



**SCIENTIFIC COMMITTEE
SEVENTEENTH REGULAR SESSION**

Electronic Meeting
11-19 August 2021

**Preliminary report on the growth and annulus formation in
otolith of bigeye and yellowfin tunas in captivity**

WCPFC-SC17-2021/SA-IP-20 (Rev.01)

Kei Okamoto¹, Kazunori Kumon², Takeshi Eba², Takayuki Matsumoto¹, Hiroki Yokoi³ and Keisuke Satoh³

¹ National Research and Development Agency, Japan Fisheries Research and Education Agency, Fisheries Resources Institute, Shimizu Field Station, Shizuoka-shi, Japan

² National Research and Development Agency, Japan Fisheries Research and Education Agency, Fisheries Technology Institute, Amami Field Station, Setouchi-cho, Japan

³ National Research and Development Agency, Japan Fisheries Research and Education Agency, Fisheries Resources Institute, Yokohama-shi, Japan

Preliminary report on the growth and annulus formation in otolith of bigeye and yellowfin tunas in captivity

Kei Okamoto¹, Kazunori Kumon², Takeshi Eba², Takayuki Matsumoto¹, Hiroki Yokoi³ and Keisuke Satoh³

Summary

Bigeye and yellowfin tunas (fork length (FL): 31.1–54.2 cm) were caught by a pole and line fishery at the coastal waters of Amami archipelago, Japan (27°36'N–27°56' N, 129°24'E–129°43'E) in October 2020 and they were reared for an experiment at a sea cage (28°09'N, 129°15'E). Fish length was measured and oxytetracycline (OTC) was injected when they were released into the cage. After 57 to 60 days and 152 to 181 days, the fish were pulled out once or twice from the cage, and FL was measured and OTC was injected again, then they were released into the other cage. One bigeye and 12 yellowfin tunas of them were implanted an archival tag (LAT2810L, Lotek Wireless Inc.) into peritoneal cavity to obtain information about ambient temperature, peritoneal cavity temperature and swimming depth during the rearing experiments for estimating daily food consumption. Three bigeye and 69 yellowfin tunas were continuously reared over 30 days, the growth rates of them were 0.04 ± 0.06 cm/day (growth increment -0.04–0.08 cm; rearing period 41–154 days) and 0.06 ± 0.02 cm/day (0.00–0.14 cm; 42–181 days) for bigeye and yellowfin tunas, respectively. The sagittal otoliths were extracted from died fish and the fluorescent band formations by OTC injections were confirmed. As of July 7, 2021, the information about the archival tags of one bigeye and 3 yellowfin tunas were obtained.

Introduction

Stock status of bigeye and yellowfin tunas in the western and central Pacific Ocean (WCPO) have been strongly affected by growth parameters. In terms of bigeye tuna, the estimated stock status was largely changed according to updates of the parameters associated to the growth curve (Davies et al. 2014; Harley et al. 2014; McKechnie et al. 2017; Tremblay-Boyer et al. 2017; Ducharme-Barth et al. 2020; Vincent et al. 2020). During 10th scientific committee (SC) of the Western and Central Pacific Fisheries Commission (WCPFC) in 2014 indicated that the bigeye stock was overfished and recommended that fishing mortality on this stock should be reduced. This stock assessment was based on the growth model using propagation of modes of size composition in catch. In 2017 the stock status had drastically changed at the next stock assessment. It was indicated that the stock is not experiencing overfishing and it appears that the stock is not in an overfished condition throughout its history after 1950 to present. Several factors of this stock status change were indicated during the 10th SC meeting including changes of sub-area definition and growth model. The changes of the growth model could explain a half of the stock status

¹ National Research and Development Agency, Japan Fisheries Research and Education Agency, Fisheries Resources Institute, Shimizu Field Station, Shizuoka-shi, Japan

² National Research and Development Agency, Japan Fisheries Research and Education Agency, Fisheries Technology Institute, Amami Field Station, Setouchi-cho, Japan

³ National Research and Development Agency, Japan Fisheries Research and Education Agency, Fisheries Resources Institute, Yokohama-shi, Japan

changes according to the changes of time series of the depletion rate of SSB (McKechnie et al. 2017). The growth models used in 108 out of 144 scenarios of stock assessment in 2017 were based on otolith growth increments, and its subsequent stock assessment in 2018 was indicated robustness of the stock status of the 2017 stock assessment after updating the otolith growth model according to SC13 advices for improving the growth model, such as adding otolith from larger than 130 cm FL and only using specimen with high confidence readable otolith annuli. In the latest stock assessment in 2020, several growth models were applied to the stock assessment model, such as the otolith increments reading (Farley et al. 2020a), combination of otolith increments reading dataset and bigeye tuna tag-recapture data (Vincent et al. 2020), and conditional age-length dataset constructed from the combined daily and annual otolith dataset were tested (Eveson et al. 2020). Afterall, the further updated otolith growth model was determined as inappropriate for the stock assessment because the stock assessment model predicted very high levels of biomass. Therefore, only the combined dataset of otolith increments reading and tag-recapture was employed to the stock assessment, although the stock was similar to the previous one. In terms of yellowfin tuna in the WCPO, the stock assessment of this species in 2017 applied the growth model based on size composition data, while the latest stock assessment in 2020 examined three kinds of growth models which are conditional catch at age, length compositions, and otolith growth increments. The stock status of yellowfin tuna had substantially changed to more optimistic (Vincent et al 2020). Although the influence of the growth model changes seems not to be large one like bigeye tuna, the growth parameters showed a positive impact for the optimistic situation according to the results of changes of spawning potential of the developing process of reference case (Vincent et al. 2020).

Numerous growth studies were applied to the commercial important species of bigeye and yellowfin (e.g., tagging experiment: Clear et al. 2000; otolith: Farley et al. 2020) including recent approaches using a bomb radiocarbon otolith dating to determine the fish age and to get the precise growth curve for the bigeye and yellowfin tunas (Andrews et al. 2020; Farley et al. 2020b). Some tag-release and recapture studies with marking by oxytetracycline (OTC) or strontium chloride (SrCl) have been conducted for the wild tropical tunas, and the correlation between the number of otolith annulus and days at liberty after recapturing the fish have been discussed based on the results (Clear et al. 2000; Hallier et al. 2005; Schaefer and Fuller 2006; Wild et al. 1995). However, the mechanism of the daily and annual ring formation and the relationship between the width of increment and ambient environmental conditions for these species are not elucidated. Another approach is needed to investigate mechanism to form the increment and eventually verify the appropriate ageing methods for these species. In this study, we conducted raring experiment of bigeye and yellowfin tuna juveniles caught at the Japanese coastal waters to accumulate the knowledge related to the otolith growth daily and annual increment formation, and to examine influence of the oceanographic condition on the formation.

Materials and methods

Bigeye and yellowfin tunas were caught by pole and line fisheries at the coastal waters of Amami archipelago (27°36'N–27°56' N, 129°24'E–129°43'E) in October 2020. The fish were transported to the sea cages (18m and

20m diameters each) of Japan Fisheries Research and Education Agency, Fisheries Technology Institute, Amami Field Station (28°09'N, 129°15'E) with keeping in the fish tank on the fishing vessel. Fork length was measured, and body weight was estimated from unpublished data in Fisheries Resources Institute. The fish were released into a sea cage after a shot of OTC whose amount was determined based on the estimated body weight as body concentration should be more than 27 ppm (Wild et al. 1995). Under the rearing condition, the fish were fed mainly sand eels (Ammodytidae) and occasionally krill (Euphausiidae) for 1 to 4 times / day except for the officer's holidays. After 57 to 60 days and 152 to 181 days, the fish were pulled out once or twice from the cage, and FL was measured and OTC was injected again, then they were released into the other cage. One bigeye and 12 yellowfin tunas of them were implanted an archival tag (LAT2810L, Lotek Wireless Inc.) into peritoneal cavity to obtain information about ambient temperature, peritoneal cavity temperature and swimming depth every 10 seconds during the rearing experiments for estimating daily food consumption. Retrieval of dead individuals were conducted every day except for the officer's holidays, then the sagittal otoliths were extracted from died fish after kept in frozen. Otoliths were kept in light-shielded microtube, and then the fluorescent band formations by OTC injections were examined under UV lighting microscope.

Results

A total of 7 bigeye and 241 yellowfin tunas were fished and released into a sea cage. The fork length was 44.6 ± 6.0 cm (mean \pm SD; range: 38.0–54.2 cm, N=7) and 38.1 ± 2.6 cm (31.1–46.2 cm, N=241) for bigeye and yellowfin tunas, respectively. The estimated body concentration of OTC to the estimated body weight should be 28.7–67.2 ppm and 40.0–85.1 ppm for bigeye and yellowfin tunas, respectively. As regarding to the information of archival tags, one bigeye and 3 yellowfin tunas implanted archival tag were retrieved until July 7, 2021. The relationship between time series and the difference of ambient and peritoneal cavity temperatures for the entire period and a day were shown in Figures 1 and 2, respectively. Time series of temperature difference was zigzag with showing some spikes during entire period (Figure 1). The timings of these spikes mostly correspond to one of the feeding times (Figure 2).

As for July 7, 2021, the rearing period are 2–154 days and 1–266 days for bigeye and yellowfin tunas, respectively, and approximately 50 yellowfin tunas are continuing to be reared. Three bigeye and 69 yellowfin tunas were continuously reared over 30 days, the growth rates of them were 0.04 ± 0.06 cm/day (growth increment per day: -0.04–0.08 cm; rearing period: 41–154 days) and 0.06 ± 0.02 cm/day (0.00–0.14 cm; 42–181 days) for bigeye and yellowfin tunas, respectively. One or two fluorescent bands formations according to the times of OTC injections were observed in the sagittal otolith of all fishes investigated (Figure 3). Growth increment of alive fishes after the release into a sea cage to the first retrieval in 57–60 days was 5.1 cm and 6.4 cm for 2 bigeye and 4.06 ± 1.39 cm (range: 0.30–8.10 cm, N=68) for yellowfin tunas, respectively. From the first retrieval to the second retrieval, it was 4.04 ± 1.33 cm (1.50–6.60 cm, N=23) in 94–123 days for yellowfin tuna.

Discussion

We reported preliminary results of the growth rates associated with rearing experiment of the WCPO bigeye and yellowfin tunas. Growth rate of both species shows considerable individual variability. Some dead individuals are quite lean compared to the body condition at the catch and there is no stomach content. Therefore, the variation is assumed to be associated to their foraging conditions.

As for the yellowfin tuna, the IATTC has conducted rearing experiments in the Achotines Laboratory in Panama (Margulies et al. 2007). They have also succeeded to be captive breeding, and thus spawning biology, growth and survival rates of larvae have been investigated (Wexler et al. 2003). Also in Japan, artificial fertilization by using the eggs from wild-caught yellowfin female and studies related to primary growth and survival rates of larvae and spawning biology have been reported (Harada et al. 1971; Harada et al. 1980; Kaji et al. 1999; Shimizu and Shiozawa 2004). In contrast, only a few studies of captive reared experiment especially related to growth in bigeye tuna have been reported (Matsumoto et al. 2004; Yasutake et al. 1973). Therefore, it is required any additional knowledges even if there are very few individuals in bigeye tuna. As regarding to formation of otolith daily rings, some studies have confirmed that the rings are formed daily in wild bigeye tuna (Clear et al. 2000; Schaefer and Fuller 1996). The limited study has indicated that the rings were formed daily in the juveniles of bigeye and yellowfin tunas at least 55 days maximum under rearing condition as well (Matsumoto et al. 2004). In this study, the fish have been reared for longer period for both species. Especially yellowfin tuna, we have reared more than 9 months and more than 50 individuals survive. The number of OTC marks corresponding to the number of injections for the fish were observed, also obvious growth increments were observed in sagittal otolith between the two fluorescent bands or the first mark to the edge. At this stage, there was no chance to count the daily rings and verify the daily formation comparing with the number of days reared, which will be addressed in near future.

The daily time series variation of difference between ambient and peritoneal cavity temperatures acquired from the archival tag shows stable trend except for some spikes. The time of the highest spike corresponds to the timing of feeding and the lowest peak was caused by the temperature of bait which are kept in refrigerator until feeding, thus it is assumed that the signal is providing explicit evidence of foraging. Here, there is insufficient information to discuss the relationship among the growth, otolith annulus formation, and elapsed rearing days. However, we have already retrieved some otoliths which are included fluorescent marks. In addition, the time series information of ambient and peritoneal cavity temperatures has also been collected from some individuals. It is expected to be ascertained some factors affecting otolith daily and annuli formation by using data from direct rearing experiment and archival tags.

Acknowledgements

We would like to thank the staffs of Fisheries Technology Institute, Amami Field Station for their support in daily rearing of fish. We also thank to K. Gen, T. Takashi, K. Okita, K. Higuchi, T. Hayashida, and H. Minami for their help to conduct this study smoothly. Setouchi Fisheries Cooperative Associations made enormous contributions for

coordinating this project and their vessels, “Meisei-maru”, “Toyoshima-maru”, and “Wakita-maru” caught and treated fish effectively for the experiment. The Japan Fisheries Agency financially supported this work.

References

- Andrews, A.H., Pacocco, A., Allman, R., Falterman, B.J., Lang, E.T., and Golet, W. (2020). Age validation of yellowfin (*Thunnus albacares*) and bigeye (*Thunnus obesus*) tuna of the northwestern Atlantic Ocean. *Canadian Journal of Fisheries and Aquatic Science* 47: 637–643 (dx.doi.org/10.1139/cjfas-2019-0328).
- Clear, N., Davis, T., and Carter, T. (2000). Developing techniques to estimate the age of bigeye tuna and broadbill swordfish off eastern Australia: a pilot project [online]. Final report No. 98/113 for the Fisheries Research Development Corporation Project, Australia.
- Davies, N., Harley, S., Hampton, J., and McKechnie, S. (2014). Stock assessment of yellowfin tuna in the Western and Central Pacific Ocean. Technical Report WCPFC-SC10-2014/SA-WP-04.
- Ducharme-Barth, N., Vincent, M., Hampton, J., Hamer, P., Williams, P., and Pilling, G. (2020). Stock assessment of bigeye tuna in the western and central Pacific Ocean. WCPFC-SC16-2020/SA-WP-03-Rev3.
- Eveson, P., Vincent, M., Farley, J., Krusic-Golub, K., and Hampton, J. (2020). Integrated growth models from otolith and tagging data for yellowfin and bigeye tuna in the western and central Pacific Ocean. WCPFC-SC16-2020/SA-IP-03.
- Farley, J., Krusic-Golub, K., Eveson, P., Clear, N., Rouspard, F., Sanchez, C., Nicol, S., and Hampton, J. (2020a). Age and growth of yellowfin and bigeye tuna in the western and central Pacific Ocean from otoliths. Technical Report SC16-SA-WP-02.
- Farley, J., Andrews, A., Clear, N., Hampton, J., Ishihara, T., Krusic-Golub, K., MacDonald, J., Okamoto, K., Satoh, K., and Williams, A. (2020b). Report on the bomb radiocarbon age validation workshop for tuna and billfish in the WCPO. WCPFC-SC16-2020/SA-IP-17-Rev.01.
- Hallier, J., Stequert, B., Maury, O., and Bard, F. (2005). Growth of bigeye tuna (*Thunnus obesus*) in the eastern Atlantic Ocean from tagging recapture data and otolith readings. *ICCAT Collective Volume of Scientific papers* 57: 181–194.
- Harada, T., Mizuno, K., Murata, O., Miyashita, S., and Hurutani, H. (1971). On the artificial fertilization and rearing of larvae in yellowfin tuna. *Memoir, Faculty of Agriculture, Kinki University, Japan* 4: 145–151.
- Harada, T., Miyashita, S., and Yoneshima, H. (1980). Effect of water temperature on yellowfin tuna hatching. *Memoir, Faculty of Agriculture, Kinki University, Japan* 13: 29–32.
- Harley, S. J., Davies, N., Hampton, J., and McKechnie, S. (2014). Stock assessment of bigeye tuna in the Western and Central Pacific Ocean. WCPFC-SC10-2014/SAWP-01.
- Kaji, T., Tanaka, M., Oka, M., Takeuchi, H., Ohsumi, S., Teruya, K., and Hirokawa, J. (1999). Growth and morphological development of laboratory-reared yellowfin tuna *Thunnus albacares* larvae and early juveniles, with special emphasis on the digestive system. *Fisheries science* 65: 700–709.

- Margulies, D., Suter, J.M., Hunt, R., Olson, R.J., Scholey, V.P., Wexler, J.B., and Nakazawa, (2007). Spawning and early development of captive yellowfin tuna *Thunnus albacares*, Fishery Bulletin 105: 249–265.
- Matsumoto, T., Mizoguchi, M., and Okuhara, M. (2004). Validation of otolith daily increments for yellowfin and bigeye tunas by injection of fluorescent substances. Handbook and abstract of the third International Symposium on Fish Otolith Research and Application, Townsville p.63.
- McKechnie, S., Pilling, G., and Hampton, J. (2017). Stock assessment of bigeye tuna in the western and central Pacific Ocean. Technical Report WCPFC-SC13-2017/SA-WP-05.
- Schaefer, K.M. and Fuller, D.W. (2006). Estimates of age and growth of bigeye tuna (*Thunnus obesus*) in the eastern Pacific Ocean, based on otolith increments and tagging data. Inter-American Tropical Tuna Commission Bulletin 23: 32–76.
- Shimizu, H. and Shiozawa, S. 2004. Allometry and development of caudal skeleton of hatchery-reared yellowfin tuna *Thunnus albacares*. Bulletin of Fisheries Research Agency 10: 1–7.
- Tremblay-Boyer, L., McKechnie, S., Pilling, G., and Hampton, J. (2017). Stock assessment of yellowfin tuna in the Western and Central Pacific Ocean. Technical Report WCPFC-SC13-2017/SA-WP-06.
- Vincent, M., Ducharme-Barth, N., Hamer, P., Hampton, J., Williams, P., and Pilling, G. 2020. Stock assessment of yellowfin tuna in the western and central Pacific Ocean. WCPFC-SC16-2020/SA-WP-04-Rev3.
- Wexler, J.B., Vernon, P.S., Olson, R.J., Margulies, D., Nakazawa, A., and Suter, J.M. (2003). Tank culture of yellowfin tuna, *Thunnus albacares*, developing a spawning population for research purpose. Aquaculture 220: 327–353.
- Wild, A., Wexler, J.B., and Foreman, T.J. (1995). Extended studies of increment deposition rates in otoliths of yellowfin and skipjack tunas. Bulletin of Marine Science 57: 555–562.
- Yasutake, H, Nishi, G., and Mori, K. (1973). Artificial fertilization and rearing of bigeye tuna (*Thunnus obesus*) on board, with morphological observations on embryonic through to early post-larval stage. Bulletin of the National Research Institute of Far Seas Fisheries 8: 71–78.

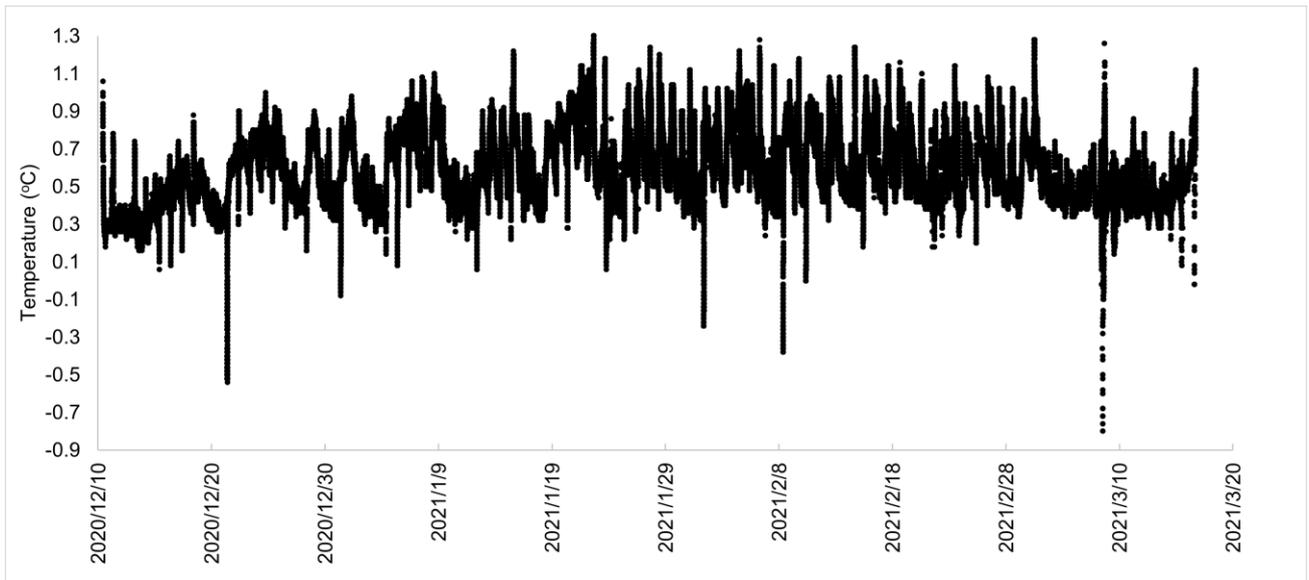


Figure 1. Time series variation of difference between ambient and peritoneal cavity temperatures in bigeye tuna acquired from the archival tag.

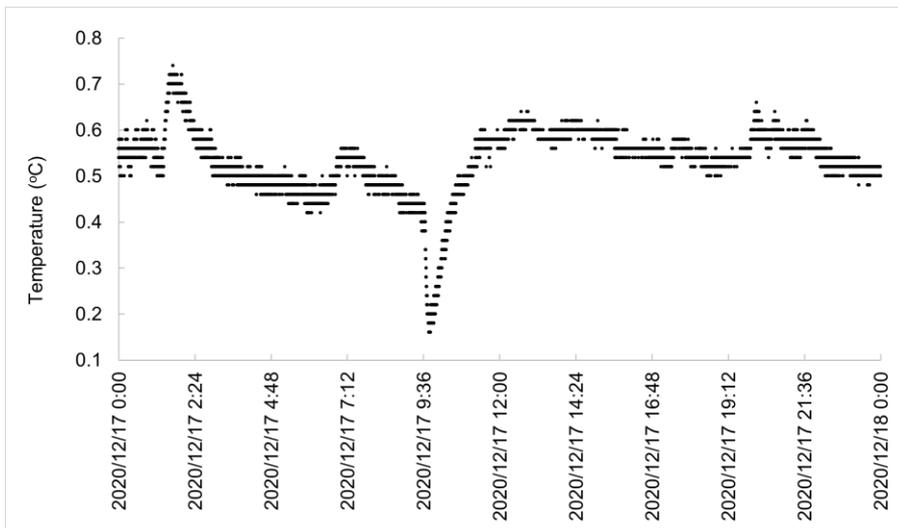


Figure 2. Daily time series variation of difference between ambient and peritoneal cavity temperatures in bigeye tuna acquired from the archival tag.



Figure 3. Sagittal otolith of yellowfin tuna with two fluorescent marks by OTC injections observed under UV-lighting microscopy.