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Relationship between Gill Raker Number and Early Growth of Pacific Bluefin Tuna *Thunnus orientalis*

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Abstract

This study examined the variation in the gill raker number (GRN: a meristic count) of Pacific bluefin tuna *Thunnus orientalis* in relation to its early growth using otolith microstructure analysis. A total of 360 age-0 fish (fork length: 102 mm-369 mm) in which the gill raker should have been fully developed were analyzed. The GRN on the upper limb, lower limb, and in total (including one gill raker at the joint of two limbs) ranged from 9-13, 21-26, and 32-40, respectively. GRN was negatively related to early growth, as indicated by the mean otolith increment width. Fish with fewer gill rakers attained better growth during the early life stage than fish with more gill rakers. This relationship was especially evident for growth from 15-19 days after hatching soon before metamorphosis. These results suggest that the early-stage growth of bluefin tuna is an important determinant of morphology. Furthermore, GRN can be an alternative tool for monitoring early growth and may also be related to survival and recruitment success.

Discipline: Fisheries
Additional key words: growth rate, juvenile, Scombridae

Introduction

Gill raker number (GRN) is a meristic count unique to each fish species and related to feeding ecology (e.g., Amundsen et al. 2004, Gerking 1994). GRN generally increases during early life stages and then stops; so, a growth model that has a plateau (i.e., a von-Bertalanffy growth curve) is used to describe this development (Villallobos & Rodríguez-Sánchez 2002). However, GRN can vary within a given species even during this plateau. GRN also often reflects the population structure of a species (e.g., Kisney et al. 1994, Wilk et al. 1980). These variations are typically due to ecological and environmental factors. For example, intra-population variation in stickleback (genus *Gasterosteus*) is explained by adaptation to the type of available prey (Hosoki et al. 2019). Meanwhile, the effect of temperature during the early life stages on GRN and other meristic counts has also been inferred by experimental studies (e.g., Lindsey 1988, Murray & Beacham 1989). However, since the relationships between potential factors and meristic counts vary among species (Lindsey 1988), detailed ecological studies for a given species of interest are required.

Pacific bluefin tuna (PBF) *Thunnus orientalis* is a highly migratory pelagic species that is widely distributed in the Pacific Ocean. Its spawning grounds have been observed only in the northwestern Pacific Ocean and adjacent waters. PBF then grows around Japanese coastal regions in their early life stages. To morphologically identify species during juvenile stage, the change in the GRN of PBF relative to body size has been studied using a growth model (Tanaka et al. 2020). Notably, the total GRN of PBF plateaus above 100 mm fork length (FL). Additionally, the GRN of the lower limb develops earlier compared with the upper limb, and the threshold of the plateau for the lower limb is estimated at 50 mm FL (Tanaka et al. 2020). However, GRN varies above these thresholds for unknown reasons. The ranges in Tanaka et al. (2020) above 100 mm FL are 32-39, 10-13, and 21-25 for a total, upper limb, and lower limbs, respectively. Additionally, the total GRN has been reported as 34-43 by Collette & Nauen (1983) and 32-43 by Nakabo & Doiuchi (2013). Although there are multiple spawning grounds in the western North Pacific, PBF are recognized to have only a single stock (Nakatsuka 2020). The main

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differences in spawning fish among spawning grounds are attributed to the size and age of spawning fish (e.g., Ashida et al. 2015, Ohshimo et al. 2018, Okochi et al. 2016). Therefore, the genetic influence on morphology can be disregarded, and the variation in the GRN of PBF should reflect differences in ecological characteristics during the early stages of life, specifically the environmental factors during these stages.

Otolith microstructure analysis is commonly used in fisheries to estimate hatch date and growth trajectory during early life stages (e.g., Secor et al. 1995). While otolith growth reflects body growth, it has been used to clarify the growth history and survival processes before recruitment (e.g., Campana 2005). Like many other species, this method has been applied to studies on PBF to estimate several ecological characteristics such as growth-selective survival and geographical variation in growth rate (e.g., Ishihara et al. 2019, Tanaka et al. 2006, 2020; Watai et al. 2017, 2018). Therefore, it can be assumed that the combined analysis of GRN and otolith microstructure will clarify the relationship between early growth and the GRN of PBF. If such relationship was observed, it should demonstrate that the variation in GRN is an ecophenotypic characteristic of PBF. Additionally, the stages to determine GRN could be clarified.

Accordingly, this study aimed to clarify whether GRN, a meristic count, is related to the early growth of PBF. As such, age-0 fish (which have a fully developed GRN) were collected around Japan and analyzed. Both the GRN and otolith microstructure were examined for all specimens. First, GRN and growth were compared between the Pacific Ocean and the Sea of Japan to examine the geographical variations with respect to spawning and nursery areas. This is because the samples collected from the Pacific Ocean (i.e., off Kochi) could have only originated from one spawning ground (around the Nansei Islands in the Pacific side), whereas samples collected from the Sea of Japan could have contained offspring from two spawning grounds (around the Nansei Islands and the Sea of Japan) (Fig. 1). After performing these geographical comparisons, the relationship between GRN and growth was examined among all samples to determine whether GRN has an ecophenotypic characteristic and critical stage to determine GRN. Finally, the effect of early growth on GRN is discussed.

Materials and methods

Samples were collected by coastal fisheries operating off the Shimane and Ishikawa prefectures in the Sea of Japan and off Kochi Prefecture in the Pacific Ocean during 2018-2020 (Fig. 1). Additionally, samples collected by research cruises using a surface-trawl (NST-520, Nichimo, Japan) around Sado Island in the Sea of Japan during 2018-2020 were used for analysis (see Sato et al. 2021, Tanaka et al. 2020). The mouth opening and mesh size of the cod-end of the trawl net were 30 m × 30 m and 11 mm, respectively. Fish were frozen at each port or on board before laboratory analysis. In the laboratory, FL was measured to the nearest millimeter using calipers (CFC-60GL, Mitsutoyo, Japan; CD-S15C, Mitsutoyo, Japan; PC-110KD, Niigataseiki, Japan). Gill arches and otoliths were then extracted for analysis. Since the increase in GRN with body growth stops around 100 mm FL (Tanaka et al. 2020), samples exceeding this threshold were used for analysis. A total of 360 juveniles with a FL ranging from 102 mm-369 mm were analyzed.

Left gill arches were used to count of GRN. As GRN does not differ significantly between the left and right arches with respect to body growth (Tanaka et al. 2020), the selection of arch would not have influenced the results. On the left gill arch, GRN was separately counted for the upper and lower limbs. One gill raker at the joint of these two limbs was not counted in either two limbs but was included in the total GRN.

Otolith microstructure analysis was performed according to the methods of Watai et al. (2017, 2018) and Tanaka et al. (2020). Otoliths were embedded in resin and sectioned using a low-speed saw (ISOMET; Buhler, Japan) parallel to the postrostral (longitudinal) axis. The core region of the otoliths in this axis was then exposed by polishing sectioned samples with a series of 400-2400-grit abrasive paper (Relex NACM; Meiwafosis, Japan) and by etching with 0.5 N HCl. The surface of the sample was photographed by a scanning electron microscope (TM3030; Hitachi, Japan) at 400-1,200× magnification. Finally, the increments and widths on these photographs were counted and measured using the RATOC otolith measurement system (ARP/W+RI; Ratoc System Engineering, Japan) along the direction of maximum otolith growth. Daily age (i.e., days after hatching, DAH) was calculated as the number of increments plus three, because the first daily ring is formed on the 4th day after hatching (Itoh et al. 2000).

GRN was compared between the Sea of Japan and the Pacific Ocean using the Wilcoxon rank-sum test because the normality of the data assumption was not supported by the Shapiro–Wilk test. Since otolith increment width (IW) changes drastically in relation to DAH during the early period of life, the mean IW at 5-day intervals (i.e., 5-9, 10-14, 15-19, 20-24, and 25-29 DAH) was also compared between the two seas (Watai et al. 2018). IW was analyzed using either the Student's *t*-test or the Wilcoxon rank-sum test based on

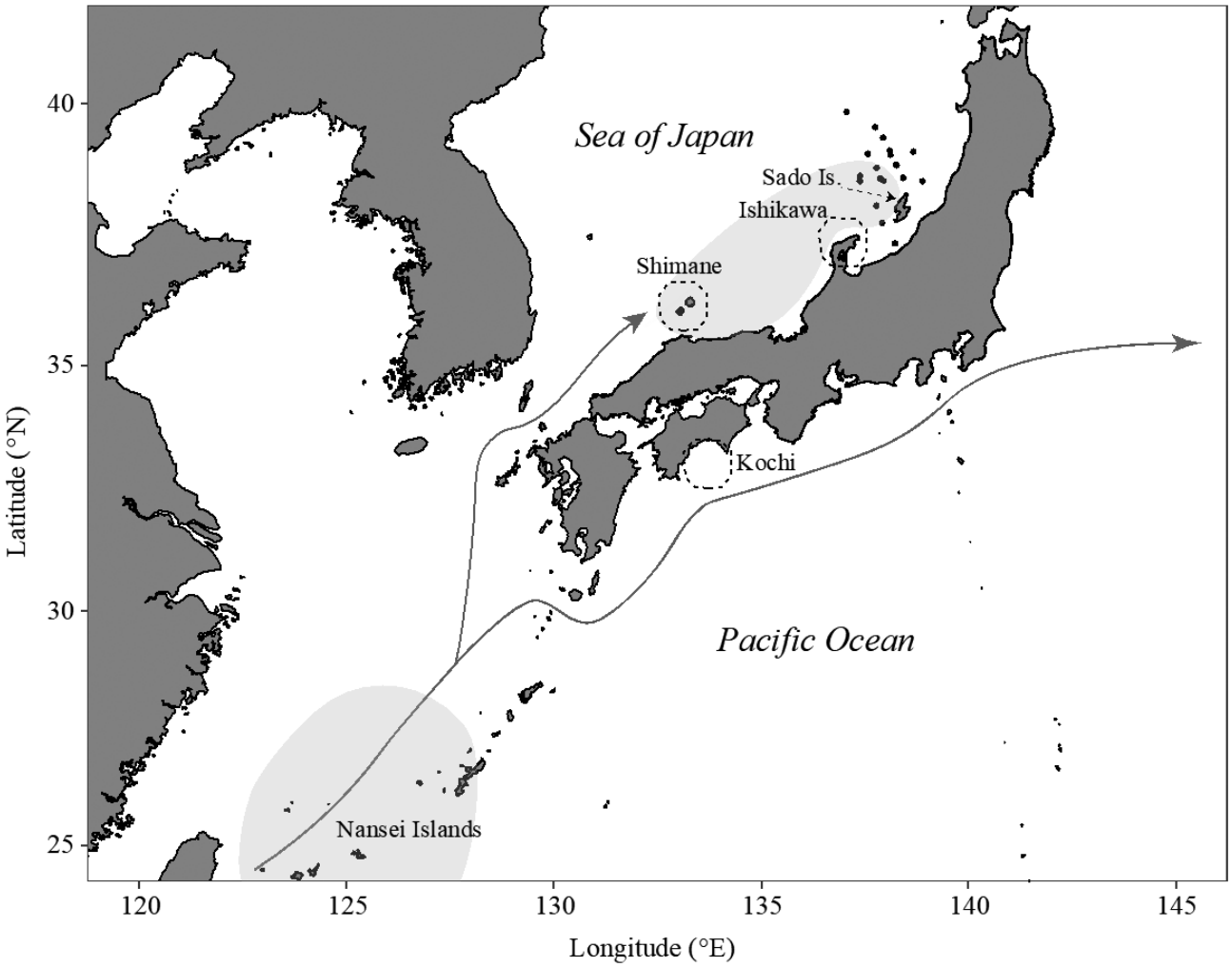

Fig. 1. Map of the sampling area (around Sado Island and off Kochi, Shimane, and Ishikawa prefectures)
Potential spawning areas (around the Nansei Islands and Sea of Japan) of the Pacific bluefin tuna samples are also shown as shaded areas, and current outlines are represented as arrows.

the significance determined by the Shapiro–Wilk test.

These IWs were then directly compared with GRN using all samples in the following steps. For each DAH interval, samples were divided into quartiles with respect to IW (i.e., 0-25, 25-50, 50-75, and 75-100%). Then, GRN (i.e., total, lower limb, and upper limb) was compared among these growth-based quartiles by the Kruskal–Wallis rank-sum test because the assumption of normality was not supported by the Shapiro–Wilk test. If a significant difference was found among groups, the Steel–Dwass test was performed for multiple comparisons among groups.

All statistical analyses were performed using R-version 3.6.3 (R core team 2020). The NMS3 package (Schneider et al. 2021) was used for the Steel–Dwass test. The level of statistical significance was set at $P < 0.05$.

Results

The total, lower limb, and upper limb GRNs ranged from 32-40, 21-26, and 9-13, respectively (Fig. 2). The GRNs for the lower limb differed significantly between the two seas (Wilcoxon rank-sum test, $P = 0.039$), whereas those for the upper limb ($P = 0.594$) and total ($P = 0.316$) did not. The GRN of the lower limb in the Pacific Ocean was greater than that in the Sea of Japan (Fig. 2). The daily age of samples at a catch ranged 33-105 DAH. IW was as follows: 1.10 μm-10.79 μm, 3.74 μm-41.25 μm, 8.01 μm-67.37 μm, 24.43 μm-69.61 μm, and 20.92 μm-72.19 μm at 5-9 DAH, 10-14 DAH, 15-19 DAH, 20-24 DAH, and 25-29 DAH, respectively (Fig. 3). IW differed significantly between the two seas at 5-9, 10-14, and 15-19 DAH (Wilcoxon rank-sum test,

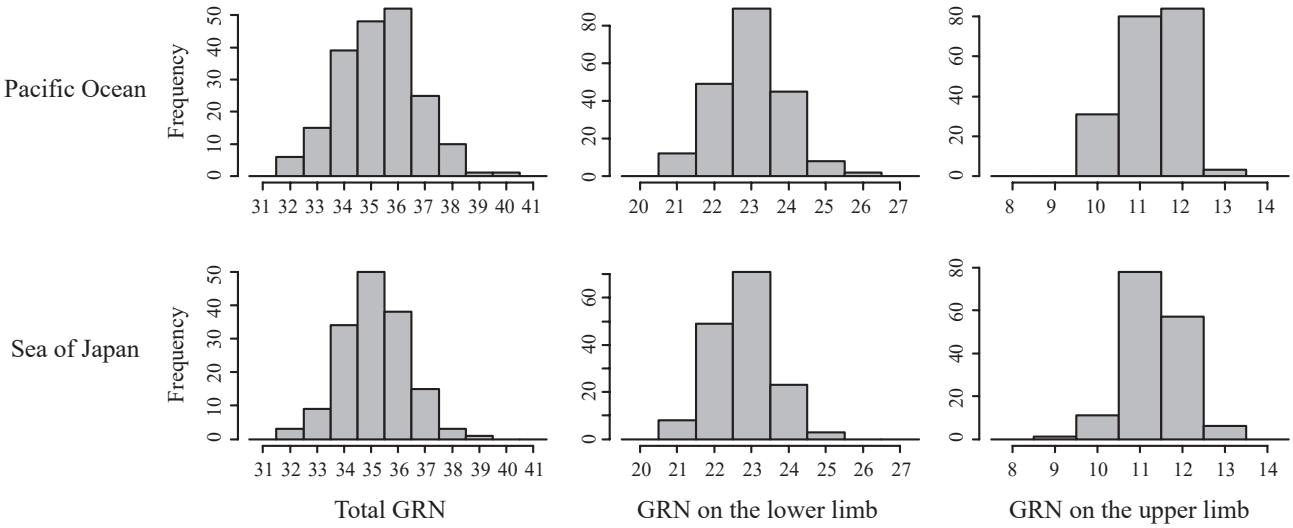

Fig. 2. Histograms showing the gill raker numbers (GRNs) of Pacific bluefin tuna
The total, lower limb, and upper limb GRNs of Pacific bluefin tuna are shown. Samples from the Pacific Ocean and the Sea of Japan are shown in the top and bottom 3 panels, respectively.

all $P < 0.001$), but not at 20-24 DAH (*t*-test, $P = 0.050$) or 25-29 DAH (Wilcoxon rank-sum test, $P = 0.982$). For the 5-9, 10-14, and 15-19 DAH group, IW was higher in the Sea of Japan than in the Pacific Ocean.

Among IW quartiles, there were significant differences in total GRN and GRN on the lower limb at 15-19 DAH (Kruskal–Wallis rank-sum test, $P = 0.002$ and 0.006×10^{-1}, respectively) and in the GRN on the lower limb at 10-14 ($P = 0.029$) and 25-29 DAH ($P = 0.034$) (Table 1). At 15-19 DAH, there were significant differences between the first and third quartiles of IW (Steel–Dwass test, $P = 0.009$ for total GRN, $P = 0.003$ for lower limb GRN) and between the first and fourth quartiles of IW ($P = 0.013$ for total GRN, and $P < 0.001$ for lower limb GRN) (Fig. 4). Samples with a larger IW tended to have a lower GRN. The GRN on the lower limb was significantly different only between the first and third quartiles of IW at 10-14 DAH (Steel–Dwass test, $P = 0.049$) (Fig. 5). There were no significant differences in the GRN of the lower limb by the multiple comparisons at 25-29 DAH (Steel–Dwass test, $P > 0.05$ for all pairs) (Fig. 5).

Discussion

The range of GRN was similar to foregoing findings (Collette & Nauen 1983, Nakabo & Doiuchi 2013, Tanaka et al. 2020). Geographical analyses revealed that both GRN and IW differed between the Sea of Japan and the Pacific Ocean (Figs. 2, 3). These results might suggest that growth rates in the early period of life could be related to GRN. Although IW in the Sea of Japan was larger than that in the Pacific Ocean from 5-19 DAH (Fig. 3), Watai et al. (2018) reported that the growth rate of PBF in the Sea of Japan was not always higher than that in the Pacific Ocean from 2011-2015. Ishihara et al. (2019) examined the otoliths of larval samples also collected from 2011-2015 and reported that the growth rate before the flexion stage was faster in the Pacific Ocean than that in the Sea of Japan. Therefore, the difference in the findings of growth rate between the present and previous studies might be due to differences in the study years or stage of fish. Accordingly, the geographical difference in the GRN of PBF between the two seas may be inconsistent characteristic.

The quartile-based analysis of IW corroborates the notion that the growth rate of PBF in early life determines their GRN. Specifically, faster early growth results in lower GRN. This pattern indicates that the duration wherein the differentiation of gill rakers occurs decreases when the body growth is faster. Meristic counts are associated with the temperature during the early period of life (e.g., Lindsey 1988). Sfakianakis et al. (2011) reported that lower temperature results in higher meristic counts of the zebrafish *Danio rerio* in a rearing experiment from the half-epiboly stage until after metamorphosis. Other studies report similar relationships between temperature and meristic counts (e.g., Murray and Beacham 1989). Since temperature is generally associated with growth rates, these findings also imply that growth rate and meristic counts are negatively associated. However, in the case of PBF, the temperature

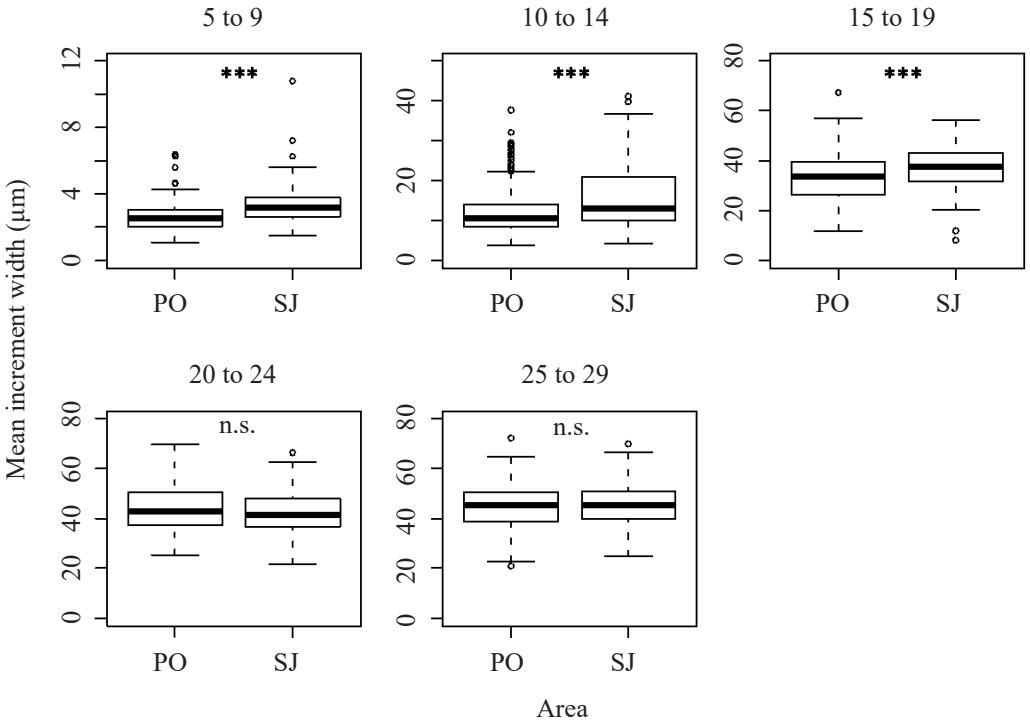

Fig. 3. Mean increment width of otoliths of samples from the Pacific Ocean (PO) and the Sea of Japan (SJ)
Data are shown for each age group (in days after hatching, noted above each panel). Asterisks and superscript letters indicate the significance of differences in each pair (*** $P < 0.001$, n.s.: not significant) by the *t*-test (20-24 days after hatching) or the Wilcoxon rank-sum test (other panels).

in the early period of life is generally higher in the Pacific Ocean (along with the Kuroshio current) than that in the Sea of Japan. For example, the temperature where the larvae were captured in the Pacific Ocean (around the Nansei Islands) and the Sea of Japan were approximately 25°C-29°C and 23°C-28°C, respectively (Ishihara et al. 2019). Similarly, juveniles in 15.0 mm-29.7 mm standard length were collected at temperatures from 26.5°C -27.5°C in the Pacific Ocean (Tanabe et al. 2022) conversely to their habitat temperature of approximately 24°C-25°C in the Sea of Japan at around 50 mm FL (Tanaka et al. 2020). Therefore, the growth rate of PBF may reflect other environmental factors aside temperature. Accordingly, a rearing experiment can be an alternative tool to examine potential factors affecting growth and meristic counts.

The GRN on the lower limb, except on the upper limb, differed significantly between the two seas. One possible reason for this difference is that there the GRN on the upper limb was smaller with less variance (0.529) than that on the lower limb (0.847) (Fig. 2). Another possible reason is the difference in the development of gill rakers between the two limbs. The GRN on the lower limb attains to the constant at a smaller body size (~50 mm FL corresponds to ~25-30 DAH) than that on the upper limb (~100 mm FL corresponds to ~30-40 DAH) (Tanaka et al. 2020). This difference in the speed of development could be a cause of the difference in the effect of body growth on GRN.

The growth from 15-19 DAH is one of the most important periods that determines GRN (Table 1). This period corresponds to the postflexion stage and shortly before metamorphosis in previous rearing experiments (Tanaka et al. 2007). The adult-type digestion system of PBF, which includes the development of a pyloric caecum and digestive enzymes, is established at 12-15 DAH in laboratory-reared fish (Kaji et al. 1996, Miyashita et al. 1998, Tanaka & Suzuki 2016). Additionally, the expression of gastric function-related genes is significantly elevated at around 15 DAH (Yasuike et al. 2021). Therefore, the condition and growth around 15 DAH could be a crucial determinant of the body structure with respect to feeding and hence the survival of PBF.

Current results further indicate that GRN can be a conventional metric for monitoring the early growth of PBF, which is usually determined by time-consuming

Table 1. *P*-values of the Kruskal–Wallis rank-sum test which compared gill raker number (GRN) among samples divided by quartile of otolith increment width for each age group (in days after hatching)

Days after hatching	Total GRN	GRN on the lower limb	GRN on the upper limb
5 to 9	0.407	0.448	0.745
10 to 14	0.086	**0.029**	0.638
15 to 19	**0.002**	**0.006×10^{-1}**	**0.320**
20 to 24	0.956	0.182	0.352
25 to 29	0.325	**0.034**	0.426

Significant values ($P < 0.05$) are shown in bold.

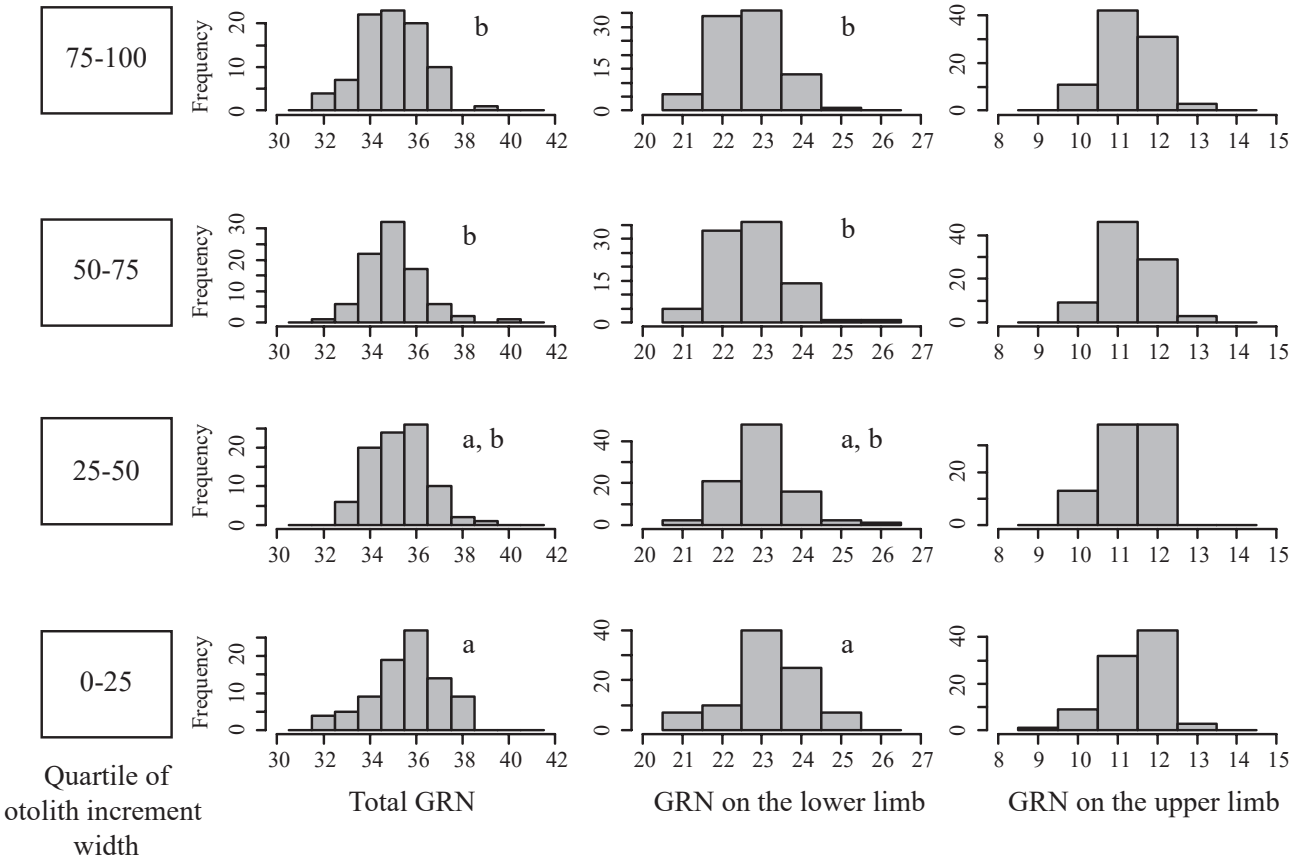

Fig. 4. Histograms showing gill raker numbers of Pacific bluefin tuna with respect to otolith increment width at 15-19 days after hatching

The total (left column), lower limb (center column), and upper limb (right column) gill raker numbers (GRNs) are shown. Data are presented for quartiles (noted in the boxes on the left) of otolith increment width at 15-19 days after hatching. Superscript letters indicate grouping determined by the Steel–Dwass test for multiple comparisons.

otolith examination before observation. Moreover, recruitment success can also be monitored by the GRN of age-0 fish if there is a clear relationship between early growth and the level of recruitment. Accordingly, future studies investigating GRN, growth, and recruitment would advance our knowledge about the recruitment success of many species.

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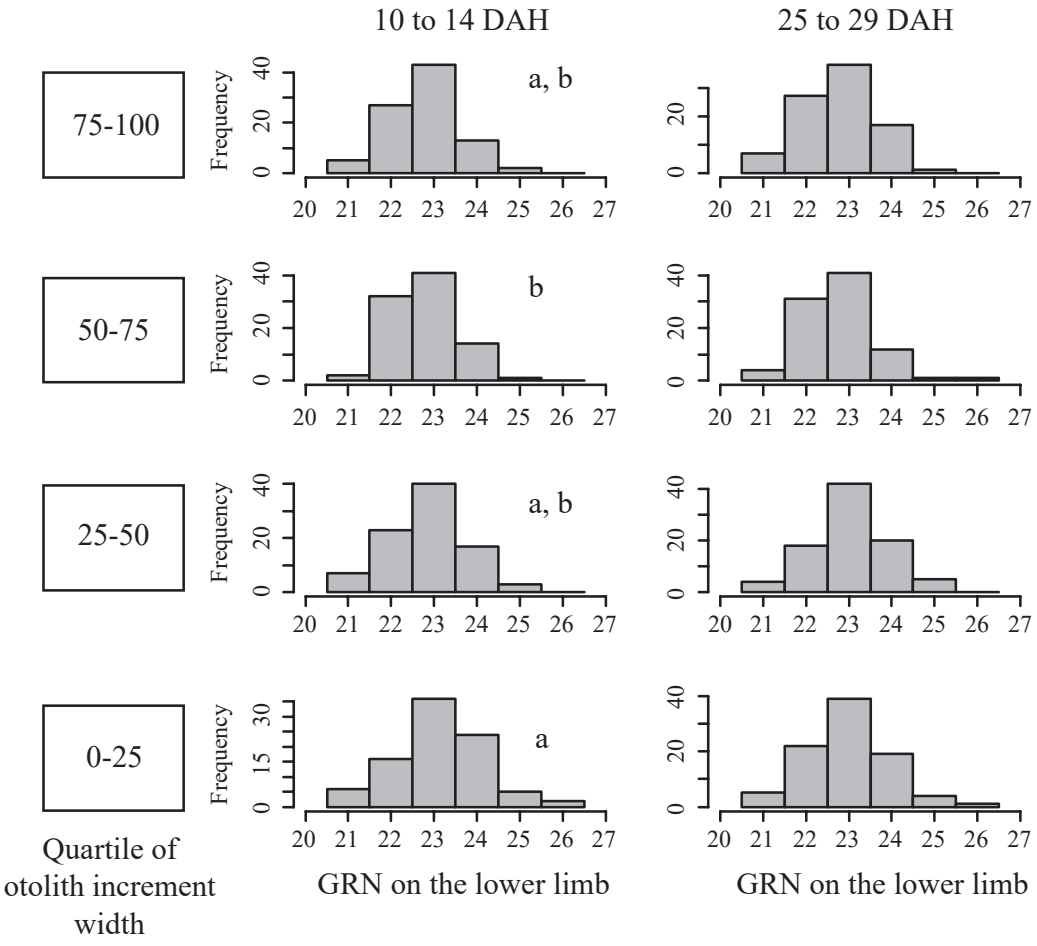

Fig. 5. Histograms showing gill raker numbers on the lower limb of Pacific bluefin tuna with respect to otolith increment width
Data are presented for quartiles (noted in boxes on the left) of otolith increment width at 10-14 (left column) and 25-29 days after hatching (right column). Superscript letters indicate grouping determined by the Steel–Dwass test for multiple comparisons.

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