrete circular tank of 1,300 m^3. This broodstock has spawned almost year-round on a near-daily basis. Fertilized eggs were collected shortly after spawning in the broodstock tank during June, 2011. The eggs were carefully rinsed in a plastic bucket and transferred to 20 *l*-volume

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Table 1. Embryonic developmental stage of yellowfin tuna *Thunnus albacares* and elapsed time (HH:MM) until hatching under different incubation temperatures

Developmental stages	Incubation temperature (℃)						Equation between time and incubation temperature $T = a + b\theta + c\theta^2 + d\theta^3$ (T = time: hr., θ = incubation temperature: ℃)
	22	24	26	28	30	32	
2 cell	-	-	-	0:00	0:00	0:00	
4 cell	0:00	0:00	0:00	0:25	0:25	0:20	
16 cell	0:50	0:30	0:30	0:35	0:35	0:35	$T = 51.909 - 5.555\theta + 0.1987\theta^2 - 0.0024\theta^3$ ($r^2 = 0.950$)
Morula (late Cleavage)	1:40	1:20	1:00	1:10	1:10	1:05	$T = 60.814 - 6.314\theta + 0.2218\theta^2 - 0.0026\theta^3$ ($r^2 = 0.919$)
Early gastrula		6:00	5:15	3:50	3:35	3:20	$T = -85.02 + 11.499\theta - 0.4643\theta^2 + 0.006\theta^3$ ($r^2 = 0.978$)
Appearance of embryonic shield		8:15	7:35	5:35	5:20	4:35	$T = -126.47 + 16.164\theta - 0.626\theta^2 + 0.0078\theta^3$ ($r^2 = 0.960$)
Appearance of Kupffer's vesicle		12:00	10:00	8:35	7:20	6:45	$T = 65.301 - 3.0748\theta + 0.0269\theta^2 + 0.0004\theta^3$ ($r^2 = 1.00$)
Beginning of heart beat		20:30	16:00	14:05	12:05	11:05	$T = 477.32 - 44.729\theta + 1.457\theta^2 - 0.0161\theta^3$ ($r^2 = 0.973$)
First hatch		25:15	19:45	17:50	16:20	15:20	$T = 939.3 - 91.743\theta + 3.052\theta^2 - 0.034\theta^3$ ($r^2 = 0.997$)
Hatching		29:15	22:00	20:50	20:05	18:35	$T = 1845.4 - 187.68\theta + 6.4312\theta^2 - 0.0734\theta^3$ ($r^2 = 0.994$)

experimental tanks. These tanks were provided moderate aeration during the experiment from a single diffuser. In Experiment-1 (Exp-1), fertilized eggs were obtained at a water temperature of 28.7℃ and at 28.5℃ in Experiment-2 (Exp-2), respectively. Other eggs were used to estimate the normal hatching rates measured in up-flowing 100 *l*-volume cylindrical tanks and the hatching rates were 98.9% (28.5℃) in Exp-1 and 92.6% (28.8℃) in Exp-2, respectively.

A total of 6 different temperatures were set (Exp-1: 22, 24 and 26℃; Exp-2: 28, 30 and 32℃) in order to describe the embryonic development and the incubation period until hatching at each temperature. Approximately 1,500 eggs were used for each experimental tank both in Exp-1 and Exp-2, and the elapsed time from the first observation until each embryonic stage was recorded. In Exp-1, the developmental stage at collection was 4-cell stage, whereas it was 2-cell stage in Exp-2. Ten to 20 eggs were sampled every 5–15 min, and their development was examined under a microscope using egg development descriptions of PBT (Miyashita et al. 2000). Determination of egg developmental stage was based on the stage observed for the majority of eggs (over 50%) in a single sample.

In Experiment-3, we further investigated the hatching time of YFT eggs (0.94 ± 0.02 mm in diameter and 0.22 mm of oil goblin) at 23–32℃ (at approximately 1℃ intervals) in order to estimate the relationship between incubation temperature and the time required from spawning until hatching by regression analysis. These eggs were collected in June, 2012 and similar methods of egg collection and incubation protocols of Exp-1 and 2 were used. In addition, temperature characteristics (μ, a) were calculated according to Arrhenius's formula (μ) and Higurashi and Tauchi's (1925) formula; $\ln D = K - a\,T$, where D denotes the time (h), T denotes the temperature (℃) and a, K denote the constant values.

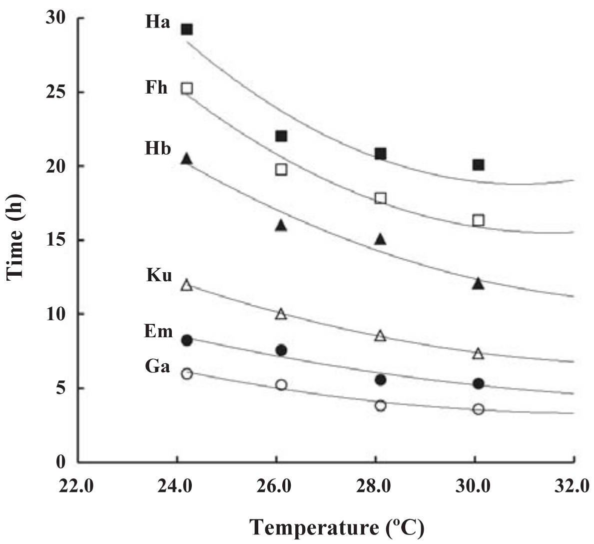

Fig. 1. Relationship between incubation temperature and embryonic developmental stages of yellowfin tuna *Thunnus albacares*. Ga, early gastrula stage; Em, appearance of embryonic shield; Ku, appearance of Kupffer's vesicle; Hb, beginning of heart beat; Fh; first hatch; Ha, hatching.

Q_{10} values of constants $\{Q_{10} = (T_2/T_1)^{[10/(\theta_1-\theta_2)]}\}$ were also calculated in order to compare these values with those of YFT inhabiting the WCPO (Masuma 2013).

Embryonic stages and elapsed time from the 2- and 4-cell stages until hatching (HH:MM) under different incubation temperatures is presented in Table 1. At 24℃, it took 0.5 h to proceed until the 16-cell stage, 20:30 h until the beginning of the heart beat stage, and 29:15 h until hatching. Elapsed time until hatching was shorter at higher incubation temperatures. The shortest elapsed time until hatching occurred at 32℃ (18:35 h). The acceleration of development at higher temperatures was much clearer after the gastrula stages to beginning of heart beat stage (Fig. 1). Hatching was observed at 24, 26, 28, 30 and 32℃, however, embryonic development stopped after the morula stage (late cleavage) at 22℃. The relationship between elapsed time until the first hatching and incubation temperature was expressed by the regression equation: $T = 0.2008\,\theta^2 - 12.327\,\theta + 207.899$ ($r^2 = 0.9458$, $n = 11$), where T represents time until first hatching (h), and θ represents incubation temperature (℃) (Fig. 2). As a result of converting the elapsed time at the completion of hatching into natural logarithm, two linear regression lines were obtained (Fig. 3). These two linear regression lines intersected at 26.9℃ and the coefficients [−0.1077 ($r^2 = 0.9448$, $n = 6$), −0.0288 ($r^2 = 0.667$, $n = 6$)] were found to be significantly different (ANCOVA, $P < 0.01$).

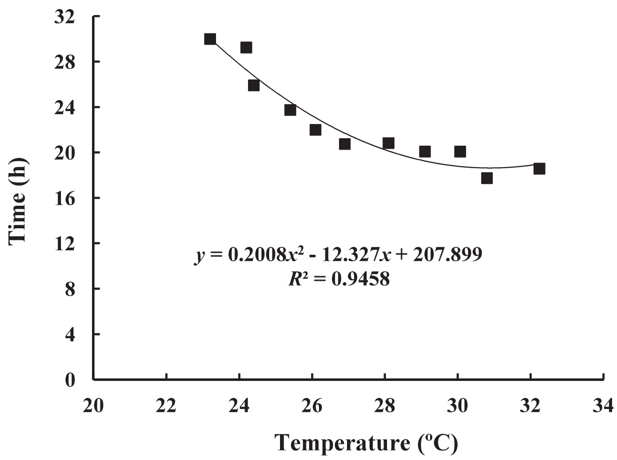

Fig. 2. Relationship between incubation temperature and elapsed time until hatching.

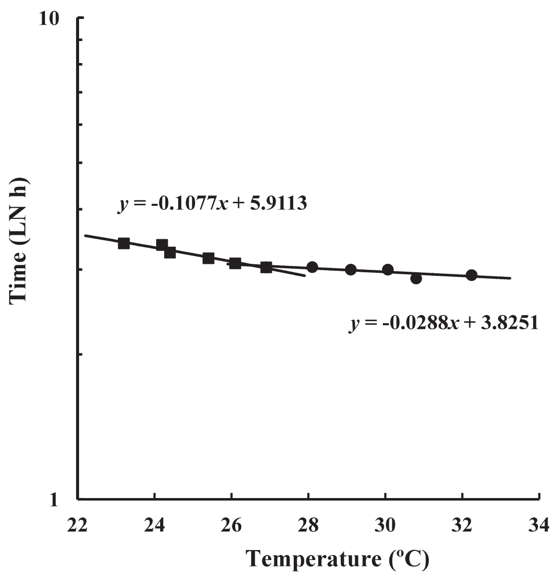

Fig. 3. Two linear regression lines derived from converting the elapsed time at the completion of hatching into natural logarithm and the inflection point of its relationship with incubation temperature.

The temperature characteristics and values of the constants for the relationship between incubation temperature and the time required for hatching are shown in Table 2. A comparison of μ, a, and Q_{10} values showed a tendency for higher values at incubation temperatures of 23.2–26.9℃ (μ: 18,959, a: 0.11, Q_{10}: 2.92) compared to those for 26.9–32.2℃ (μ: 4,986, a: 0.03, Q_{10}: 1.23).

This study elucidated the stage-specific development of YFT inhabiting the EPO, under wide range of temperatures. This information is useful in the study of early life history and aquaculture of tunas to estimate the hatching time and/or calculate spawning time. The embryonic development of YFT was similar to that of PBT (Miyashita et al. 2000); however, several differences were observed. For example, in the final stage of egg development shortly before hatching, the embryo covered three quarters of the yolk in YFT, whereas it covered the yolk completely in PBT. Regarding the relationship between incubation temperature and development, Miyashita et al. (2000) reported that the inflection point in the linear regression of incubation temperature and hatching time was 25℃ in PBT. The regression lines intersected at 26.9℃ in this study for YFT. The existence of an inflection point is related to enzyme reactions associated with hatching (Yasunaga 1988). This inclination of the inflection point at a higher temperature is possibly explained by the fact that YFT inhabit warmer water areas than PBT. In addition, the temperature characteristics (μ, a) and values of Q_{10} indicated that these values were higher at temperatures below the inflection point at 26.9℃ compared to those at 26.9–32.2℃, which is coincident with YFT inhabiting the WCPO (Masuma 2013). Therefore, the developmental speed of YFT inhabiting the EPO suggests that their eggs are also susceptible to lower temperatures. Masuma (2013) further reported that the average temperature constant values of YFT are much higher than those of PBT which is also confirmed in this study. Therefore, these results might confirm some

Table 2. The temperature characteristics (μ, a) and values of constants (Q_{10}) for relationship between incubation temperature and time required for hatching of YFT eggs

Incubation temperature (℃)	23.2–26.9	26.9–32.2	23.2–32.2
μ	18,959	4,986	9,774
a	0.11	0.03	0.05
Q_{10}	2.92	1.23	1.7

μ: temperature characteristic of Arrhenius's formula (Higurashi and Tauchi 1925)
a: temperature characteristic of Higurashi and Tauchi's formula (Higurashi and Tauchi 1925)

differences in distribution areas of PBT and YFT, and similarities in distribution of YFT stocks in the EPO and WCPO.

The embryonic development of YFT from fertilization to hatching at 27℃ was also investigated by Margulies et al. (2007) and Wexler et al. (2011) for the EPO, and these durations were similar to the present study (i.e. about 22–23.5 h at 26–27℃). On the other hand, Harada et al. (1980) reported that the time required for hatching of WCPO YFT was longer at 26:40–42:00 h at an average water temperature of 26.4℃. This may be explained by the differences in the methods for obtaining fertilized eggs [i.e. artificial insemination and lengthy transport of eggs until incubation (Harada et al. 1980) versus natural spawning]. Therefore, the different methods of fertilization could affect the developmental speed. The required time from fertilization until hatching of YFT was about 22 h at 26℃ in this study. In comparison with other congeneric species, egg stage durations of bigeye tuna *T. obesus* were 30 h at 25.5–27.5℃ (Yasutake et al. 1973), and 24:4 h at 26.5℃ in PBT (Miyashita et al. 2000). Masuma (2013) reported duration of 20.6 h until the beginning of hatching at 26.7℃ for YFT inhabiting the WCPO, and this duration is similar to that found in this study (19.8 h). Therefore, YFT may have a slightly shorter egg-stage duration compared to that of other *Thunnus* species.

In conclusion, the rate of embryonic development of YFT inhabiting the EPO is temperature dependent, similar to other marine teleosts including other tunas such as *T. obesus,* PBT, and YFT inhabiting the WCPO. However, YFT required a shorter time for hatching compared to that of other *Thunnus* species.

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References

Harada, T., S. Miyashita and H. Yoneshima (1980) Effect of water temperature on yellowfin tuna hatching. *Mem. Fac. Agri. Kinki Univ.,* **13**, 29–32 (in Japanese with English abstract).

Higurashi, T. and M. Tauchi (1925) Gyoran no fukanisuu to ondo tono kankei. *Suisankousyuujyosikenhoukoku,* **21**, 11–17 (in Japanese).

Kawahara, S., A. J. Shams, A. A. Al-Bosta, M. H. Mansoor and A. A. Al-Baqqal (1997) Effects of incubation and spawning water temperature, and salinity on egg development of the orange-spotted grouper (*Epinephelus coioides,* Serranidae). *Asian Fish. Soc.,* **9**, 239–250.

Lehodey, P. and B. Leroy (1999) Age and growth of yellow fin tuna (*Thunnus albacares*) from the western and central Pacific ocean as indicated by daily growth increments and tagging data. In "*Report of the Twelfth Meeting of the Standing Committee on Tuna and Billfish*", Oceanic Fisheries Programme, Secretariat of the Pacific Community, Noumea, New Caledonia, Working paper YFT-2, 1–21.

Margulies, D., J. M. Suter, S. L. Hunt, R. J. Olson, V. P. Scholey, J. B. Wexler and A. Nakazawa (2007) Spawning and early development of captive yellowfin tuna (*Thunnus albacares*). *Fish. Bull.,* **105**, 249–265.

Masuma, S. (2013) Studies on broodstock management and spawning ecology of bluefin and yellowfin tuna in captivity. *Bull. Fish. Lab. Kinki Univ.*, **13**, 37–236 (in Japanese with English abstract).

Miyashita, S., Y. Tanaka, Y. Sawada, O. Murata, N. Hattori, K. Takii, Y. Mukai and H. Kumai (2000) Embryonic development and effects of water temperature on hatching of the bluefin tuna, *Thunnus thynnus*. *Suisanzoshoku*, **48**, 199–207 (in Japanese with English abstract).

Wexler, J. B., D. Margulies and V. P. Scholey (2011) Temperature and dissolved oxygen requirements for survival of yellowfin tuna, *Thunnus albacares*, larvae. *J. Exp. Mar. Biol. Ecol.,* **404**, 63–72.

Yasunaga, Y. (1988) Studies on physiology and ecology of larvae and juveniles of plaice *Paralichthys olivaceus*. *Bull. Nat. Res. Ins. Fish. Eng.*, **9**, 9–164 (in Japanese with English abstract).

Yasutake, H., G. Nishi and K. Mori (1973) Artificial fertilization and rearing bigeye tuna (*Thunnus obesus*) on board, with morphological observations on embryonic through to early post-larval stage. *Bull. Far Seas Fish. Res. Lab.*, **8**, 71–78 (in Japanese with English abstract).