Study on the effect of broodstock diet of yellowfin tuna, *Thunnus albacares* on spawning performance and nutrients transition to eggs and larvae

(キハダマグロの親魚用餌の産卵成績への影響および卵・仔魚への栄養素移行に関する研究)

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Successful spawning of yellowfin tuna, *Thunnus albacares* under land-based concrete tank facilities has been started almost two decades ago at the Achotines Laboratory of the Inter-American Tropical Tuna Commission (IATTC), Las Tablas, Los Santos, Panama. However, very little attempt has been made to study nutritional aspects of this valuable species. The quality of eggs and their offspring completely relies on quality of broodstock diet. In addition, broodstock diet together with some environmental factors regulates the spawning performance, fecundity, hatching and larval performance. Two trials were conducted from May 22 - June 26 (Trial 1) and November 1 - December 13 (Trial 2), 2011. Time to spawn, fertilized eggs, hatching rate and water parameters were collected on regular basis. Similarly, egg and oil globule diameter, length of newly hatched larvae (NHL) were measured and eggs and NHL were sampled for proximate analysis. The water quality parameters during the experimental period were fairly constant and may have no effect on spawning or hatching rate. The size of eggs was found very similar in both trials under the existing condition. The fecundity was found to have direct effect with the feed composition, and spawning was delayed by five minutes each day while the feed composition was in changing state in trial 1 (phase 1). The effect has clearly observed with low protein and lipid content in eggs and NHL in phase one. The results from this study indicated the delay of spawning might be due to low levels of lipid and protein transferred in eggs from diet. It seems that the prevailing environmental condition in Achotines laboratory is suitable for rearing broodstock; however, providing correct nutrition to the broodstock, the egg quality, spawning performance, hatching rate and larval performance could be improved in certain extent.

Key words: Yellowfin tuna; Broodstock diet; Eggs and larvae; Spawning performance; Proximate composition

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Yellowfin tuna, (YFT, *Thunnus albacares*) is a very important species in tuna fisheries around the globe due to its occurrence throughout most of the tropical and subtropical areas of the world oceans (Nishikawa et al. 1985). According to statistics in 2008, global catch of YFT accounts approximately 27% of the total global catch of tuna by stock (FAO 2011). Schaefer (1998) demonstrated a similar geographical pattern of spawning by yellowfin tuna in the eastern Pacific Ocean from the histological analysis of reproductive status. In all oceans, the occurrence and collection of yellowfin larvae has been done most often at sea surface temperatures of 24° to 30°C (Richards and Simmons 1971; Suzuki 1994). In last few decades, several attempts have been made for culture and captive propagation of YFT (Harada et al. 1971, 1980; Masuma et al. 1993; Kaji et al. 1999a; Wexler et al. 2003). The spawning frequency has been recorded close to daily, predominantly within the sea surface temperature range of 26° to 29°C in case of purse-seine caught (Schaefer 1996, 1998) and tank-reared (Bayliff 1998) yellowfin in the eastern Pacific and pen-reared yellowfin in the western Pacific (Masuma et al. 1993). Previously spawning and rearing of yellowfin tuna have completely depended upon artificial fertilization of eggs from wild fish or natural spawning of broodstock from net pens, and the success of these methods mostly depends on either the collection of mature fish timely, the physico-chemical parameters of sea water and the uncontrolled variability of physical conditions in sea pens (Wexler et al. 2003). In contrast, as an alternative to those methods, the land-based culture facility provides more control of rearing methods and physical conditions. To investigate the early life histories of tunas, the Inter-American Tropical Tuna Commission (IATTC) maintaining land-based culture facilities at the Achotines Laboratory in Los Santos Province, Republic of Panama since 1985 (Olson and Scholey 1990; Margulies 1993; Lauth and Olson 1996). With the agreement between the IATTC, Overseas Fishery Cooperation Foundation (OFCF) of Japan and the government of the Republic of Panama started maintaining yellowfin tuna broodstocks in tanks in 1993. Later on, the first successful spawning started in October 1996. Spawning was intermittent for first 2 months and occurred daily thereafter (IATTC, unpublished data). The spawning performance and feeding practice has been clearly described by Wexler et al. 2003. However, no investigation has done yet to evaluate the nutritional transition from broodstock diet to eggs and larvae and their effect on spawning performance, egg quality and hatching success of this valuable species.

Therefore, this study was conducted to evaluate the spawning performance, egg quality, larvae and hatching success in relation to the broodstock nutrition under the present feeding

regime practiced in Achotines laboratory of the Inter-American Tropical Tuna Commission, Panama.

Materials and methods

Brood stock maintenance

Our observation of yellowfin tuna broodstock population maintained at the Inter-American Tropical Tuna Commission's (IATTC) Achotines Laboratory in Los Santos Province, Republic of Panama was divided into two different time periods of the year 2011. Therefore, the feeding and management activities during those periods have been described here only. The broodstock was fed with sardine, *Sardinops sagax* (California big scale sardine), squid, *Illex argentinus* (Argentine shortfin squid), pellet (Complete feed for salmon and trout, Bio-Oregon, ME, USA) and tuna vitamin (Thomas Products, LLC, Madera, CA). In trial 1, the duration was 36 days from May 22- June 26, 2011, and this trial was divided into two phases. The first phase was 10 days in which squid was added gradually until the ratio of squid and sardine reached 50:50. In phase two, the ratio of sardine and squid was maintained at 50:50 for next 26 days. The second trial duration was 42 days from November 1- December 12, 2011 and the feeding ratio of squid and sardine was maintained at 50:50. The inclusion of pellet and vitamin mix to the diet remained same in both trials. The broodstock management systems and other husbandry conditions have been elaborately described in Wexler et al. (2003).

Spawning, egg collection and hatching (experimental procedures)

Spawning of YFT brood stock was confirmed every night by scooping the eggs from the collector net set beside the rearing tank. The spawning time was recorded at the same time. Fertilized eggs were collected in a 20 L plastic bucket 2 h after spawning. Upon collecting the eggs, oil globule and egg diameters were measured using an ocular micrometer on an inverted microscope. The stage of egg development was also recorded daily. Fifty thousand eggs were transferred into each of 280 L incubation tanks, and three tanks in each time were considered for measuring hatching rate. Hatching started approximately 18-20 h of spawning in both trials. Hatching was recorded almost every day in trial 1; however, it was measured 3-4 times a week

in second trial. Water temperature for both the broodstock tank and hatching tank was recorded regularly.

Sampling

Eggs were collected in a 20 L plastic bucket just 2 h after spawning from the naturally spawning broodstock tanks of Achotines laboratory and transferred to funnel having mesh 100 µm. Similarly newly hatched larvae were siphoned from the incubation tanks and collected into scope net and blotted on a filter before weighing. Total length was measured taking thirty fresh individuals in each time of sampling. All samples were freeze dried and stored under -80°C and finally transferred to Uragami experiment station of Kinki University, Wakayama, Japan for all nutritional analysis.

Chemical analyses

Proximate composition of all samples was analyzed by standard methods of the Association of Official Analytical Chemists (AOAC 1995). Crude protein content was determined using semi micro-Kjeldahl, moisture content was dried in an oven (110°C for 24 h), crude lipid by Soxhlet extraction with diethyl ether, and ash content by a muffle furnace (600°C for 24 h).

Statistical analysis

Data are expressed as the means ± standard error of mean (SE) of triplicates. All data were subjected to one-way analysis of variance (ANOVA). When the factor was detected to be significant, the means among the treatments were compared using Tukey's test of multiple comparison with a 95 % level of significance. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) program for Windows (v. 17.0, Chicago, IL, USA).

Results

The egg and oil globule diameter, spawning and hatching performance data is presented in Table 1. The frequency of spawning was observed one time each day. The average egg diameter was 0.98 and 0.99 mm for trial 1 and trial 2, respectively. The oil globule diameter was very similar in both trials. The mean total length of the newly hatched larvae (NHL) was 3.15 and

3.25 mm for trial 1 and trial 2, respectively. The average hatching rate for trial 1 and trial 2 was 84.83 and 85.02 %, respectively. The average fecundity was estimated 397195 and 258601 day^{-1} for trial 1 and 2, respectively. The average broodstock tank temperature was 28.55°C for trial 1 and 27.80°C for trial 2. In both trials the hatching tank temperature was lowered by approximately 0.4°C.

Table 1. Measurement of egg and oil globule diameter, larval length, hatching rate, fecundity and temperatures observed during the experimental periods.

Parameter	Trial 1	Trial 2
Egg diameter (mm)	0.98 ± 0.015	0.99 ± 0.026
Oil globule (mm)	0.22 ± 0.006	0.22 ± 0.007
NHL (mm)	3.15 ± 0.126	3.25 ± 0.095
Hatching rate (%)	84.83 ± 11.3	85.02 ± 10.9
Fecundity (eggs spawned/day)	397195	258601
Temperature of broodstock tank (°C)	28.55 ± 0.36	27.80 ± 0.20
Temperature of hatching tank (°C)	28.15 ± 0.35	27.45 ± 0.25

Daily fecundity was estimated for both trials and presented in Fig. 1. In 1st phase of trial 1 average daily fecundity gradually reduced and it went upto next five days in phase 2 and then increased again and later tended to be equal every day. The average fecundity was recorded 397195 day^{-1} for trial 1. On the other hand, in trial 2 average daily fecundity showed asymptotic pattern except 8-10 days where there was a big drop down of total eggs. The average fecundity 258601 day^{-1} was 0.5 times lower than that observed in trial 1.

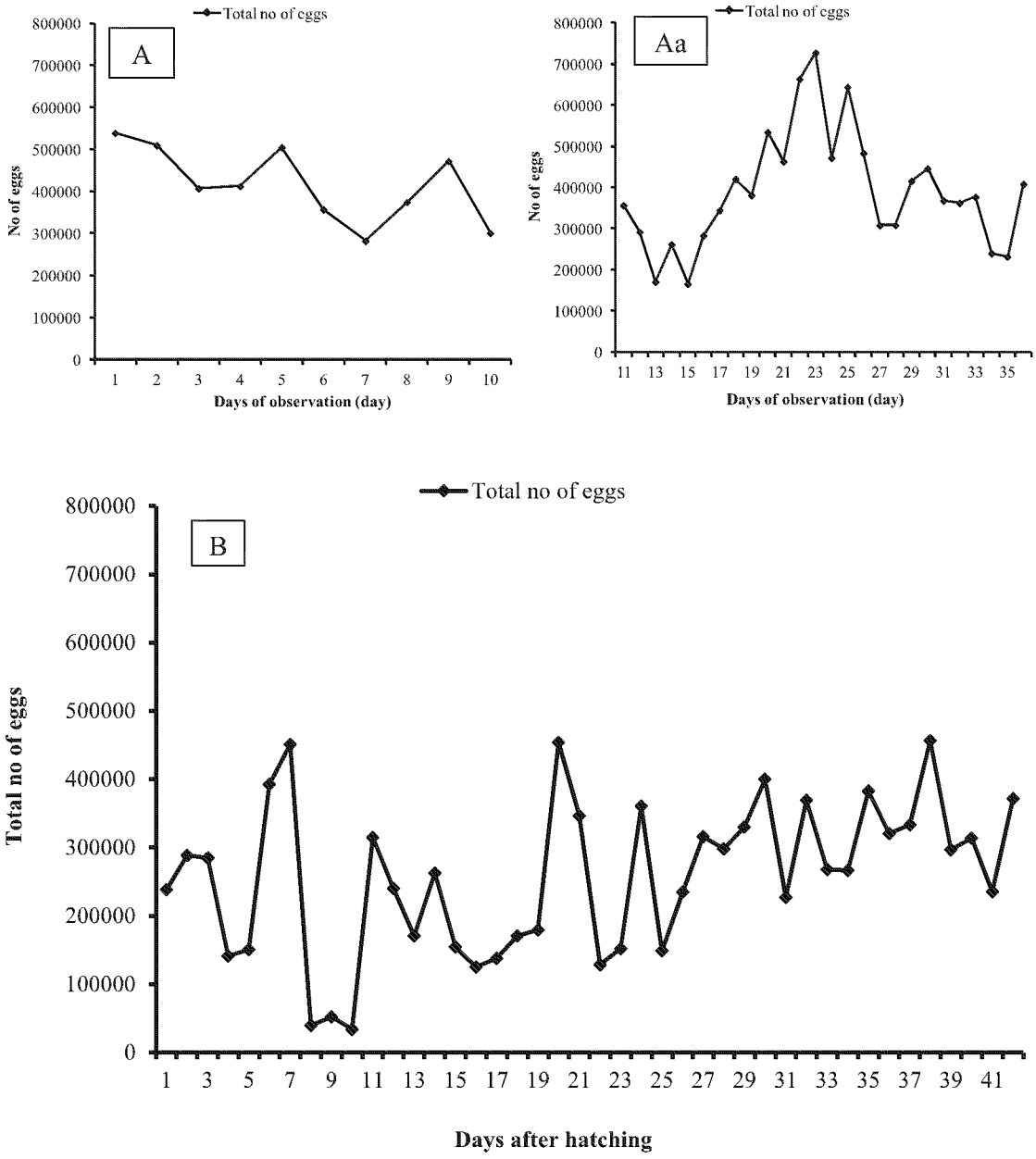

Fig. 1. Total no of eggs spawned daily during the experimental period. **A**: first phase (10 days) of trial 1 from May-June 2011. **Aa**: 2nd phase (35 days) of trial 1 from May-June 2011. **B**: trial 2 (42 days) from November-December 2011.The average number of eggs spawned 255349 per day.

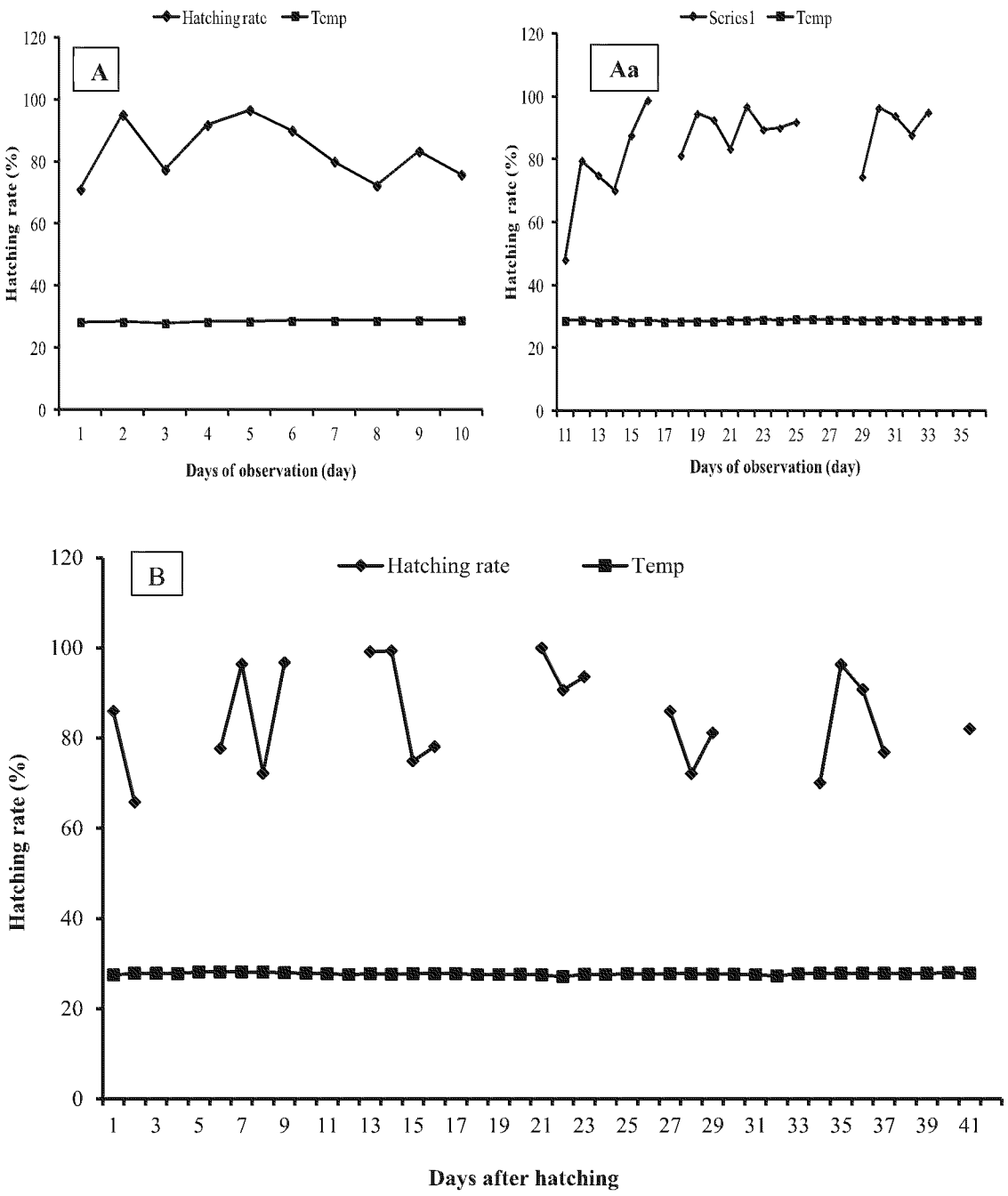

Fig. 2. Relation between the water temperature and hatching rate. **A**: first phase (10 days) of trial 1 from May-June 2011. **Aa**: 2nd phase (35 days) of trial 1 from May-June 2011. **B**: trial 2 (42 days) from November-December 2011. Average temperature was 27.8 ± 0.2°C and hatching rate was ca. 85.1 % during the experimental period.

The relation between the tank water temperature and hatching rate is presented in Fig. 2. In both trials hatching rate was very similar ca. 85% although the water temperature reduced by 1°C in trial 2.

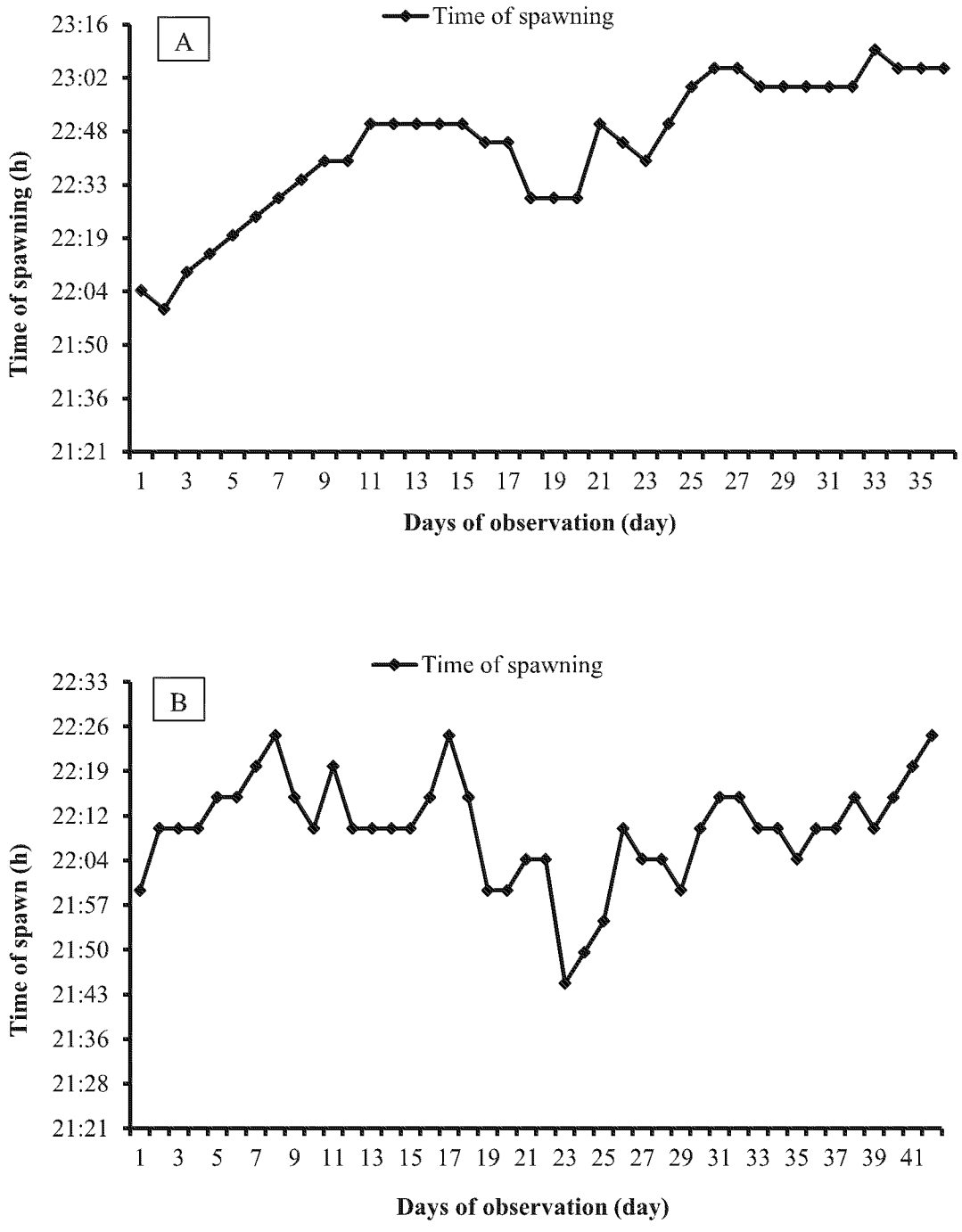

Fig. 3. The time of spawning during the experimental periods. **A:** indicates the observation during the period of May-June 2011, and **B:** indicates the observation took place during November-December 2011.

Table 2. Proximate composition (% on wet weight basis) of eggs and newly hatched larvae collected during the experimental periods.

	Moisture	Crude protein	Crude lipid	Crude ash
Egg (Trial 1, 1st phase)	87.82 ± 1.4	5.21 ± 0.2^{b}	1.03 ± 0.1^{c}	3.64 ± 0.2^{a}
Egg (Trial 1, 2nd phase)	87.72 ± 1.5	4.63 ± 0.2^{c}	1.58 ± 0.1^{b}	2.73 ± 0.2^{b}
Egg (Trial 2)	87.87 ± 1.6	6.42 ± 0.3^{a}	1.67 ± 0.1^{a}	0.98 ± 0.1^{c}
NHL (Trial 1, 1st phase)	89.47 ± 1.3	3.54 ± 0.1^{d}	1.09 ± 0.1^{c}	3.43 ± 0.1^{a}
NHL (Trial 1, 2nd phase)	89.62 ± 1.4	4.88 ± 0.2^{c}	1.49 ± 0.1^{b}	2.33 ± 0.2^{b}
NHL (Trial 2)	90.57 ± 1.8	5.09 ± 0.2^{b}	1.82 ± 0.1^{a}	0.96 ± 0.1^{c}

* Trial 1 includes 1st phase and 2nd phase.

Time of spawning was recorded daily for both trials and presented in Fig. 3. In 1st phase of trial 1, spawning time was delayed by 5 min every day until the feeding ratio of squid and sardine reach 50:50. Henceforth, the spawning time variation reduced considerably. In case of trial 2, the variation of spawning time was very little as compared with trial 1; however, spawning time was delayed from day 23-25 days of observation.

Frequent sampling of eggs and newly hatched larvae was done in both trials is presented in Table 2. There were no differences in moisture content in eggs and NHL in both trials. Crude protein content was significantly higher in eggs in trial 2 followed by eggs in 2nd phase in trial 1 and NHL in trial 2. Crude lipid content was significantly higher in both eggs and NHL in trial 2. Significantly lower crude ash was found in both eggs and NHL in trial 2.

Discussion

The eggs and oil globule diameter were found very similar in two trials which are consistent with previous studies on YFT conducted in laboratory condition (Margulies et al. 2001; Buentello et al. 2011). Although the average temperature in trial 2 slightly decreased; however,

the variation was relatively very low. The range of temperature observed in both trials was very optimal for rearing of yellowfin eggs and yolk-sac that has been confirmed by previous studies (Margulies et al. 2007; Wexler et al. 2011). The average total length of newly hatched larvae was very similar from both trials ca. 3.20 mm and the length is somehow 0.6 mm bigger than that observed in YFT reared in net pen in Ishigaki Island, Okinawa, Japan (Kaji et al. 1999b; Margulies et al. 2001). The average hatching tank water temperature in Achotines laboratory was ca. 28 °C, whereas the average tank temperature in Yaeyama station was 26.4 °C, and therefore, the differences in temperature may be one of the important reasons for size differentiation. The variation in size of newly hatched larvae due to temperature differences has been proved in case of Atlantic cod, *Gadus morhua* (Pepin et al. 1997) and Atlantic silversides, *Menidia menidia* (Bengtson et al. 1987); however, no studies have done yet with YFT.

The daily fecundity may consider as an index of assessing the egg production capability of brood fish has indicated direct influence of broodstock feed composition from both trials (Mourente and Tocher 2003). The daily fecundity reduced considerably while the ratio of squid and sardine was trying to equalize, and the effect continued five more days to regain the trend. On the other hand, with a fixed ratio squid and sardine in trial 2, produced almost very similar no of eggs during the whole experimental period. Several studies on rainbow trout proved that the daily and seasonal rates of feeding ration of the broodstock diets have direct effects on fecundity and egg size (Sprinate et al. 1985; Jones and Bromage 1987; Bromage et al. 1992). In addition, the mean fecundity in trial 2 lowered by 0.5 times as compared with trial 1 as because the broodstock size reduced in trial 2 as a result of unexpected death of two broods due to collision with tank walls before the start of the feeding trial. The broodstock size, one of the important determiners of fecundity estimation (Bromage et al. 1992; Richter et al. 1995), however, other reasons may be involved.

Effect of temperature and salinity on hatching rate has been intensively studied for many different fish species; however, very few studies have done for tuna on these aspects. Harada et al. (1980) mentioned the optimum range of temperatures for survival of artificially fertilized yellowfin eggs at hatching was 24-30 °C. The optimal temperature for embryonic development and hatching of Pacific bluefin tuna eggs was 25 °C (Masuma, 2009; Miyashita et al. 2000). In recent study, Wexler et al. (2011) found that the optimal range of temperatures for rapid growth and moderate to high survival at first-feeding YFT larvae was from about 26 to 31 °C. Wexler et al. (2011) have also noticed that hatching time reduced to 17 h from 48 h when the

temperature increased from 19 °C to 35 °C. Our observation for both trials ties with the optimum temperature range for YFT as mentioned earlier, and produced very similar hatching rate indicating that the temperature range in Achotines laboratory is very suitable for successful hatching for this species.

YFT broodstock has been spawning naturally in land-based culture facility at Achotines laboratory since 1996 (Wexler at al. 2003). Spawning is occurring almost daily under the prevailing condition that remains fairly constant, therefore, all the environmental parameters seems suitable for broodstock to spawn. We assumed that spawning will most like occur at similar time at night; however, it was delayed by almost five minutes every day at first phase of trial 1 while the feed composition was gradually adjusted at a ratio of 50:50 of squid and sardine. Therefore, feed composition might have direct effect in transmitting essential nutrients to egg development and spawning performance. Cerda et al. (1990) showed that changes of diet composition from natural trash fish to commercial diet directly affect the fecundity, delayed spawning, hatching rates and survivals in sea bass. This finding is consistent with our present observation in which the egg lipid content was significantly lower during first phase of trial 1 (Table 2). This finding is further supported by studies done for seabass in which the total lipid content of the eggs has been correlated with egg and larval viability following alterations in spawning time (Devauchelle et al., 1982; Carrillo et al., 1991).

Dietary composition as well as the change of protein and/or lipid fraction has proved to have direct impact on the quality of eggs, spawning performance, hatching success and larval quality for red sea bream, *Pagrus major* (Watanabe et al. 1984 a, b; 1985) and gilt- head sea bream, *Sparus aurata* (Zohar et al. 1995). From this investigation, protein and lipid fraction in both eggs and NHL was significantly higher while the broodstock were fed with 50:50 ration of squid and sardine. This may indicate that during the first phase of trial 1 nutrient transition was directly affected with the dietary composition and further protein to lipid fraction. However, long term trial is necessary to investigate more in details to find out specific effect of fatty acids and amino acids involved with nutrient transition to eggs and larvae, and further affecting spawning performance, hatching success and larval quality. The spawning time was delayed unexpectedly on 23-25 DAH this may be due to the incidence of red tide in adjacent sea on those days although the effect of red tide is unclear in case of YFT broodstock.

In conclusion, it has been revealed that broodstock diet directly affecting the nutritional status of eggs and newly hatched larvae. In addition, the change of dietary composition affected

the egg maturity, fecundity, time of spawning and the transfer of nutrients in terms of protein, lipid and ash. The rearing condition at Achotines laboratory seems very suitable for rearing broodstock tuna; however, by manipulating diet composition, the egg quality, spawning performance, hatching rate and larval quality could be improved.

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