EMBRYO DEVELOPMENT OF YELLOWFIN TUNA (*Thunnus albacares*) AT DIFFERENT INCUBATION TEMPERATURE

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ABSTRACT

The experiment was conducted in order to figure out the effect of incubation temperature on embryonic development of yellowfin tuna, *Thunnus albacares* eggs. Five different incubation temperatures were applied as treatments, i.e.: 24°C, 26°C, 28°C, 30°C, and 32°C with 3 replicate each. Ten micro plates with lid (IWAKI, Japan) were used; each has 6 well and 10 mL volumes. Five micro plates were used for experiment and five for balance on shaker. Three well of each micro plate were filled with 8 mL ultra violet sterilized sea water and 50 fertilized eggs. Temperature was set using Multi Thermo Incubator which has 5 level racks. Temperatures were set from the lowest to the highest on bottom to upper rack order. To maintain eggs dispersed in the medium, shaker on each rack was operated at 150 RPM. The embryo was monitored every 30-60 minutes depends on embryonic stage development using Microscope which was connected to Digital Camera DXM 1200F. Image analyses by Image Analyzer Program. The results showed, incubation temperature was significantly affect ($P<0.05$) embryonic development and hatching time of yellowfin tuna (*Thunnus albacares)* eggs. Optimum incubation temperature for embryo development and hatching was 28°C. Decreased on incubation temperature slows down embryo development at all stages, and vice versa, increased on incubation temperature accelerates embryo development.

KEYWORDS: embryo development, incubation, temperature, yellowfin tuna eggs

INTRODUCTION

Temperature was the main factor affect on embryo development of fish. Success of embryo development, length of incubation period and normality of hatch-out larva was de-pended on incubation temperature. Optimum incubation temperature became necessary to understand in order to get high hatching rate and normal larvae. In general, the increase of incubation temperature accelerates embryo development. On the other hand, incubation temperature decline will slow down embryo development.

Information about spawning of tuna in nature, sea pen, floating cage and in land-based tank was available (Kumai, 1997; Masuma *et al*., 2003; Wexler *et al*., 2003). Mean while, water temperature or incubation temperature was different on each location and seasons. Spawning season of tuna was estimated based on gonad stage, the present of eggs or larvae in nature. Based on gonad maturation stage, Itano (2000) predicted that in Eastern Pacific Ocean, Yellowfin tuna spawn when surface water temperature between 26°C—30°C. While based on the present of larvae, Ueyanagi (1969) conclude that Yellowfin tuna in Central and Western Pacific Ocean spawn when surface water temperature was above 26°C. Research on Bluefin tuna breeding confined in floating cage in Japan revealed that broodstock start to spawning when surface water temperature reach 23.2°C (Kumai, 1997). Masuma *et al*. (2003) reported that Bluefin tuna (*Thunnus thynnus orientalis*) reared in sea pen spawn at temperature range from 24°C—29°C. Yellowfin tuna (*Thunnus albacares*) reared in land-based tank in Panama was found to spawning when water temperature was above 24°C and ceased

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to spawning when temperature below 23°C (Niwa *et al*., 2003). It seems that spawning season of tuna is different from one location to another and affected by water temperature.

Project for the research on propagation of Yellowfin tuna in land-based tank was also conducted under research cooperation between Indonesia and Japan through Overseas Fishery Cooperation Foundation (OFCF). Spawning was started at water temperature of 28.8°C in 2004 and 28.2°C in 2005 (Hutapea *et al*., 2005). Water temperature was different compared to Japan and Panama projects but the pattern nearly the same. Spawning was started when there was a tendency of water temperature to go down and than followed by increased of temperature.

Observation of embryonic development of yellowfin tuna was conducted at room temperature (27°C-28°C) and it was found that embryo could develop normally and incubation time from fertilization to hatch out was 18 hours 55 minutes (Hutapea *et al*., 2006). Based on available data on tuna spawning that were varies on each location, it is necessary to study embryo development and incubation period of yellowfin tuna eggs at different incubation temperatures to find optimum incubation temperature and optimum hatching period of Yellowfin tuna eggs in Indonesia. The results of this study could be useful to understand how far the effect of temperature on embryo development and normality of hatch out larvae.

MATERIALS AND METHODS

The experiment was conducted at Tuna Laboratory, Research Institute for Mariculture, Bali in March 2006. Broodstock is raised in a circular concrete tank with dimension of diameter 18 m and depth 6 m, equivalent to 1,500 m^3 and fed scad mackerel and squid at 1:1 ratio. The amount of feed supply per day was range from 5%--10% of biomass. Vitamin complex, vitamin C and E were added to the feed. Water management for broodstock tank was semi-closed system, 50% filtered fresh sea water and 50% recirculated sea water pass through sand filter was supplied continuously.

Yellowfin tuna eggs used for this experiment were collected from natural spawning of broodstock in captivity. Eggs were collected directly from broodstock tank soon after spawning and stage of eggs was blastodisk stage.

Research was carried out using 5 micro plates (IWAKI, Japan) transparent with lid for treatments and 5 micro plates for balance. Each micro plate has 6 well and 3 well were filled with 8 mL filtered and UV treated sea water. Fertilized eggs were put into each hole as many as 50 eggs. Treatments were incubation temperature as followed: A. 24°C, B. 26°C, C. 28°C, D. 30°C, and E. 32°C with 3 replications (each well present as replication). Water temperatures were set using Multi Thermo Incubator (MTI-202, Tokyo Rikakikai Co., LTD) which was comprised of 5 racks and each rack was set up as treatment temperature from the lowest to the highest from bottom to upper rack consecutively. To maintain eggs disperse in the water column, shakers were used (Shaker, IKA-MTS 2/4 digital, Japan) at 150 RPM. Each rack had one shaker with 2 micro plates, one for treatment and another as balance. Observation on embryo development was started when setting temperature was reached. On each observation, 5 eggs from each treatment and replication randomly sampled and observed under Microscope (Nikon SMZ 1000 with magnification 20X; Nikon Eclipse E600 with magnification 100X) which was connected to Nikon Digital Camera DXM 1200F. Image was analyzed using Image Analyzer Program (Win Roof V.5.0 Mitani Corporation). After observed, eggs were put back into the micro plate well.

Parameters observed were embryonic stage development, duration of each stage, hatching time and hatching rate and percentages of abnormal larvae under different incubation temperatures.

RESULTS AND DISCUSSION

Experiment was started at first cleavage stage (two cells). All micro plates which were filled with eggs were put into multi thermo incubator according to temperature setting. Water temperature in micro plates at initial was room temperature and need several minutes to reach equal water temperature as set up.

First observation was conducted on morula stage in which setting temperature was reached in all treatments. The incubation temperature was affect on embryo development since morula stage. To reach morula stage at 32°C was 01H:30 minutes and it was nearly twice faster compare to 24°C incubation temperature which was 02 H:30 minutes.

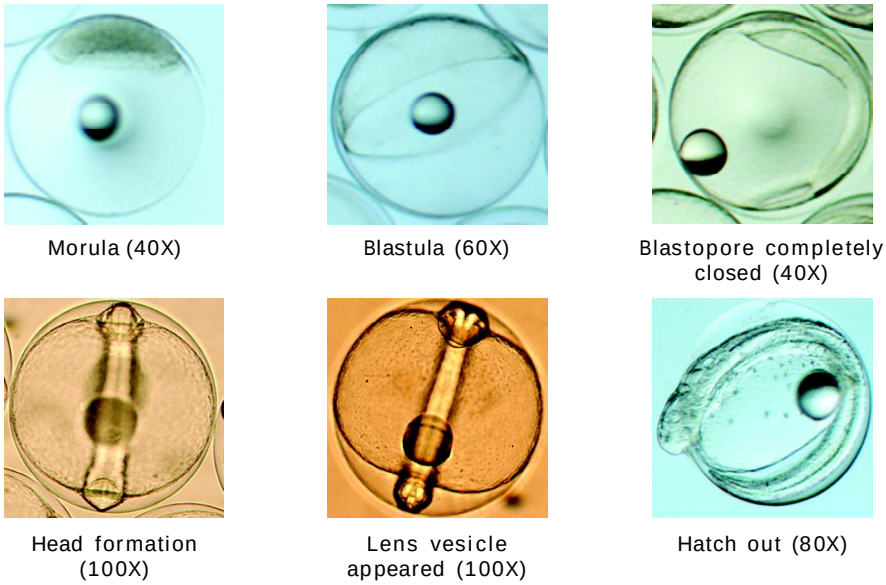


Figure 1. Embryo development stages of yellowfin tuna (*Thunnus albacares*)

Embryo stages of yellowfin tuna eggs presented in Figure 1. It was found that first critical period of embryonic development of yellowfin tuna eggs was from 8 cells to many cells (morula stage) due to fragile to handling. The second critical period was from heart start beating to just before hatch out in which eggs were tend to sink. When eggs suspended on the bottom, lack of oxygen supply and diseases infection is probably happened and then embryo will not develop and dead (Hutapea *et al*., 2006).

Incubation temperature was significantly affect on embryonic development and hatching period of yellowfin tuna eggs ($P<0.05$). Treatment A (24°C) was significantly different to treatment B (26°C). Treatment A (24°C) and B (26°C) was significantly different to treatment C (28°C), D (30°C), and E (32°C) ($P<0.05$). Treatment C (28°C) and D (30°C) was not significantly different ($P>0.05$) but both were significantly different to treatment E (32°C) ($P<0.05$). Reduce on incubation temperature was slowed down embryo development, on the other hand, increase on incubation temperature was accelerated embryo development (Table 1).

Incubation temperature was significantly affect on embryo development of Yellowfin tuna eggs all stages (Figure 2). It shows how important incubation temperature both for embryo development and hatching period on Yellowfin tuna eggs. According to Jungwirth & Winkler (1984), temperature was the main environment factor in fish eggs development.

On treatment A (24°C), some eggs stop develop at 8 cells to morula stage and some abnormal development on appearance of embryonic shield stage and caused on low hatching rate. The same results was reported by Hutapea *et al*. (2006) that one of critical period of embryonic development of yellowfin tuna was at morula stage (many cells). When Orange spotted grouper (*Epinephelus coioides*) and Marble grouper (*Epinephelus microdon*) eggs at morula stage incubated in low temperature, embryonic development stopped at gastrula stage (Melianawati *et al.,* 2001; Melianawati *et al*., 2002).

Statistical analysis on hatching rate showed that incubation at 24°C was significantly different with others treatments ($P<0.05$). At 32°C incubation temperature, embryo was developed normally but hatch out larvae were abnormal and bend. Based on this experiment, to get normal embryonic development and high hatching rate, incubation temperature for yellowfin tuna eggs were range from 26°C—30°C.

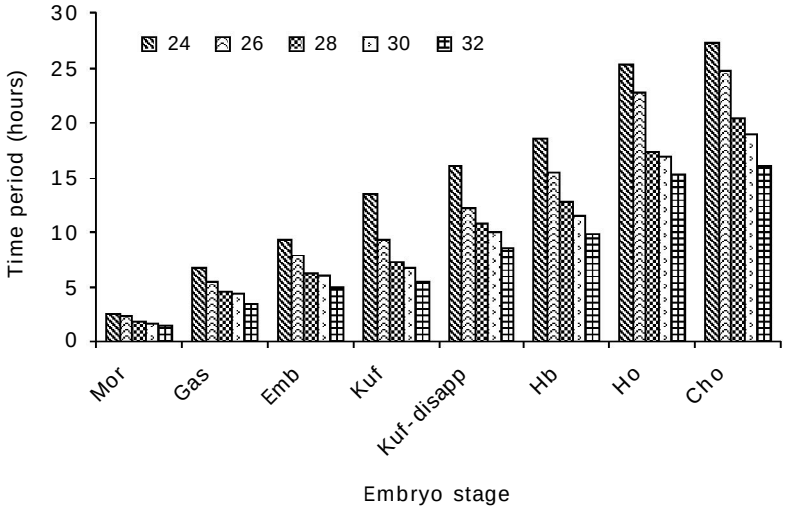
Based on this experiment for all treatments, the longest stage period was from heart beat

Table 1. Embryonic development of yellowfin tuna (*Thunnus albacares*) at different incubation temperatures

Embryo development stage	Incubation temperature (°C)				
	24	26	28	30	32
	Time after spawning (H:Min.)				
Morula	2.30	2.18	1.54	1.36	1.30
Gastrula	6.45	5.25	4.30	4.30	3.25
App. of embryonic shield	9.15	7.50	6.10	5.50	4.50
App. of kuffer vesicle	13.30	9.15	7.20	6.45	5.30
Disappeared of kuffer's	16.00	12.15	10.40	10.00	8.30
Heart beating	18.30	15.30	12.45	11.30	9.45
Start hatch out	25:15[a]	22.45[b]	17.20[c]	17.00[c]	15.15[d]
Ceased hatch out	27.15	24.45	20.20	19.00	16.00
Hatching rate (%)	38.7 ± 5.1[a]	95.30 ± 1.5[b]	94.7 ± 4.0[b]	100 ± 0.0[b]	91.0 ± 15.6[b]
Abnormal larvae (%)	12.0 ± 2.0[a]	12.7 ± 4.2[a]	0.0 ± 0.0[b]	4.0 ± 3.5[ab]	32.7 ± 15.0[c]

started to nearly hatch out stage. On this stage, embryo was fully develop, eyes and lens were develop even though no pigmentation yet, tail part actively moving, length of embryo increased progressively, black pigment appear on yolk sac, yellowish melanophore pigment at four sites on body part. According to Hutapea *et al*. (2006), hatching enzyme was active on this stage and tail moving was to help on hatching process.

Development period of embryo from each stage at different incubation temperature was presented on Figure 2. The effect of temperature on embryo development becomes clearer from morula stage to hatch out stage.



Note : **Mor** = Morula, **Gas** = Gastrula, **Emb** = Embryo formation commenced, **Kuf** = Kuffer's vesicle appeared, **Kuf-disapp** = Kuffer's vesicle dissapeared, **Hb** = Heart start beat, **Ho** = Hatch out started and **Cho** = Cease hatch out

Figure 2. Relationship between incubation temperature and embryo development of yellowfin tuna (*Thunnus albacares*) eggs

In morula stage, effect of temperature was just slightly different from one temperature to the next temperature treatments. But it was shown the differences when comparing between the highest temperature (32°C) which need 01H:30 minutes to reach morula stage and it was nearly twice faster compare to the lowest incubation temperature (24°C) which need 02 H:30 minutes.

To the further embryo development, the effect of incubation temperature becomes clearer from one temperature to another except between treatment C and D which was statistically no significantly different. The hatch out larvae from treatment C and D also showed good performance, no bend larvae or dead soon after hatch.

Based on embryo development and performance of hatch out larvae, incubation temperature at 28°C was the best. And base on this, relationship between incubation temperature and period of start and ceased hatch out larvae was presented in Figure 3. Linear regression was the fittest. The increased on incubation temperature was affected on acceleration of hatch out period of larvae and vice versa.

The effect on reduction of incubation temperature on embryo development and hatching period was presented on Figure 4. Assumed that 28°C incubation temperatures was the best and took it as based point. Reduction was more pronounce effect than the increase of incubation temperature on embryo development period. Figure showed, when incubation temperature decrease 2 or 4°C at embryo shield appearance stage for example, development was delayed for about 2 or 3 hours compared when incubation temperature increased 2 or 4°C which only accelerate to half or an hour period consecutively.

It is important to know the optimum incubation temperature in order to get the best result on hatching rate and normality of hatch out larvae. Further more, these data also useful in transportation of eggs for long distance. By reduced the temperature make it possible to transported eggs without any problem on hatch out larvae during transportation and still possible to get high percentage on normal hatch out larvae.

CONCLUSIONS

Incubation temperature at 28°C was the optimum which gave good embryo development and high normal performance hatch out larvae.

Low incubation temperature (24°C) caused on stop or abnormal development of early embryo stages of yellowfin tuna eggs and delay of hatching period.

High incubation temperature (32°C) was accelerated embryo development and hatching period but it was increased abnormal hatch out larvae.

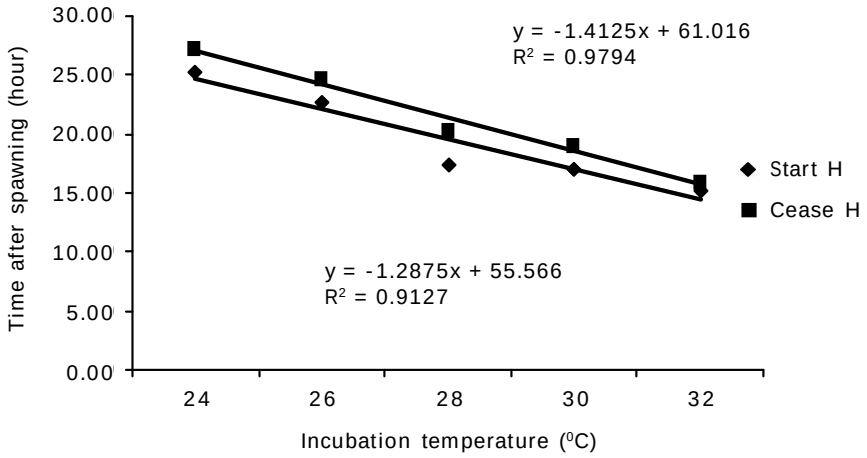


Figure 3. Relationship between incubation temperature and hatching time of yellowfin tuna eggs (*Thunnus albacares*)

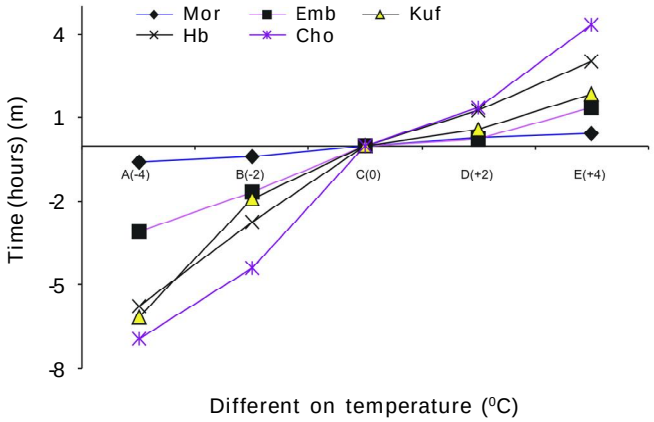


Figure 4. Effect of incubation temperatures to delay or to accelerate embryo development of yellowfin tuna (*Thunnus albacares*). Optimum incubation temperature was taken at 28°C

Reduction was more pronounce effect than the increase of incubation temperature on embryo development period.

ACKNOWLEDGEMENT

The experiment was conducted under the project for the research on the propagation of tuna species in the Republic of Indonesia between Marine Affairs and Fisheries Research Agency of Indonesia and Overseas Fishery Cooperation Foundation Japan. The OFCF provided the majority funding for this project, without which this project would have not been possible. We would also thanks to Akio NAKAZAWA (OFCF expert) for his expertise on tuna culture and to all tuna propagation staffs for their support to ensure the success of this project.

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