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Otolith-based analysis of the early growth and survival processes in Pacific bluefin tuna Thunnus orientalis(耳石分析によるクロマグロの初期成長・生残に関する研究)

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Doctoral Dissertation

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March 2018

Graduate School of Marine Science and Technology
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Summary

The spawning stock size of Pacific bluefin tuna (PBF; *Thunnus orientalis*) is now at a historically low level (about 11,000 tons) according to the International Scientific Committee for Tuna and Tuna-like Species in the North Pacific Ocean and the fraction of the PBF fisheries catch represented by immature fish has become dominant. Under such circumstances, it is crucial to obtain knowledge on the early life processes that shape recruitment into the adult population in order to carry out successful fisheries resource management. In this study I used otolith analysis-based body size back-calculation to examine various processes associated with the early growth and survival processes in PBF from two spawning grounds, namely the Nansei Islands area and the Sea of Japan. My thesis is divided in three chapters as follows.

In the first chapter, I conducted a study to: (1) evaluate the hypothesis of growth-dependent survival in young PBF from the north-western Pacific Ocean; (2) identify critical developmental stages for survival; and (3) compare interannual differences in early growth. To this end, I compared the daily growth trajectories of a large number of larvae (standard length (SL) <15 mm), juveniles (15 ≤ SL ≤ 150 mm), and young-of-year (YOY; SL >150 mm) collected between 2011 and 2015. Otolith radius (OR) and SL were highly correlated and yielded a single relationship applicable for the five year-classes. Body size back-calculation showed that only larvae with fast, steady growth successfully transitioned to the juvenile stage whereas an ontogenetic analysis of daily growth rates revealed interannual differences only in larvae, and not in the larval stage of juveniles and YOY. Neither sudden decreases nor increases in growth rates were observed during the larval stage of any of the stages, suggesting that the observed variability in larval body size may be the result of individual differences in growth rates rather than of drastic, one-time events. Overall, the results of the present study indicated that growth-dependent survival

of larvae may be the most critical for PBF recruitment.

In the second chapter, I compared the growth history of YOY PBF from five year-classes (2011-2015) that were assigned to three cohorts based on estimated hatching date and capture site of each individual, namely those born in the Nansei Islands area and grown up in the Pacific coast of Japan, born in the Nansei Islands areas and grown up in the Sea of Japan, and born and grown up in the Sea of Japan. The results were discussed in relation to the available information on water temperature profiles during the spawning season and productivity in the two areas. Larval growth rates of PBF in the Sea of Japan were highly variable compared to those in the Nansei Islands areas and were not necessarily higher as expected from the inherent higher primary and secondary productivity of the Sea of Japan. The mean SL was higher in the Sea of Japan cohort than in the two Pacific cohorts in 2012 whereas the opposite pattern was found for 2014 and 2015. The apparent uncoupling of growth rates on food abundance in larvae in the Sea of Japan may be related to the inherent thermal instability and the proximity of winter in relation to the spawning season in this area, causing larvae to experience suboptimal temperatures for growth with increasing frequency. In contrast to larvae, local-born juveniles and Nansei Islands-born YOY living in the Sea of Japan had high growth rates compared to those in the Pacific in a manner that is consistent with the higher productivity in this area. These results also suggest that the growth rates of juveniles and YOY PBF may be less sensitive to decreasing temperatures or that older fish may have lower thermal optima for growth than larvae.

The third chapter was designed to compare the growth rates of larvae born successively later during the spawning season and to analyze the correlation of larval growth rates with water temperature. Using the same specimens and methods described in the second chapter, I assigned the early- and late-born specimens identified within each year as individuals born in the Nansei Islands area and the Sea of Japan, respectively. Next, the back-calculated body size for each day

of life was used to estimate the mean instantaneous growth rates of individual larvae hatched in successive two-week periods during the spawning season in the two areas. This study revealed different growth trends between Nansei area and Sea of Japan larvae from hatching to about 20 days after hatching. Thus, while Nansei Islands-born larvae showed no clear hatch date-dependent differences in growth rates throughout the spawning season, those born in the Sea of Japan showed first an increase and then a decrease in growth rates towards the end of the spawning season. Moreover, there was a close association between the larval growth trend and the water temperature profile in the Sea of Japan but not in the Nansei area. Since the Nansei Islands area is characterized by lower primary and secondary productivity as well as more massive PBF egg/larval production than in the Sea of Japan, it is suggested the possibility that the observed hatch date and temperature effects may be food-dependent.

Overall, this study demonstrated the occurrence of growth-dependent survival during the larval stage in Nansei Islands-born PBF. In the analysis, conducted with larvae, juveniles, and YOY, it was also possible to demonstrate significant interannual differences in growth rates of larvae but not in juveniles and young-of-the-year. These evidences support the notion that the larval stage is the most critical for PBF survival. In an analysis conducted with YOY born/grown up in the Sea of Japan and in the Western Pacific, it was possible to demonstrate marked interannual variation in larval growth rates in fish born in the former but not in the latter area. This study provided evidence of a positive, likely food abundance-dependent correlation between temperature during the early larval period and larval growth rates. Finally, this study showed that the Sea of Japan supports better juvenile and YOY growth than the Pacific coast of Japan.

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General Introduction

The Pacific bluefin tuna (PBF; *Thunnus orientalis*) spawns from April to June in the area off the Philippines up to the Ryukyu Islands in the north-western Pacific Ocean and from June to August in the Sea of Japan (Yabe *et al.*, 1966; Ueyanagi, 1969; Kitagawa *et al.*, 1995; Tanaka *et al.*, 1996; Ashida *et al.*, 2015; Okochi *et al.*, 2016). Larvae and juveniles hatched in the north-western Pacific Ocean and Sea of Japan are carried by the Kuroshio Current and Tsushima Warm Current (Tanaka *et al.*, 2007) respectively, whereas young-of-year (YOY; age-0) grow off the coast of Japan. Age-0–2 fish are caught by trolling, purse seine and set net fisheries. The trolling fishery in Kochi Prefecture targets YOY PBF in summer (Ichinokawa *et al.*, 2014). The spawning stock size of PBF is now at a historically low level according to the International Scientific Committee (ISC) Pacific Bluefin Tuna Working Group (http://isc.fra.go.jp/reports/stock_assessments.html, accessed 23 February 2017). Successful management of fish resources requires knowledge on all biological processes from spawning and early larval life until successful recruitment to the fisheries and spawning stocks. Under such circumstances, for fishery resource management, the knowledge about early life history before recruitment need to be accumulated.

The first objective of this study was to examine the relation of growth history and eventual recruitment using larvae, juveniles, and YOY collected in the north-western Pacific between2011 and 2015 and evaluate the hypothesis that larval survival is growth dependent. The second objective was to compare the growth patterns of three cohorts that differed in birth or grow-up area (e.g., born and grown up in the north-western Pacific, born in the north-western Pacific and grown up in the Sea of Japan, and born and grown up in the Sea of Japan) from birth

until the YOY stage during a 5 year period. The third objective was to compare the growth rates of larvae born successively later during the spawning seasons in the Nansei Islands area and the Sea of Japan and to analyze the correlation of larval growth rates with water temperature.

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Chapter 1. Evaluation of growth-dependent survival during early stages

Introduction

The number of fish recruited into the adult fish population is determined by the abundance of eggs and newborn stages and the subsequent losses incurred at each stage of development before the age of sexual maturation. Mortality is usually high and variable during the earliest stages (Peterson and Wroblewski, 1984; Peterman *et al.*, 1988). Thus, determining the contribution of each stage to the eventual recruitment will help identify critical or key life stages in the prerecruitment period for PBF (Bradford and Cabana, 1997).

Scombrid fishes, including PBF, are generally considered to have survival strategies in the early life stages characterised by feeding on large prey and rapid growth (Hunter, 1981; Tanaka *et al.*, 1996). Tanaka *et al.* (2014) suggested that fast-growing PBF larvae subsequently show significantly faster growth after they transition to feeding on fish prey, illustrating the importance of piscivory for the growth and survival of these fish during their early life stages. Several studies have reported growth-dependent survival processes during the early life stages of fish. According to these studies, larvae with higher growth rates or larger size-at-age have a higher probability of survival (e.g. Meekan and Fortier, 1996; Takasuka *et al.*, 2003; Robert *et al.*, 2007). Tanaka *et al.* (2006) hypothesised that the survival of larval PBF depends largely on their growth during the very early stages of their life history (6–13 days after hatching; DAH), before the onset of piscivory. Moreover, Satoh *et al.* (2008) reported high natural mortality of larval PBF in the north-western Pacific Ocean, which is considered to be the main spawning ground of PBF.

The present study is part of a larger study that includes larval and juvenile surveys to determine PBF spawning and nursery grounds and to analyse the process of PBF recruitment (Fujioka *et al.*, 2014). The objective of the present study was to obtain information on recruitment of age-0 PBF based on an analysis of otolith increments and body size back-calculation. Briefly, we evaluated the hypothesis that larval survival is growth dependent (Tanaka *et al.*, 2006) by comparing the growth trajectories of PBF larvae, juveniles and YOY collected in the north-western Pacific Ocean during surveys conducted between 2011 and 2015. We also examined the statistical significance of the interannual differences in larval growth rates for the three developmental stages. The results provided a working model for the process of PBF recruitment in the north-western Pacific.

Materials and methods

Sampling and measurements

Larvae were collected with a ring net in the waters around the Ryukyu Archipelago (Fig. 1.1) between May and August of each year. This survey period was chosen based on information on the spawning season of PBF (e.g. Ashida *et al.*, 2015; Okochi *et al.*, 2016). The diameter of the mouth of the ring net was 2 m, its length was 4 m and the mesh size was 0.34 mm. The net was towed at the sea surface beside the research vessel at a speed of ~1.5 kn (~0.77 m s–1) for 10 min each time. These operating conditions have been optimised in previous studies and were shown to allow unbiased collection of PBF larvae of all sizes (see Tanaka *et al.*, 2006). Juveniles were collected by midwater trawling in the north-western Pacific Ocean between May and July of each year. The width and height of the mouth of the midwater trawl net (NBT-2P-SY; Nitto Seimo, Tokyo, Japan) were both 30 m, and the net length was 85 m. The mesh size of the inner

bag of this trawl net was 15 mm. Trawling was done at night (1900–0500 hours) at a towing depth of ~20–30 m based on the distribution of juvenile PBF determined in previous surveys (Tanaka *et al.*, 2007). Depth recorders (SBT-500; Murayama Denki, Tokyo, Japan) were attached to the net otter boards and mouth to record the depth of each sampling. Towing duration was 30 min at each station and the towing speed was ~4–5 kn. YOY were caught by the troll fisheries off the coast of Kochi Prefecture during July and August of each year.

Smaller larvae were sorted by species on-board based on morphological characteristics (Okiyama, 1974) and were preserved in 99.5% ethanol for further examination and body size measurements. Older larvae and juveniles were identified using multiplex polymerase chain reaction (PCR) genotyping analysis (Suzuki *et al.*, 2014) because PBF, yellowfin tuna (*Thunnus albacares*), and albacore (*Thunnus alalunga*) occur sympatrically in the survey area and can be difficult to distinguish morphologically. The standard length (SL) of larvae was measured to the nearest 0.1 mm using ImageJ (http://imageh.nih.gov/ij/, accessed 23 February 2017) from digital images taken under a dissecting microscope. The fork length (FL) of juveniles and YOY was measured with a calliper to the nearest 0.1 mm, and the values were converted to SL using the equation SL = 0.95FL + 0.18 (the present study: r2 = 0.996, n = 218, SL = 13.3–173 mm, FL = 14.4–182 mm).

The otoliths of all specimens were removed, washed in distilled water, dried and preserved in individually labelled tubes for subsequent analysis. For the purposes of the present study, fish were divided into three size or stage classes based on the observations of Kaji *et al.* (1996), Miyashita *et al.* (2001) and Miyashita (2002) as follows: larvae (SL <15 mm); juveniles (15 ≤ SL ≤ 150 mm); and YOY (SL >150 mm).

Otolith analysis and body size back-calculation

Otolith radius (OR) and increment width measurements, as well as increment counting, were performed using the sagittal otoliths, but the analytical protocols differed somewhat between larvae and the other two developmental stages because of differences in otolith thickness and microstructure visibility. Briefly, larval otoliths were mounted on glass slides with nail enamel and were observed without sectioning because of their transparency (Tanaka *et al.*, 2006, 2014; Satoh *et al.*, 2013; see also Fig. 1.2). For juveniles and YOY, otoliths were embedded in epoxy resin and a thick section, including the otolith nucleus and the postrostrum, was cut with a low-speed saw (Isomet; Buehler, Evanston, IL, USA). The otolith sections were mounted on glass slides with resin after one side had been trimmed to as close as possible to the nucleus with 400- to 4000-grit abrasive paper (Reflex NAC M; Meiwafosis, Tokyo, Japan). The other side of the sectioning plane was then ground with a grinding or polishing machine (Doctor-lap ML-182; Maruto Instrument, Tokyo, Japan) and abrasive paper until the sections were ~50 μm thick. The sections were then further polished with 0.3-μm alumina powder (Micropolish II; Buehler) or diamond paste (DP-Paste; Struers, Copenhagen, Denmark) in a grinding or polishing machine (LaboPol-4; Struers, Copenhagen, Denmark) to a mirror-like finish.

Otoliths were analysed under a video microscope with transmitted light at magnifications of ×100–×500 with a non-immersion lens using the RATOC otolith measurement system (ARP/W+RI; Ratoc System Engineering, Tokyo, Japan). Measurements of increment width and OR were performed along a transect that included the otolith nucleus and postrostrum margin first automatically, and then visually corroborated by an observer. Increments were enumerated and assigned the corresponding age in days (DAH), taking into consideration that increments are formed daily and that the first increment forms at 4 DAH (Itoh *et al.*, 2000). The age, in days, of the marginal increment (day of capture) was used to estimate the hatch date of

each individual. The biological intercept method (Campana and Jones, 1992; Campana, 1996) was used to back-calculate the growth trajectory in terms of body size (SL) at each day of age for each fish based on the relationship between OR and SL.

Statistical analysis

Curve fitting for the OR–SL relationship was performed using the curve-fitting functions of Prism 5 (GraphPad Software, San Diego, CA, USA) using the Akaike information criterion (AIC) to select the best model. The F-test was then used to test for interannual variation in model parameters. The significance of differences in the back-calculated body size between larvae, juveniles and YOY per daily age was assessed by analysis of variance (ANOVA) followed by Tukey's multiple comparison test using Prism 5 (GraphPad Software). For daily ages and comparisons with only two groups (e.g. juveniles and YOY), the t-test was used. Similar analyses were performed to compare the growth rates of larvae, juveniles and YOY in equivalent periods of life (5-day periods) within each year-class and the interannual differences in growth rates for each developmental stage.

Results

Body size distribution and daily age of samples

In all, 604 larvae, 315 juveniles and 1336 YOY were collected for the present study. Most larvae were collected between May and June, although a few were collected as late as in middle August. Juveniles and YOY were collected in June–July and July–August respectively. Not all individuals could be analysed for some collection dates and, in these cases, representative

individuals were chosen randomly from among the available specimens (Fig. 1.3). The estimated hatch dates of the samples ranged from May to August (Fig. 1.4). Larvae born in July and August (<4% of the total number) were excluded from subsequent analysis to limit the cohort under investigation. Details of the capture date, body size and daily age distribution of the fish analysed are given in Table 1.1. In all, 577 larvae were analysed with SLs and estimated daily ages ranging from 2.4 to 11.0 mm and from 5 to 18 days respectively. The 92 juveniles analysed had SLs and daily ages ranging from 23.5 to 145 mm and from 18 to 55 days respectively. The SLs and daily ages of the 116 YOY used in the analysis ranged from 163 to 248 mm and from 44 to 85 days respectively.

OR–body size relationship and back-calculated growth trajectory

The highest AIC (%) values of the SL–OR relationships for the five 1-year datasets were obtained with quadratic functions (>99.9%) compared with linear and exponential models (<0.1%). There were no statistically significant differences in the Y-intercept (B0) and regression coefficients (B1, B2) of the SL–OR relationships for the five years and therefore all data were combined as a single function (SL = 2.6 + 0.088OR + 0.00001OR2; r2 = 0.996; Fig. 1.5). The combined SL–OR relationship was used to back-calculate the body size for each day of life and compare the early growth trajectories of larvae, juveniles and YOY of the same year-class on a daily basis. In all year-classes, larvae, juveniles and YOY had similar range and mean body size shortly (5 days) after hatching, but within 2 weeks larvae had significantly lower means than the other developmental stages (Fig. 1.6). Significant differences between the SL of larvae and the other stages were first noted from 6, 8, 8, 9 and 12 DAH in 2011, 2012, 2013, 2014 and 2015 respectively. The magnitude of the differences between the SL of juveniles and YOY varied

greatly with the daily age or year of observation. For example, the SL of juveniles around 20 DAH was marginally larger than that of YOY in 2011, 2012 and 2013, whereas for other years there was either no difference (2014) or SL was larger in YOY (2015). Thereafter, juveniles apparently lagged behind YOY in all year-classes but, due to the few available large juveniles, statistically significant differences could be demonstrated only in 2011 and 2014 (from 40 and 28 DAH respectively).

Developmental and interannual differences in growth rates

The back-calculated body size for each day of life was then used to estimate the instantaneous growth rates of individual fish for four 5-day periods (5–9, 10–14, 15–19 and 20–24 DAH) and compare developmental stages and year-classes. At first, the between-stage comparison was conducted using all available larvae, juveniles and YOY hatched between May and June without distinction of hatch date, but it was then reassessed using a narrower hatch date range (last 10 days of May only) to confirm the results for a more defined cohort. This period was chosen because it corresponds to the maximum availability of samples of the three developmental stages (see Fig. 1.4). From 5 DAH onwards larvae had significantly lower growth rates than the other two stages (Fig. 1.7). Comparisons of the growth rates of juveniles and YOY occasionally yielded significant differences, but without a clear pattern and in general were not significant. The results obtained with a narrower hatch date range strictly corroborated those obtained with the entire dataset (Table 1.2; Fig. 1.7).

Clear interannual differences in daily growth rates within each developmental stage were only noted for larvae between 5 and 14 DAH, whereby values tended to be higher in 2011 and 2015, lower in 2013, and intermediate in the other years (Fig. 1.8). Statistical comparison for

15–19 DAH was not possible due to the few larvae available. No clear interannual trend in instantaneous growth rates could be discerned for juveniles and YOY.

Discussion

Otolith-based back-calculation of body size on a daily age scale is a powerful tool to reconstruct the growth trajectories of individual fish and to compare survival and growth relationships for different developmental stages and year-classes (e.g. Campana, 1990; Hare and Cowen, 1995). The present study reports, for the first time and in detail, the OR–SL relationship for the entire larval–juvenile–YOY phase of PBF using only wild specimens. The strong correlation of OR and SL and, more importantly, the lack of interannual differences demonstrated in the present study would make it possible in the future to perform this analysis even without specific datasets for each year-class and to use otolith increment width as a proxy of past growth rates for the most critical developmental stages of PBF for recruitment.

A consistent pattern of growth was observed in all year-classes whereby juveniles and YOY have body sizes during their larval stage that are comparable to the upper values recorded in actual larvae of the respective year-class. This fact, and the fact that the difference between the mean body size of actual larvae and the larval stages of juveniles and YOY increases gradually with daily age, suggest that growth is maintained steady only in individuals successfully transitioning to the next developmental stage (the putative survivors), whereas poorly growing larvae do not survive beyond this stage. In contrast with the clear difference between the mean larval size of larvae and the other stages, the larvae of juveniles and YOY did not differ in size in a consistent manner. This observation strongly suggests that growth-dependent survival is most critical in the larval stage and less so in the subsequent stages (juvenile and YOY). Thus, it appears

that once PBF attain the juvenile stage, size-selective mortality is markedly reduced, perhaps as a result of factors including, but not limited to, an increase in the spectrum of food prey preference (including change to piscivority and possibly cannibalism), improved starvation tolerance, increased swimming ability and predator avoidance, or their combination (Tanaka *et al.*, 2008, 2010, 2014; Fukuda *et al.*, 2014).

That only fast-growing larvae survive seems to be a foregone conclusion, but it is rarely backed up by empirical evidence. For example, Le Pape and Bonhommeau (2015) identified an observation bias whereby growth rates are skewed towards higher values because only surviving individuals are analysed; hence, growth rates appear close to optimal even when food resources are clearly limited. This bias can be obviated by the comparison of past growth rates (or body size) in multiple developmental stages (including larvae) of the same year-class using otolith-based size back-calculation. Tanaka *et al.* (2006) first used this approach on larval and YOY PBF and provided preliminary evidence that survival of PBF depends largely on fast growth rates during the late larval phase. However, their observations were based on only one year-class (2004) and a limited number of specimens. The present study, conducted for the first time with a 5-year dataset (2011–15), three developmental stages (larvae, juveniles and YOY) and a large number of specimens, provides compelling evidence supporting the larval phase growth-dependent survival hypothesis for PBF. In addition, analysis of the daily increment width of otoliths, which reflects daily patterns of somatic growth (e.g. Hare and Cowen, 1995), helps explain how these differences between 'winners and losers' were brought about. The results reveal neither sudden decreases of increment width in actual larvae nor sudden increases of increment width in the larval stage of juveniles and YOY, suggesting that the body size differences at the larval stage observed in the present study may be the result of subtle, probably daily individual differences in growth rates

rather than of drastic, one-time events (e.g. acute food deprivation or chance encounter of a food patch; Tanaka *et al.*, 2008).

This result is not surprising in view of the findings of several other studies about the temporary uncoupling of otolith and body growth with changing food conditions (see Campana and Neilson, 1985). The otolith increment width analysis also revealed interannual differences in daily somatic growth during the period considered as critical for PBF survival (5–14 DAH) according to the present study. Food availability and ambient water temperature are the most critical factors affecting the growth of marine fish larvae (e.g. Govoni *et al.*, 1985; Folkvord *et al.*, 2000; Takahashi and Watanabe, 2004), including species of tuna (e.g. Wexler *et al.*, 2007; Garcia *et al.*, 2013; Satoh *et al.*, 2013). High temperature seems to promote growth rates in Atlantic bluefin tuna (*Thunnus thynnus*) in the Mediterranean Sea (Garcia *et al.*, 2013) and PBF in the north-western Pacific Ocean (Satoh *et al.*, 2013). Ongoing studies will examine the effects of these two critical factors on the interannual variation of PBF larval growth and survival. Likewise, it is not known whether the findings of the present study (obtained for specimens born in the Pacific Ocean) will also apply to PBF born in the Sea of Japan (Okochi *et al.*, 2016). Thus, further studies must compare the mechanisms underlying survival and recruitment for PBF cohorts born in different spawning grounds to obtain a thorough understanding of this species' resources in waters surrounding Japan.

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Table 1.1. Collection date, body size, and estimated daily age of Pacific bluefin tuna larvae, juveniles, and young-of-the-year that were analyzed in this study.

Developmental stage	Year	Collection date	*N*	Standard length (mm)			Estimated age (days)		
				Mean ± SD	MIN	MAX	Mean ± SD	MIN	MAX
Larvae	2011	6 Jun – 27 Jul	101	6.3 ± 1.3	3.4	10.1	11.7 ± 2.0	7	18
	2012	25 May – 25 Jul	69	4.9 ± 1.3	2.4	8.8	10.0 ± 2.2	5	17
	2013	7 May – 18 Jun	161	4.9 ± 0.6	3.5	8.8	8.5 ± 1.3	5	16
	2014	10 Jun – 20 Aug	11	5.8 ± 1.2	4.3	8.4	10.9 ± 1.7	8	13
	2015	26 May – 23 Jun	235	4.8 ± 1.0	3.0	11.0	8.7 ± 2.0	5	16
Juveniles	2011	21 Jun – 7 Jul	20	116.9 ± 10.9	105	137	40.5 ± 4.0	34	48
	2012	2 Jun – 28 Jun	34	79.0 ± 21.7	23.5	110	30.3 ± 4.7	19	39
	2013	2 Jun – 20 Jun	16	70.6 ± 42.8	29.5	137	29.1 ± 9.4	18	46
	2014	15 Jun – 3 Jul	20	78.4 ± 26.9	31.7	118	33.1 ± 7.7	22	50
	2015	3 Jul	2	142.8 ± 3.0	141	145	54.0 ± 1.4	53	55
Young-of-the-year	2011	15 Jul – 11 Aug	30	191.8 ± 19.9	164	227	59.6 ± 8.2	44	85
	2012	13 Jul – 15 Aug	20	191.9 ± 18.9	163	229	60.2 ± 4.5	51	71
	2013	25 Jul – 9 Aug	23	200.9 ± 19.2	164	227	60.8 ± 9.0	47	80
	2014	18 Jul – 22 Aug	20	198.4 ± 22.7	164	230	57.4 ± 6.4	46	68
	2015	20 Jul – 6 Aug	23	200.5 ± 24.4	167	248	61.2 ± 8.6	46	82

Table 1.2. Instantaneous growth rates for 5 DAH periods of PBF larvae, juveniles, and YOY hatched in late May between 2011 and 2015. Results are shown as mean ± standard deviation. Developmental stages within each daily age interval with different superscripts have significantly different means ($p<0.05$) in the ANOVA/Tukey's Multiple Comparison Test (comparison of larvae, juveniles, and YOY) or the T test (comparison of juveniles and YOY). N/A: not available.

		Growth rate (mm/day)							
Year	Developmental stage	5-9 DAH	n	10-14 DAH	n	15-19 DAH	n	20-24 DAH	n
2011	Larvae	0.21 ± 0.05 a	18	0.53 ± 0.15 a	17	0.53 ± 0.31 a	4		
	Juveniles	0.32 ± 0.10 b	10	1.37 ± 0.43 b	10	3.29 ± 0.87 b	10	4.21 ± 0.66 a	10
	YOY	0.34 ± 0.10 b	15	1.08 ± 0.24 b	15	2.71 ± 0.80 b	15	4.13 ± 0.72 a	15
2012	Larvae	0.19 ± 0.09 a	4	0.42 N/A	1				
	Juveniles	0.34 ± 0.10 b	28	1.62 ± 0.52 a	28	3.89 ± 0.69 a	28	4.56 ± 0.76 a	27
	YOY	0.27 ± 0.08 ab	8	1.20 ± 0.45 a	8	3.27 ± 0.76 a	8	4.55 ± 0.85 a	8
2013	Larvae	0.18 ± 0.05 a	6	0.26 ± 0.15 a	4	0.47 N/A	1		
	Juveniles	0.47 ± 0.07 b	6	1.84 ± 0.25 b	6	3.51 ± 0.31 a	6	2.72 ± 0.56 a	3
	YOY	0.31 ± 0.09 c	7	1.34 ± 0.63 b	7	2.99 ± 1.21 a	7	3.74 ± 0.55 a	7
2014	Larvae	0.24 ± 0.06 a	9	0.27 ± 0.07 a	8				
	Juveniles	0.33 ± 0.10 b	8	1.42 ± 0.50 b	8	3.24 ± 0.91 a	8	3.89 ± 0.76 a	8
	YOY	0.37 ± 0.06 b	8	1.73 ± 0.58 b	8	3.62 ± 0.91 a	8	4.15 ± 0.78 a	8
2015	Larvae	0.13 ± 0.02 a	8	0.92 N/A	1				
	YOY	0.32 ± 0.12 b	9	1.38 ± 0.37 N/A	9	3.41 ± 0.63 N/A	9	4.11 ± 0.59 N/A	9

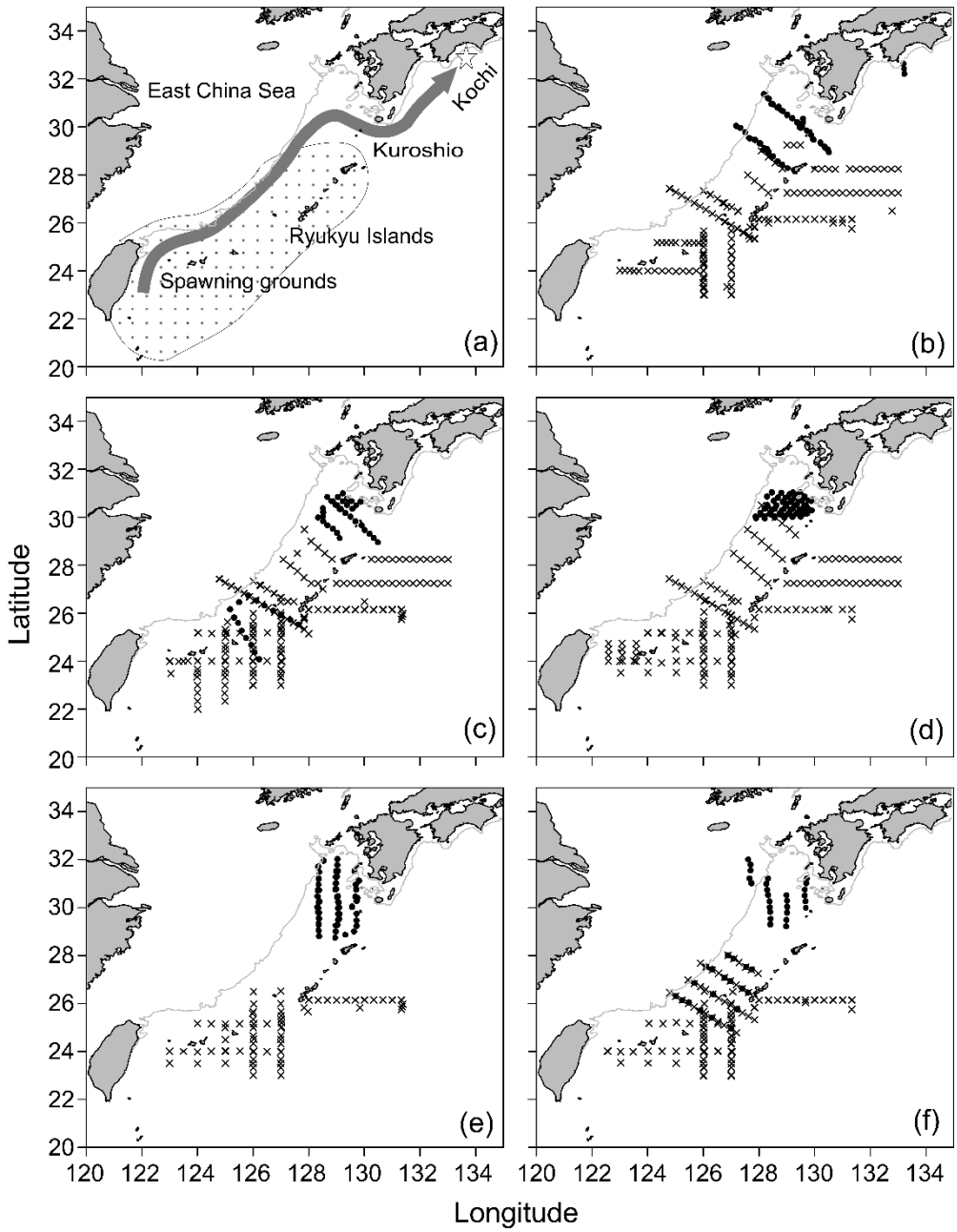

Fig. 1.1. (*a*) Schematic showing the spawning grounds (dotted areas) and the transport route of Pacific bluefin tuna larvae and juveniles in relation to the Kuroshio Current (shaded line) and the Kochi fishing grounds (star) to which young-of-year recruit. (*b*–*f*) Stations sampled for larvae and juveniles in the north-western Pacific Ocean in 2011 (*b*), 2012 (*c*), 2013 (*d*), 2014 (*e*) and 2015 (*f*). Crosses and circles respectively represent the sampling stations of larvae and juveniles.

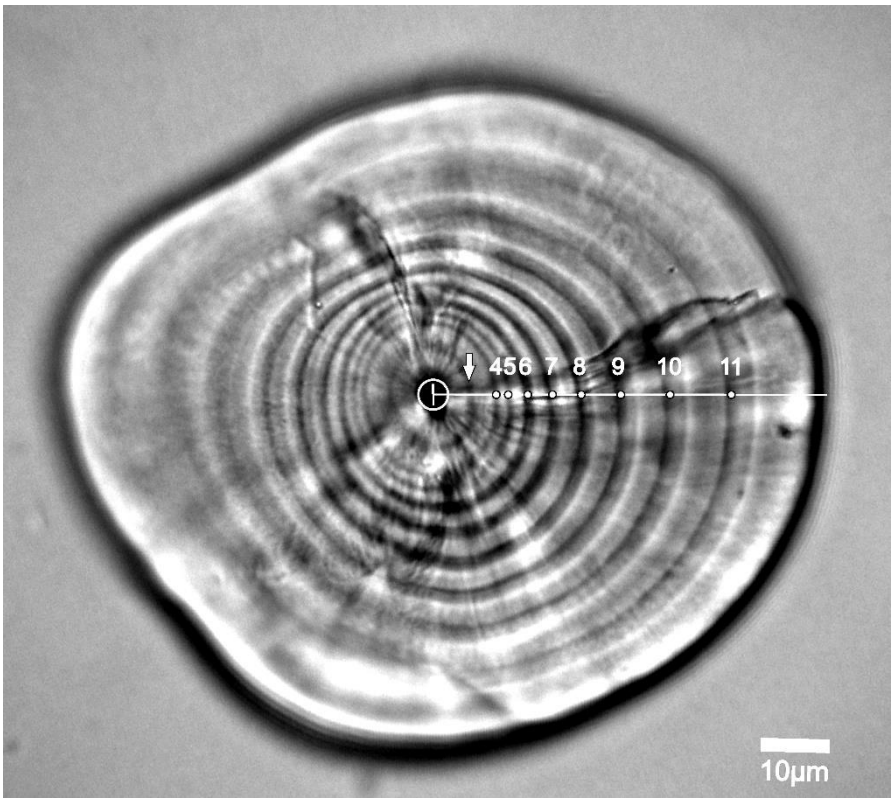

Fig. 1.2. Light microscopic visualisation of growth increments in the sagittal otolith of a Pacific bluefin tuna larvae (standard length 8.2 mm). Arrow and numbers respectively represent the diffused D-zone and the increments corresponding to Days 4–11.

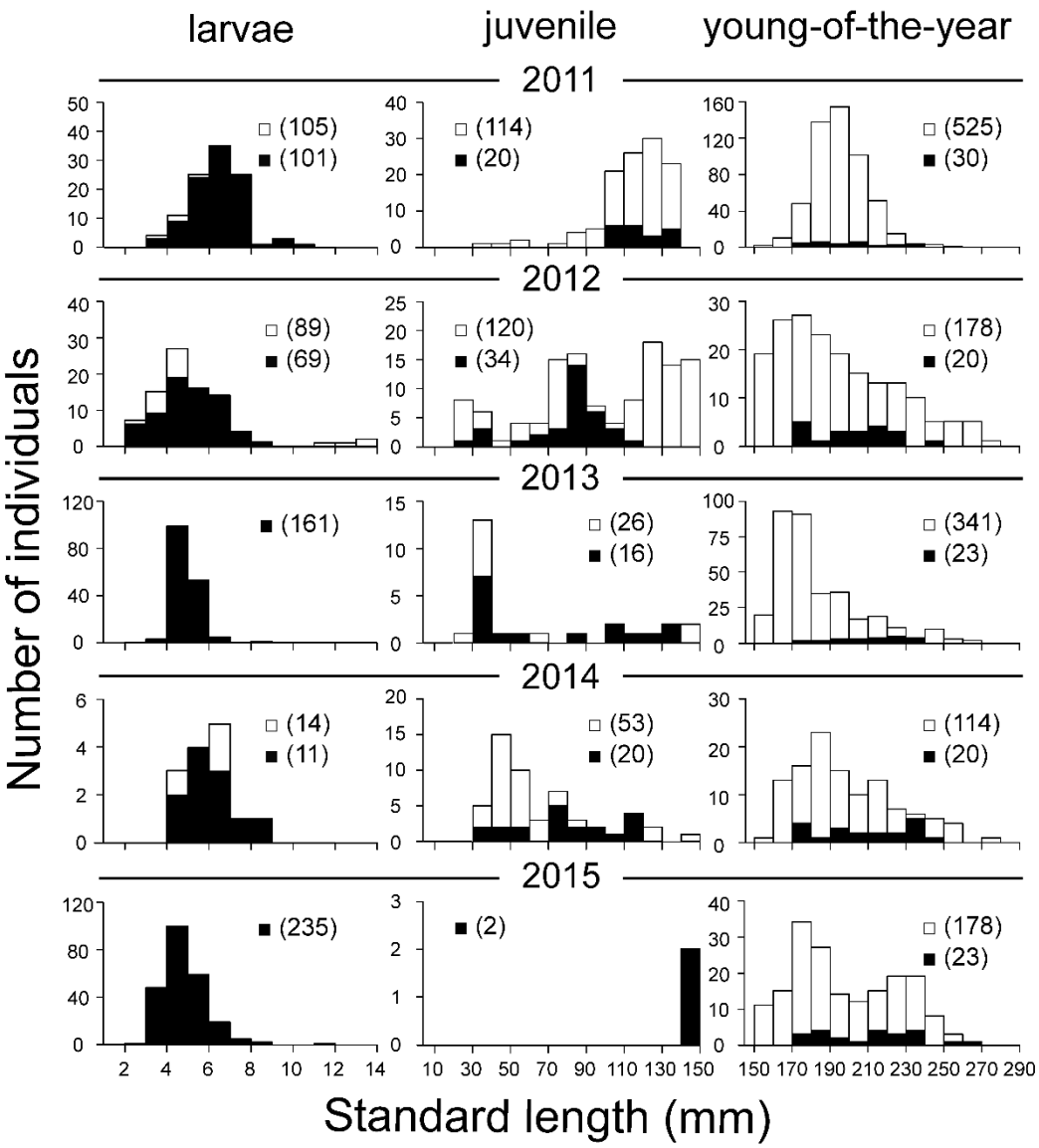

Fig. 1.3. Distribution of the standard lengths of Pacific bluefin tuna collected in the north-western Pacific Ocean from 2011 to 2015 (open bars; background) and those analysed in the present study (shaded bars; forefront). (*a*) 2011, (*b*) 2012, (*c*) 2013, (*d*) 2014 and (*e*) 2015. YOY, young-of-year.

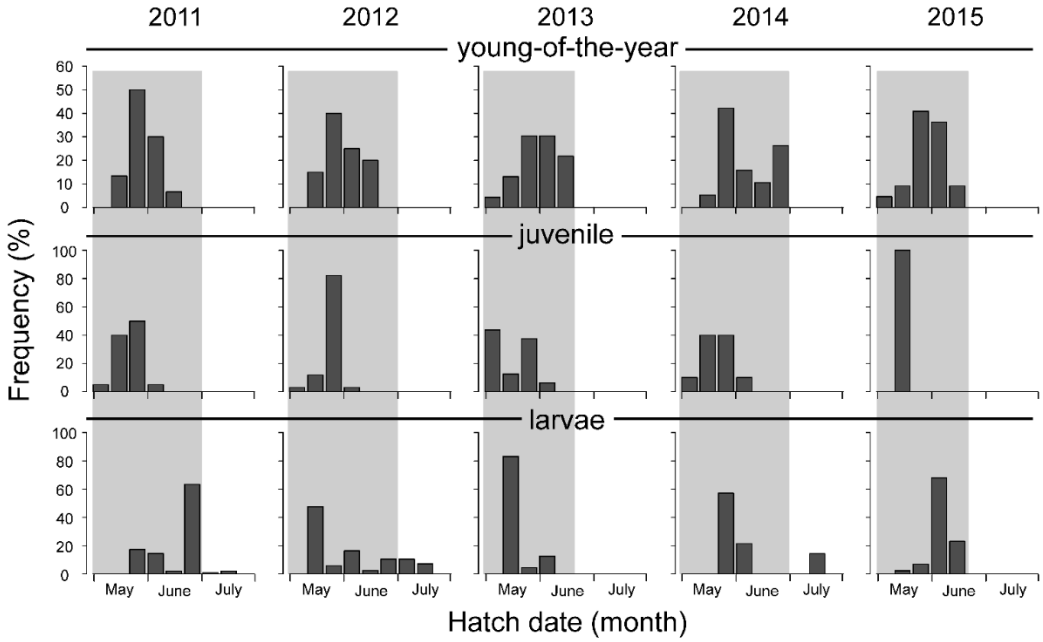

Fig. 1.4. Distribution of estimated hatch dates of (*a*) larvae, (*b*) juveniles and (*c*) young-of-the-year (YOY) of Pacific bluefin tuna collected for the present study between 2011 and 2015 as estimated from otolith daily increment analysis. Hatch dates within each month were divided in three 10-day intervals and the shaded area represents the range of hatch dates used in the growth analysis.

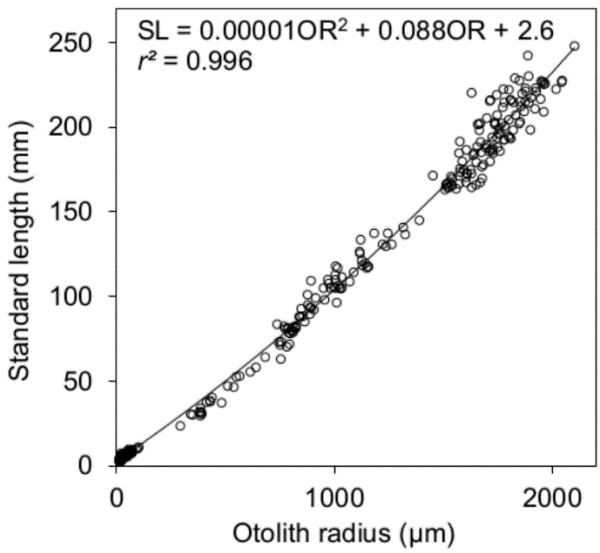

Fig. 1.5. Relationship between standard length and otolith radius of Pacific bluefin tuna larvae, juvenile and young-of-the-year from the 2011–15 year-classes.

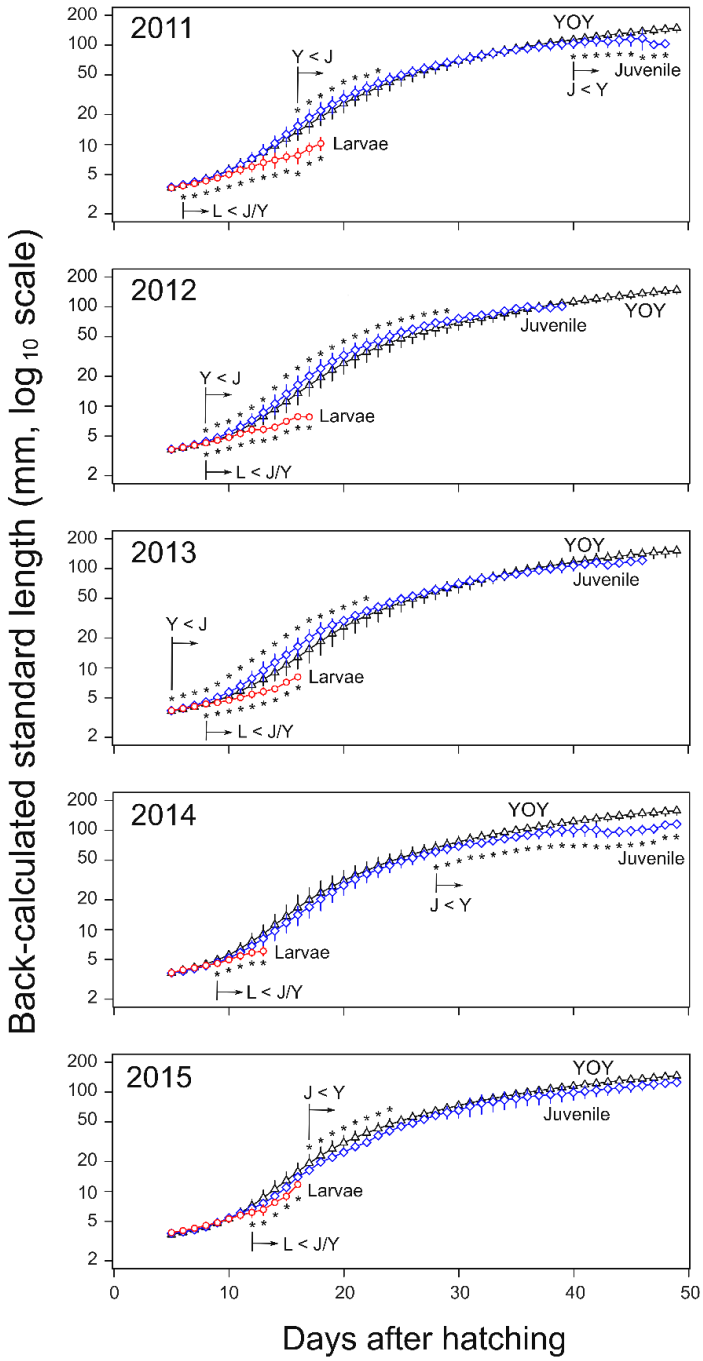

Fig. 1.6. Comparison of back-calculated standard length (mean ± s.d.) of Pacific bluefin tuna larvae (L; red circles), juvenile (J; blue squares) and young-of- year (Y and YOY; black triangles) for the 2011–15 year-classes. Asterisks represent statistically significant differences ($P < 0.05$, *t*-test) between larvae and the other stages or between juveniles and YOY.

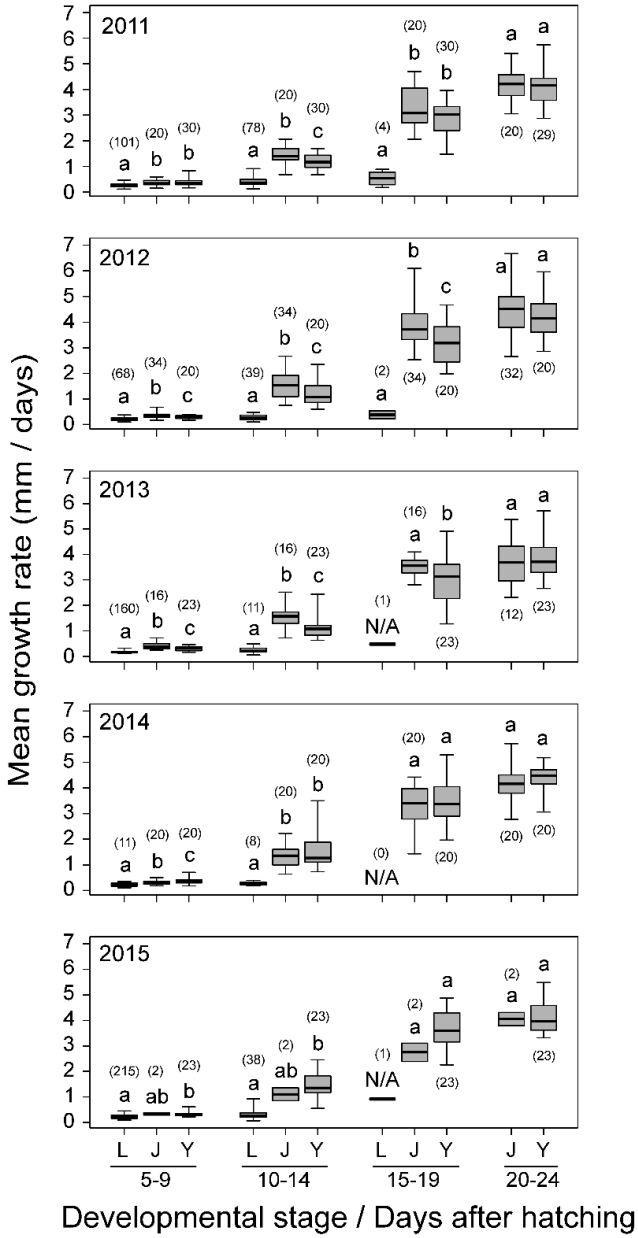

Fig. 1.7. Comparison of the mean growth rates of larvae (L), juveniles (J) and young-of- year (Y) Pacific bluefin tuna of five year-classes (2011–15) for five daily age intervals (5–9, 10–14, 15–19, 20–24 days after hatching). The plots show median values (horizontal lines within boxes), the standard deviation (boxes), maximum and minimum values (whiskers). Mean values for developmental stages within each daily age interval with different letters differ significantly different ($P < 0.05$, ANOVA followed by Tukey's multiple comparison test (for comparisons of L, J and Y) or *t*-test (for comparisons of J and Y). N/A, not available.

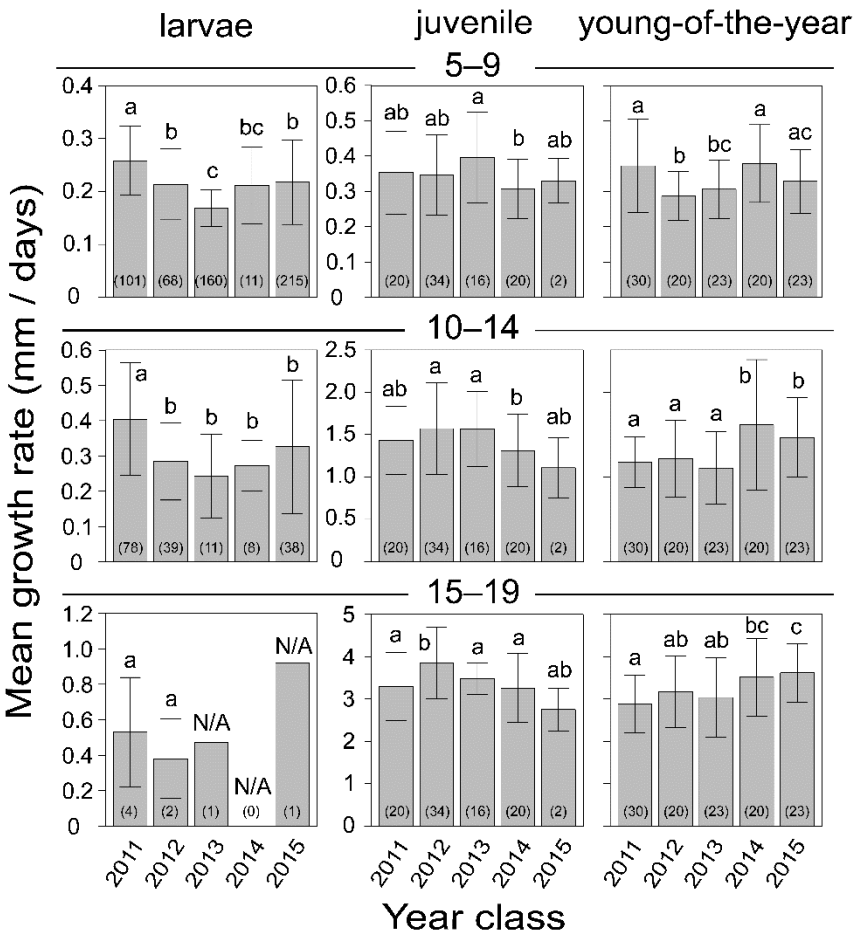

Fig. 1.8. Interannual comparison of the mean (±s.d.) growth rates of larvae, juveniles and young-of-year (YOY) Pacific bluefin tuna of five year-classes (2011–15) for three daily age intervals, namely (*a*) 5–9, (*b*) 10–14 and (*c*) 15–19 days after hatching. Mean values for year-classes within each developmental stage and daily age interval with different letters differ significantly ($P < 0.05$, ANOVA followed by Tukey's multiple comparison test). N/A, not available.

Chapter 2. Otolith-based analysis of the early growth history on different spawning grounds and nursery areas

Introduction

At present, two distinct spawning grounds of PBF have been identified, each with a characteristic spawning period. The first is observed between April and June in the Nansei Islands area from nearly the Philippines to the Ryukyu Islands and the second between July and August in the Sea of Japan (Ashida *et al.*, 2015; Ohshimo *et al.*, 2017 Okochi *et al.*, 2016). Fish born in the former spawning ground start moving northward at some time after birth and reach the Japanese East and West coasts as juveniles following the Kuroshio and Tsushima Warm Currents, respectively (Satoh *et al.*, 2008; Kitagawa *et al.*, 2010; Masujima *et al.*, 2014). PBF born in the Sea of Japan grow in that area until the YOY stage and then start migrating to the East China Sea (Itoh *et al.*, 2003). Hence, the Nansei Islands area, the Pacific coast of mainland Japan and the Sea of Japan are important nursery grounds for PBF larvae and juveniles (Tanaka *et al.*, 2006; Tanaka *et al.*, 2007). However, both nursery grounds show substantially different environmental conditions such as thermohaline structure and biological productivity, with the Sea of Japan being considered as colder, less saline, and more productive than the Pacific (Kodama *et al.*, 2015).

The analysis of growth and survival of each developmental stage can help identify critical or key life stages in the pre-recruitment period of fish (Bradford and Cabana, 1997). Natural mortality of larval PBF in the north-western Pacific Ocean spawning grounds has been shown to be high (Satoh *et al.*, 2008). In fact, in a study utilizing larvae, juveniles, and YOY born in the Nansei Islands area, Watai *et al.* (2017) showed that the survival of PBF born in the Nansei Islands area depends largely on their growth rates during the very early stages of life (6–13 DAH),

before the onset of piscivory. Moreover, their analysis of the past larval growth rates of young PBF born between 2011-2015 by the otolith growth back-calculation method revealed significant inter-annual differences in growth rates, which likely reflect yearly differences in environmental conditions even for the same spawning ground. As noted above, the two known spawning grounds for PBF differ in their physical and biological characteristics and as such may be associated with differential growth and survival, and consequently differential contribution to the adult and fisheries stocks of PBF. However, the survival processes of PBF born in the Sea of Japan remain largely unknown so it is its contribution to PBF stocks. In the present study, we applied the same methodology of otolith microstructure analysis employed by Watai *et al.* (2017) to study the past growth rates of YOY PBF captured between 2011 and 2015 in the Pacific Ocean on the East coast of Japan and in the Sea of Japan. Furthermore, the YOY PBF in this study were classified in three cohorts following criteria described by Itoh (2009), namely those 1) born and grown up in the north-western Pacific (hereafter referred to as P/P), 2) born in the north-western Pacific and grown up in the Sea of Japan and (J/P), and 3) born and grown up in the Sea of Japan (J/J), so this study was designed to compare the growth patterns of these three cohorts from their birth until the YOY stage during a 5 year period.

Materials and methods

Sampling and body size measurement

Age-0 PBF were caught by the commercial fisheries using trolling lines, purse seines or set nets off the coast of Kochi, Nagasaki, Yamaguchi, Shimane, Ishikawa and Toyama Prefecture between July and December each year from 2011 to 2015 (Fig. 2.1, Table 2.1). The FL were measured with a caliper to the nearest 0.1 mm and the values were converted to SL by the equation SL = 0.976FL-1.926 (this study; R^2 = 0.99, n = 514, 11.9-543.0 mm FL). In all, 4653 specimens

were collected for the present study and about 10% of these were randomly chosen for otolith analysis. Details of the capture date, body size and daily age (see details below) of the 429 fish that were analyzed are given in Table 2.1.

Otolith analysis and hatch date estimation

The sagittal otoliths were removed on site, washed in distilled water, dried and preserved in individually labelled tubes for subsequent analysis in the laboratory. Different analytical protocols, e.g. scanning electron microscope (SEM) and optical microscope, were used for YOY collected after and before winter, respectively, because the otolith increments formed in winter are very thin and are only discernible by SEM.

The otoliths of samples collected after winter were mounted in epoxy resin and sectioned with a low-speed saw (Isomet; Buehler, Evanston, IL, USA). The cross-section blocks were then polished with a series of 400-4000 grit abrasive paper (Reflex NAC M; Meiwafosis, Tokyo, Japan) to expose the core region and etched with 0.5 or 2N HCl until all increments and the otolith nucleus could be easily distinguished. Specimens were then examined and photographed in a SEM (Hitachi TM 3030; Hitachi Ltd, Tokyo, Japan). The otoliths of samples collected before winter were processed in the same way until exposing the core region. The otolith blocks were then glued to slide glasses and the reverse side of the block was further ground with abrasive paper in a grinder/polisher (Doctor-lap ML-182; Maruto Instrument, Tokyo, Japan) until the sections were ~50 μm thick. The specimens were further polished with 0.3-μm alumina powder (Micropolish II; Buehler) or diamond paste (DP-Paste; Struers, Copenhagen, Denmark) in a polisher (LaboPol-4; Struers) to a mirror-like finish. Otoliths were then observed under a video microscope with transmitted light and non-immersion lenses at magnifications of 100-500X.

The photographs and live video images in case of SEM and optical microscope observation, respectively, were then analyzed using the RATOC otolith measurement system (ARP/W+RI; Ratoc System Engineering, Tokyo, Japan). Measurement of increment widths were performed along the direction of maximum otolith growth connecting the otolith nucleus and the postrostrum edge and typically included two mild inflection points where the otolith growth axis shifted (see Fig. 2.2). Increment identification was done first automatically by the software and then visually corroborated by an observer. Increments were enumerated and assigned the corresponding age in days (DAH), taking into consideration that increments are formed daily and that the first increment forms at 4 DAH (Itoh *et al*., 2000). The age, in days, of the marginal increment (day of capture) was used to estimate the hatch date of each individual.

Hatch date/fishing ground-based cohort assignment

Cohort (PP, PJ, or JJ) assignment was based on fishing ground and hatch date information following the method of Itoh (2009). Briefly, a Gaussian mixture model (GMM) was first fit to the hatch date-frequency distributions for all five years using the package MCLUST (Fraley and Raftery, 2009) and the statistical computing program R (R Development Core Team 2008) to discriminate cohorts of "early-" and "late-born" individuals. Peak hatching date for each cohort was obtained from the GMM model as the average hatching date. The reference date separating each cohort within each year was then calculated as the middle point between the average hatching dates for the two cohorts (Fig. 2.3). Based on previous information on the spawning period and spawning area of the PBF (Kitagawa *et al.* 1995; Tanaka *et al.* 1996; Itoh, 2009; Ashida *et al.* 2015; Okochi *et al.* 2016; Ohshimo *et al.* 2017), the "early-" and "late-born" individuals were assumed to represent cohorts of fish hatched in the Nansei Islands area in the north-western Pacific Ocean (hereinafter referred to as "P/") and in the Sea of Japan ("J/"). To

this, information on the fishing ground (considered as the nursery area for young fish; "/P" for fish captured in the Pacific side of Japan and "/J" for fish captured in the Sea of Japan) was combined to define the three cohorts of PBF as P/P, P/J, and J/J for growth comparison.

OR-SL relationship and growth back-calculation

The OR of each individual was calculated as the sum of all increment widths from otolith nucleus to edge regardless of the presence of two mild inflexion points, as noted previously. The growth history for each individual in terms of the body size (SL) at each day of age was then back-calculated based on the increment widths and the relationship between OR and SL that was obtained as follows. First, the possibility of differences in the OR-SL relationships for the three cohorts (P/P, P/J, and J/J) separated by the GMM model and for different years of observation were tested, giving a total of 15 outcomes (3 cohorts vs. 5 years). Curve fitting was performed as described in Watai *et al*. (2017) using the Akaike information criterion (AIC) function of Prism 5 (GraphPad Software, San Diego, CA, USA) to select the best model. Data for larval and early juvenile specimens from the Nansei Islands area and the Japan Sea from Watai *et al.* (2017) and Ishihara *et al.* (unpublished observations), respectively, were added to the corresponding data sets to compensate for the lack of younger individuals/smaller otoliths in the current study. The preliminary analysis showed no significant differences between P/P, P/J, and J/J within the same year (results not shown). Furthermore, as demonstrated previously for a five year data set from the Nansei Islands cohort (Watai *et al*., 2017; equivalent to P/P in this study), this study also showed no significant inter-annual differences within each cohort (results not shown) and therefore all data sets were combined to select a single OR-SL model.

Water temperature profiles during the spawning season

The water temperature profiles during the spawning seasons in the Nansei Islands area (April to June) and in the Sea of Japan (July to August) for the five years of this study were estimated from high resolution (0.25 degree latitude x 0.25 degree longitude) data available from National Oceanic and Atmospheric Administration Advanced Very High Resolution Radiometer (NOAA/AVHRR) for the areas determined as spawning grounds by Ohshimo *et al*. (2017) and indicated in Figure 2.1. Surface temperature in the Nansei Islands area was calculated as the average of 169 grid cells within 22.875-25.875 degrees latitude and 123.625-128.125 degrees longitude. For the Sea of Japan, the average included 48 grid cells located within 35.875-37.125 degree latitude and 133.625-136.375 degree longitudinal. At the time the study was conducted data for 2015 had not yet been released.

Statistical analysis

The significance of the differences in back-calculated body size per daily age between fish assigned to the three cohorts (P/P, P/J, and J/J) was assessed by analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The t-test was used for comparisons with only two groups (e.g. P/J and J/J). Similar analyses were performed to compare the growth rates of the three cohorts in equivalent periods of life (5-day periods) within each year-class and of the same cohort in different years of observation.

Results

Hatching date estimation and cohort assignment

The estimated hatch dates of the specimens ranged from April to September (Fig. 2.3, Table 2.1). As described above, we assumed that the early- and late-born groups of specimens separated by the GMM model in each year represented individuals born in the Nansei Islands area

and the Sea of Japan, respectively. The average hatching dates for PBF from the Nansei Islands area and the Sea of Japan in 2011, 2012, 2013, 2014, and 2015 were 31 May and 1 August, 15 June and 23 August, 6 June and 26 July, 4 June and 29 July, and 1 June and 30 July, respectively, and the reference dates for separating the cohorts in each year were 1 July, 19 July, 1 July, 2 July, and 30 June, respectively. Thus, specimens captured in the Japan Sea were a mix of fish born in the Nansei Islands area (cohort P/J) and locally (cohort J/J) whereas those captured in the Pacific Ocean were all born in the Nansei Islands area (cohort P/P).

Otolith radius – standard length relationship and back-calculated growth history

As expected from Watai *et al*., (2017), a quadratic function gave the best fit to the SL-OR data as follows: $SL = 1.9 + 0.1OR + 0.00002OR^2$ ($R^2 = 0.99$; Fig. 2.4). Back calculation of the daily growth history of individual fish using this SL-OR relationship and daily otolith increment data showed that in general, all cohorts, regardless of the year of observation, had similar SLs at the first recognizable increment (corresponding to 5 DAH; Fig. 2.5). Between 5 and 20 DAH, the mean SL was higher in the "late-born" (J/J) cohort than in the two "early-born" cohorts (P/P and P/J) in 2012 whereas the opposite pattern was found for 2014 and 2015. There were no noticeable differences for the same period between the three cohorts in 2011 and 2013. From 20 to about 75 DAH, the last point in time when the three cohorts could be compared, SLs clearly became smaller in fish growing in the Pacific (P/P) compared to those growing in the Japan Sea (J/J and P/J). The mean SLs between 100 and 150 DAH for the P/J cohort were significant higher than those for the J/J cohort in 2012. In other years the differences were not significant but a similar trend of lower SLs in J/J fish at the end of this period was observed also in 2011, 2013, and 2014.

Developmental and inter-annual differences in growth rates

The back-calculated body size for each day of life was then used to estimate the mean instantaneous growth rates of individual fish in five-day periods between 5 and 54 and 110 and 139 DAH (Fig. 2.6). The trends agree in general with those of back-calculated SL and help explain the origin of the differences in body size between cohorts described in Figure 2.5. For example, the "late-born" (J/J) cohort showed faster growth rates between 5 and 19 DAH than the "early-born" cohort (P/P) in 2012 whereas the opposite pattern was found for 2014 and 2015. Meanwhile, in 2011 and 2013 there were no clear trends during this period for any of the three cohorts. From about 20 to 34 DAH, the J/J cohort had faster growth rates than P/P fish in all years except 2014 whereas P/J fish generally had intermediate values. Significantly faster growth rates in either P/J or J/J cohorts compared to P/P fish were observed sporadically between 35 and 54 DAH in 2013-2015 but not in 2011 and 2012. The P/J cohort consistently showed significantly faster growth rates after 110 DAH than the J/J cohort in all years except 2013 (Fig. 2.6).

Significant inter-annual differences in growth rates for equivalent 5-DAH segments were noted in all cohorts but in different periods depending on the cohort and year (Figs. 2.7, 2.8). For example, in the P/P cohort, growth rates were particularly slow between 5 and 9 DAH in 2012 and faster between 10 and 19 DAH in both 2014 and 2015. Interestingly, growth rates in the J/J cohort were faster between 5 and 24 DAH in 2012 and slower between 5 and 19 DAH in both 2014 and 2015. The P/J cohort only showed significant inter-annual variation after 50 DAH and the growth rates between 110 and 134 DAH were consistently slower in 2013 compared to other years.

Water temperature changes during the spawning season

The water temperature profiles in the Nansei Islands area and the Sea of Japan during

the spawning seasons between 2011 and 2015 are shown in Figure 2.9. The range of temperatures during the spawning season in the two areas is similar, with a minimum of 22ºC and a maximum of 29ºC. However, while the temperature in the Nansei Islands area increases steadily along the spawning season, that in the Sea of Japan decreases towards the end of the spawning season and shows abrupt changes and marked inter-annual variation.

Discussion

Two main spawning grounds of PBF have been identified, one near the Nansei Islands in the north-western Pacific Ocean and one in the Sea of Japan, but the spawning season in these two areas differ considerably in time to the point that both hardly overlap (Ashida *et al.*, 2015; Ohshimo *et al.*, 2017 Okochi *et al.*, 2016). Thus, spawning in the former area occurs earlier, usually from April to June, whereas in the latter it occurs mainly between July and August (Ashida *et al.*, 2015; Ohshimo *et al.*, 2017 Okochi *et al.*, 2016). PBF born around the Nansei Islands remain in the natal area for about 2-3 weeks after hatching and then move northward with the Kuroshio Current (Satoh *et al.*, 2008; Kitagawa *et al.*, 2010; Masujima *et al*., 2014). Most of the Nansei Islands-born PBF that move north reach the Pacific coast of Japan where they spend their first year (Kitagawa *et al.*, 2010; Masujima *et al*., 2014; Furukawa *et al*., 2016; Fujioka *et al*., in press) but part of the fish enter the Sea of Japan and eventually mix with local-born individuals. The longer and earlier spawning season in the Nansei Islands suggests that PBF from this spawning ground constitute the majority of the recruits each year and that they are larger than individuals born later in the Sea of Japan (Itoh, 2009). However, very little is known about the growth and survival processes of PBF born in these two grounds. Recent studies using otolith analysis to reconstruct the growth history of individual PBF born in the Nansei Islands revealed the occurrence of growth-dependent larval survival, that is, only larvae showing the fastest growth

rates successfully became juveniles and subsequently YOY (Tanaka *et al.*, 2006; Watai *et al.*, 2017). Whether a similar process occurs in the Sea of Japan remains unknown. In this study, we took advantage of the different spawning seasons between cohorts, which are reflected in different birth dates, to discriminate between fish born in the two spawning grounds and compare their past growth rates using otolith-based growth back-calculation analysis. Unlike in the previous study, which compared the growth history of larval, juvenile, and YOY specimens within the same cohort, in this study we compared the past growth rates in critical life stages of YOY PBF that were 1) born and grown up in the Pacific (cohort P/P), 2) born in the north-western Pacific and grown up in the Sea of Japan (cohort P/J), and 3) born and grown up in the Sea of Japan (cohort J/J). The three life stages analyzed were the larval, juvenile, and recruitment/pre-recruitment stages.

The first and most critical stage is from hatching to about 20 DAH, when mortality is usually high and when growth rates have the highest impact on subsequent survival (Tanaka *et al.*, 2006; Watai *et al.*, 2017). Growth back-calculation revealed that the size-at-daily age and corresponding growth rates of Sea of Japan-born (J/J) fish at the larval stage were significantly higher than in the north-western Pacific-born cohorts (P/P and P/J) in 2012 but lower in 2014 and 2015. In the two other years (2011 and 2013), the growth rates in all cohorts were similar. This inconsistency appears to be chiefly associated with marked inter-annual variations in the growth rates of J/J larvae before 20 DAH (e.g., highest and lowest in 2012 and 2014-2015, respectively) rather than in north-western Pacific-born larvae, which show little or no variation during this period across the years. It is noteworthy that the Sea of Japan is considered as more productive than the north-western Pacific in terms of phytoplankton and zooplankton production (Chiba *et al.*, 2005; Hsiao *et al.*, 2011; Kodama *et al.*, 2011, 2015; see Taniguchi 1977 and Satoh *et al.* 2013 for information on the Nansei Islands area spawning ground), although this higher productivity is

not always associated with the abundance of suitable food for PBF larvae (see Kodama *et al.* 2017). On the other hand, the surface water temperature was found to decrease before the end of the spawning period in the Sea of Japan, a fact not seen in the Nansei Islands area (this study). Likewise, the Sea of Japan is known to have weak vertical stability and to reflect local meteorological events, resulting in marked, sometimes abrupt short-term temperature fluctuations (Toba *et al.*, 1982; Yamada *et al.*, 2006). In fact, the temperature profiles determined in this study support the relative thermal instability of the Sea of Japan spawning grounds compared to the Nansei Islands area between 2011 and 2015. Moreover, PBF larval growth seems to be positively affected by increasing temperature within the range observed during the spawning season (Satoh *et al*., 2008) whereas a decrease of only 3ºC causes a sharp drop in growth rates (Kimura *et al.*, 2010; Tanaka *et al*., 2010; see also similar information for the Mediterranean bluefin tuna in Garcia *et al.*, 2013). In this context, the variable growth rates of PBF larvae born in the Sea of Japan are very likely a reflection of the late spawning season and proximity of winter, associated with the inherent high variability of water temperatures in this area.

The juvenile period between 20 and 75 DAH for Nansei Islands-born PBF includes the northward migration and is characterized by marked individual variation in growth rates probably associated with the transition from planktophagy to ichthyophagy (Tanaka *et al*., 2014). In contrast, to Nansei Islands-born PBF, fish born in the Sea of Japan are thought to spend this period locally (Itoh, 2009). A numerical simulation of the northward transport process of Nansei Islands-born PBF suggested that fish reach a branch point off the southern tip of Kyushu Island at about 50 DAH, from where they either continue on the Kuroshio Current and into the east Pacific coast of Japan or enter the Tsushima warm current and into the Sea of Japan (Masujima *et al.*, 2014). It is noteworthy that the oceanographic conditions near the branch point between October and January are highly variable, changing sometimes in a matter of hours (Nakamura *et al.*, 2003;

Kodama *et al*., 2015). Thus, juveniles growing up in different areas conceivably encounter different environmental conditions that might affect their growth. In fact, the results of this study consistently show higher growth rates in the Sea of Japan throughout the juvenile stage, which supports the general notion that the Sea of Japan is highly productive as discussed above. This is particularly clear in the comparison between Nansei Islands-born fish that took different routes whereby P/J juveniles had higher growth rates than P/P juveniles at almost any time point. The actual temperatures experienced by fish that took different northward routes are not known but the higher growth rates of juveniles entering the Sea of Japan suggest either that temperature was not as limiting for growth in the juvenile stage of PBF as it was in the larval stage or that food abundance compensated for any unsuitable temperature the larvae may have experienced. Interestingly, the differences between P/J and P/P fish became evident already from about 20 DAH. This suggests either that Nansei Islands-born PBF arrive at the branching point earlier than mathematically estimated (e.g. around 20 DAH and not 50 DAH) or that fish entering the Sea of Japan take a yet unknown, different route than the mainstream Kuroshio Current. These possibilities should be scrutinized in further studies.

Individuals that successfully recruited into the YOY (100-150 DAH) cohorts in the Sea of Japan and the Pacific coast of Japan remain in these areas for some time and are eventually exploited by the troll fisheries (Itoh *et al*., 2003; Tanaka *et al*., 2007; Itoh 2009; Fujioka *et al*., in press). Unfortunately, YOY could not be collected in the Pacific coast of mainland Japan so information on the growth rates of P/P fish is only available up to the juvenile stage. YOY from the two spawning grounds that mix in the Sea of Japan (P/J and P/P) produce a bimodal size distribution in which the immigrants, born 50-60 days earlier in the northern-western Pacific, are assumed to be larger than those born later in the Sea of Japan (Itoh, 2009; Uematsu *et al.*, 2018). In fact, the results of this study show that 100-150 DAH J/J YOY consistently had inferior growth

rates than same-daily aged P/J fish. However, it must be noted that the two cohorts differ in average daily age by about two months when they co-inhabit the Sea of Japan and that temperatures in the area tend to drop sharply towards the end of the spawning period. Thus, it can be surmised that J/J juveniles and YOY experience lower, growth unfavorable thermal conditions than P/J at comparable ages, and this ultimately causes the size different reported by Itoh (2009). In fact, some of the catches analyzed in this study included such mix of early-born/relatively larger, presumably P/J fish, and late-born/smaller, presumably J/J fish (results not shown), so future studies should examine if the inherent size advantage of the early-born P/J fish confer them also a competitive advantage over J/J fish when in coexistence.

In conclusion, the results of this study suggest that the relative growth of Nansei Islands- and Sea of Japan-born PBF cohorts until the YOY stage are dependent on complex interactions of birth date and the environmental conditions experienced by the fish at different developmental stages. For example, larvae born in the Sea of Japan do not necessarily benefit from the high primary and secondary productivity in this area, although this is observed occasionally as in 2012. This uncoupling of growth rates on food abundance in larvae is probably related to the inherent thermal instability and the proximity of winter in relation to the spawning season in this area, causing larvae to experience suboptimal temperatures for growth with increasing frequency. On the other hand, the high growth rates shown by local-born juveniles and Nansei Islands-born YOY living in the Sea of Japan are consistent with the higher productivity in this area and also suggest that either the growth rates of older fish are less sensitive to decreasing temperatures or that older fish have lower thermal optima for growth than larvae. Further studies should examine the effects of temperature on growth in relation to developmental stage of PBF and to examine the actual correlation of growth rates in different developmental stages with the strength of each year-class.

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Table 2.1. Fishing ground, year-class, collection date, estimated hatch date, body size, and estimated daily age of Pacific bluefin tuna young-of-the-year that were analyzed in this study. Results are shown separately for “early-” and “late-born” cohorts that were discriminated with a Gaussian mixture model (see text for details).

Fishing ground	Year-class	Early-born cohort						Late-born cohort					
		N	Collection date	Estimated hatch date	SL (mm)			N	Collection date	Estimated hatch date	SL (mm)		
					Mean ± SD	Min	Max				Mean ± SD	Min	Max
Kochi	2011	29	2011/7/15 - 2011/8/11	2011/5/14 - 2011/6/18	194.8 ± 20.0	165.7	229.9						
	2012	20	2012/7/13 - 2012/8/15	2012/5/13 - 2012/6/16	194.0 ± 19.3	164.6	231.6						
	2013	23	2013/7/25 - 2013/8/9	2013/5/6 - 2013/6/14	203.2 ± 19.6	165.0	229.3						
	2014	20	2014/7/18 - 2014/8/22	2014/5/20 - 2014/6/30	200.6 ± 23.1	165.9	232.8						
	2015	22	2015/7/20 - 2015/8/6	2015/5/6 - 2015/6/12	202.7 ± 25.5	168.8	251.0						
Nagasaki	2012	3	2012/10/22 - 2012/10/22	2012/6/14 - 2012/6/28	467.1 ± 3.4	463.9	470.6	1	2013/1/9	2012/8/20	366.2		
	2015	5	2015/9/3 - 2015/12/6	2015/4/29 - 2015/6/17	461.8 ± 81.6	325.5	522.8	10	2015/10/8 - 2016/1/16	2015/7/4 - 2015/8/17	428.9 ± 56.1	323.6	525.9
Yamaguchi	2011	19	2011/11/6 - 2012/1/18	2011/4/24 - 2011/6/29	483.4 ± 31.2	439.5	534.4	17	2011/11/30 - 2012/1/29	2011/7/4 - 2011/8/29	415.5 ± 54.4	313.8	483.6
	2012	11	2012/11/18 - 2012/12/4	2012/5/25 - 2012/7/14	501.4 ± 7.7	490.3	514.3						
	2013	5	2013/11/25 - 2013/12/17	2013/5/11 - 2013/6/17	483.3 ± 28.2	452.9	522.1	8	2013/11/25 - 2013/12/17	2013/7/6 - 2013/9/7	471.1 ± 17.2	457.1	511.3
	2014	6	2014/11/25 - 2015/1/14	2014/5/24 - 2014/6/28	489.4 ± 27.2	445.2	512.8	19	2014/11/25 - 2015/1/14	2014/7/3 - 2014/8/28	454.5 ± 11.8	442.0	477.5
	2015	7	2015/11/7 - 2015/11/7	2015/5/12 - 2015/6/24	476.1 ± 5.3	468.5	481.2	1	2015/11/7	2015/7/5	476.5		
Shimane	2011							11	2011/11/1 - 2011/11/14	2011/7/13 - 2011/8/20	334.2 ± 10.1	323.6	352.5
	2012							8	2012/11/15 - 2012/11/30	2012/8/6 - 2012/8/26	342.0 ± 5.8	329.1	346.6
	2013	4	2013/10/10 - 2013/12/17	2013/6/22 - 2013/6/29	431.0 ± 37.9	398.3	466.7	30	2013/10/2 - 2013/12/17	2013/7/6 - 2013/8/17	331.4 ± 45.2	264.5	466.7
	2014							17	2014/10/26 - 2014/11/19	2014/7/22 - 2014/8/25	307.3 ± 27.1	244.0	338.0
	2015	1	2015/10/3	2015/6/26	354.6			19	2015/9/21 - 2015/11/30	2015/7/1 - 2015/9/3	298.0 ± 53.3	223.4	359.6
Ishikawa	2011							6	2011/12/21 - 2011/12/21	2011/7/16 - 2011/8/20	344.8 ± 5.6	339.3	352.4
	2012	8	2012/12/21 - 2013/1/30	2012/5/26 - 2012/7/17	521.9 ± 14.4	497.0	540.0	18	2012/11/8 - 2013/1/30	2012/7/22 - 2012/9/17	372.3 ± 55.5	341.5	524.4
	2013	2	2013/12/9 - 2013/12/11	2013/6/26 - 2013/6/28	451.1	450.1	452.1	16	2013/10/26 - 2013/12/11	2013/7/4 - 2013/8/25	386.9 ± 35.7	336.9	436.5
	2014	13	2014/11/21 - 2015/1/5	2014/4/11 - 2014/6/30	521.6 ± 8.8	507.8	535.1	5	2014/12/15 - 2015/1/21	2014/7/16 - 2014/8/25	307.3 ± 27.1	244.0	338.0
	2015	3	2015/12/7 - 2016/1/14	2015/5/1 - 2015/6/13	532.2 ± 30.1	514.3	567.0	6	2015/12/8 - 2016/1/21	2015/7/6 - 2015/8/11	414.0 ± 32.4	380.8	455.0
Toyama	2011							16	2011/9/29 - 2011/10/28	2011/7/23 - 2011/8/18	287.2 ± 29.9	245.8	330.8
	2012	8	2012/12/28 - 2013/1/21	2012/6/8 - 2012/7/14	517.0 ± 11.9	497.0	536.9	4	2012/12/19 - 2012/12/19	2012/8/20 - 2012/8/30	360.8 ± 1.0	359.3	361.3
	2013							4	2013/12/19 - 2013/12/19	2013/7/26 - 2013/8/15	350.1 ± 7.1	340.1	356.6
	2015	2	2015/12/8 - 2015/12/8	2015/5/24 - 2015/6/1	520.5	509.4	531.6	2	2015/12/4 - 2015/12/4	2015/8/5 - 2015/8/11	354.4	352.3	356.4

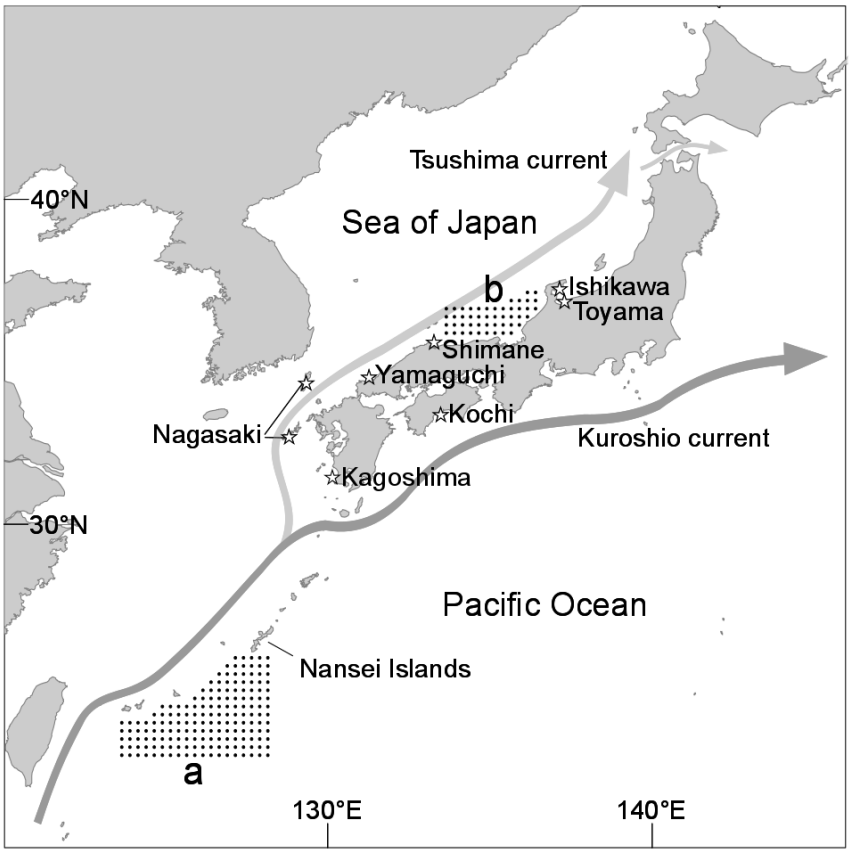

Fig. 2.1. Approximate location of the capture sites (stars) of age 0 year Pacific bluefin tuna for this study and of the spawning grounds in the Nansei Islands (a) and Sea of Japan (b) with the corresponding grids of 0.25 degrees of latitude x longitude cells used to estimate sea surface temperatures from NOAA/AVHRR data. Capture sites are represented by the names of the prefectures in whose fishing port the fish were landed.

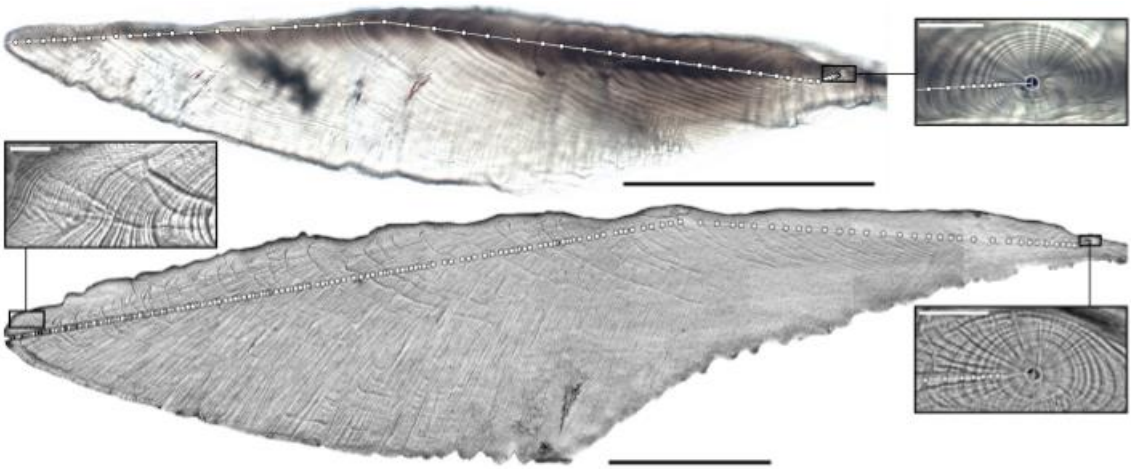

Fig. 2.2. Appearance of otolith preparations and growth increments in the sagittal otoliths of Pacific bluefin tuna under light microscopy (top; 57 days old, 171 mm SL) and scanning electron microscope (bottom; 237 days old, 499 mm SL) analysis. Dots on the surface of the otoliths indicate the approximate position of each increment. Insets show higher magnifications of the otolith nuclei and the marginal area of the otolith. Black and white bars represent 500 and 20 um, respectively.

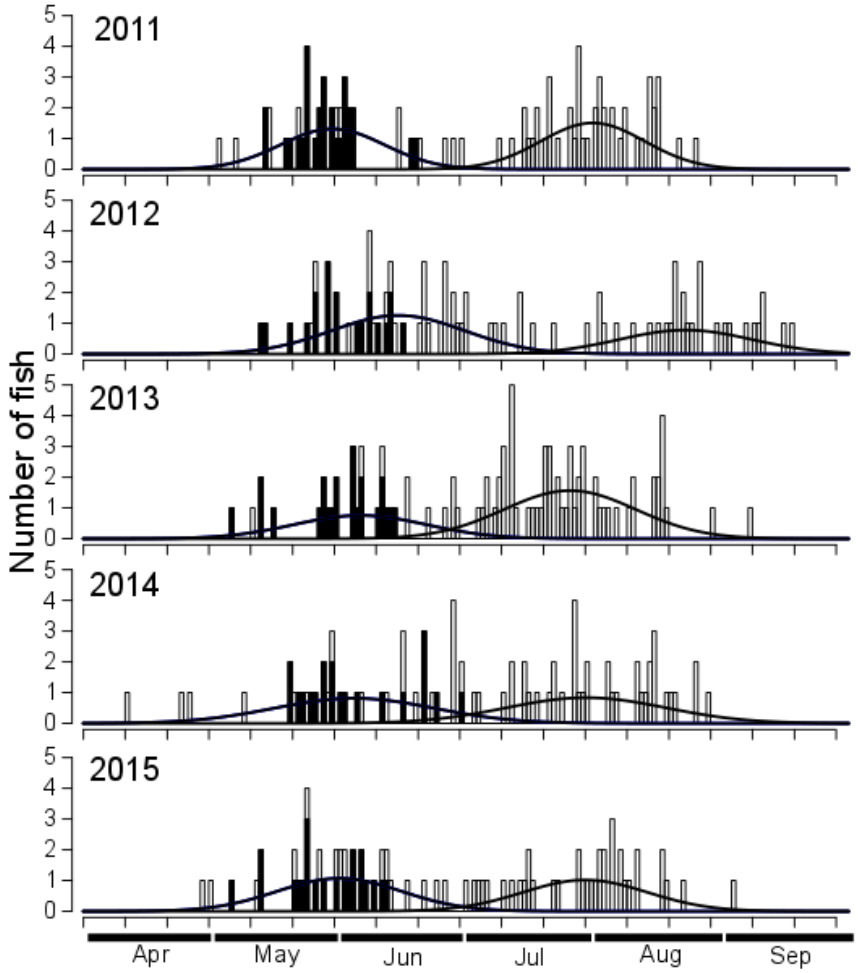

Fig. 2.3. Distribution of hatch dates of Pacific bluefin tuna collected for this study between 2011 and 2015 as estimated from otolith daily increment analysis. Black and white bars represent fish collected in the north-western Pacific Ocean and Sea of Japan fishing grounds, respectively. The smooth lines represent the distribution of hatch dates for the "early-" and "late-born" cohorts that were discriminated with a Gaussian mixture model (see text for details).

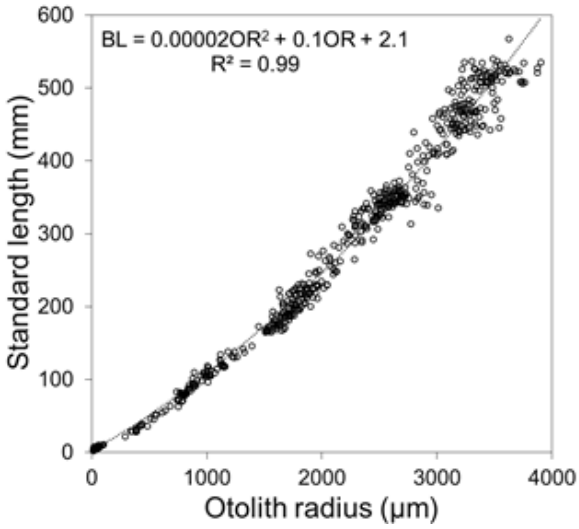

Fig. 2.4. Relationship between body length and otolith radius of Pacific bluefin tuna collected for this study between 2011 and 2015.

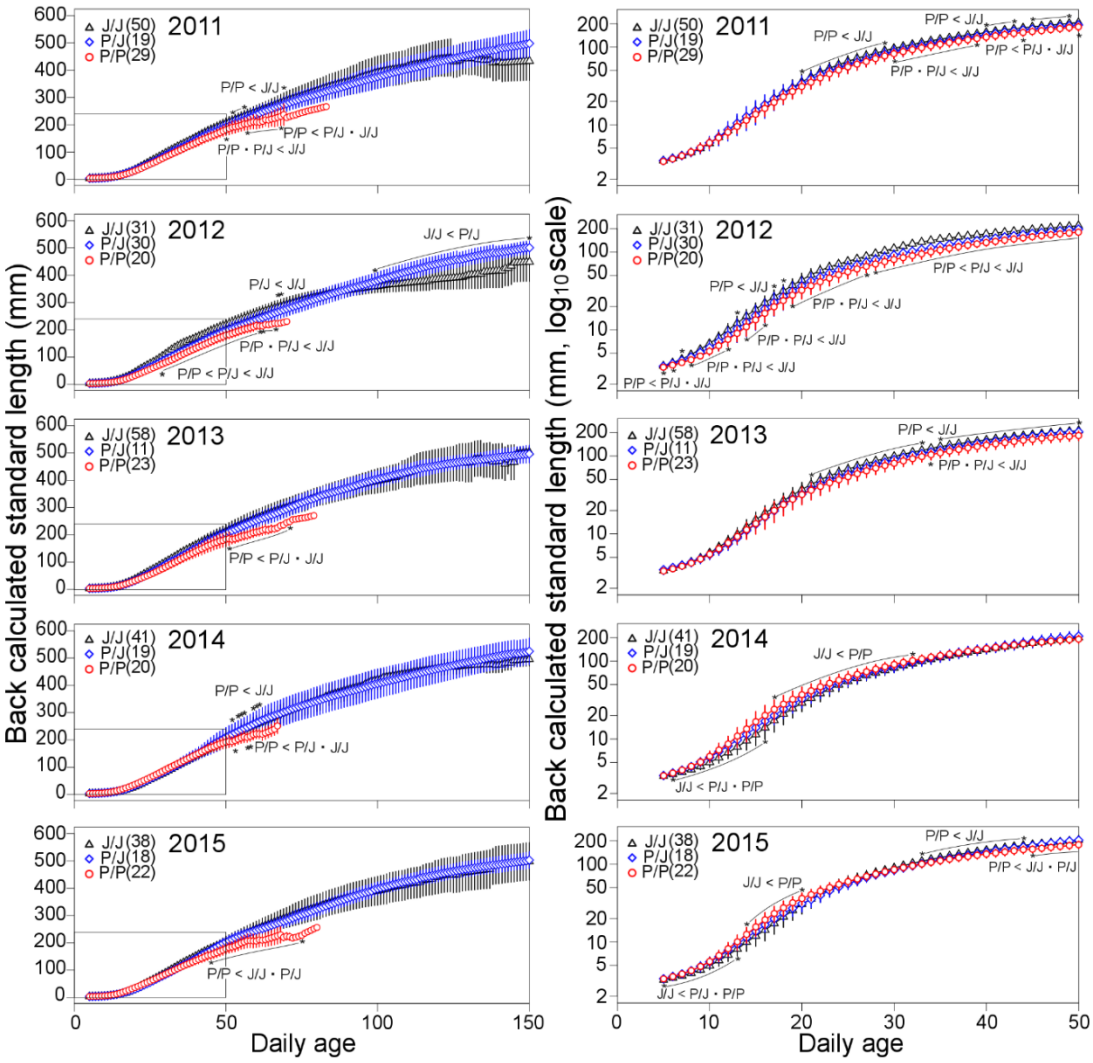

Fig. 2.5. Comparison of the back-calculated standard lengths (mean values ± standard deviation) of Pacific bluefin tuna from five year-classes (2011-2015) that were assigned to three cohorts, namely those born in the Nansei Islands area and grown up either in the Pacific coast of Japan (P/P; red) or in the Sea of Japan (P/J; blue) and those born and grown up in the Sea of Japan (J/J; black). Right panels show the details for 0-50 DAH. Asterisks represent statistically significant differences (ANOVA followed by the Tukey's Multiple Comparison Test, $p < 0.05$) between P/P, P/J and J/J.

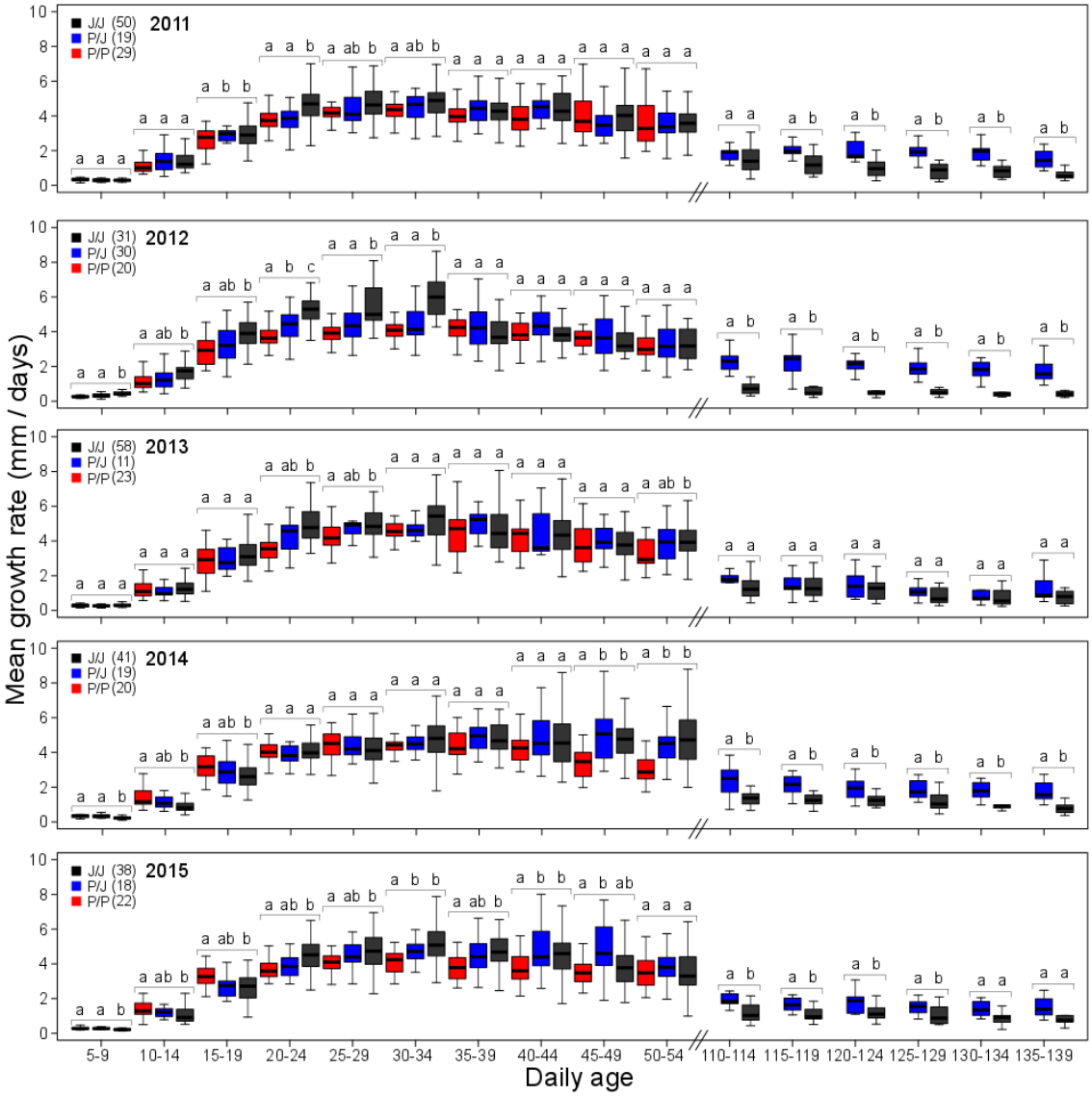

Fig. 2.6. Comparison of the mean growth rates (whisker plots with median, ± standard deviation, maximum and minimum values for specified 5-days intervals) of Pacific bluefin tuna from five year-classes (2011-2015) that were assigned to three cohorts, namely those born in the Nansei Islands area and grown up either in the Pacific coast of Japan (P/P; red) or in the Sea of Japan (P/J; blue) and those born and grown up in the Sea of Japan (J/J; black). Mean values for cohorts within each daily age interval with different superscripts differ significantly ($p<0.05$; ANOVA followed by the Tukey's multiple comparison test for comparisons of P/P, P/J, and J/J, or t-test for comparisons only between P/J and J/J).

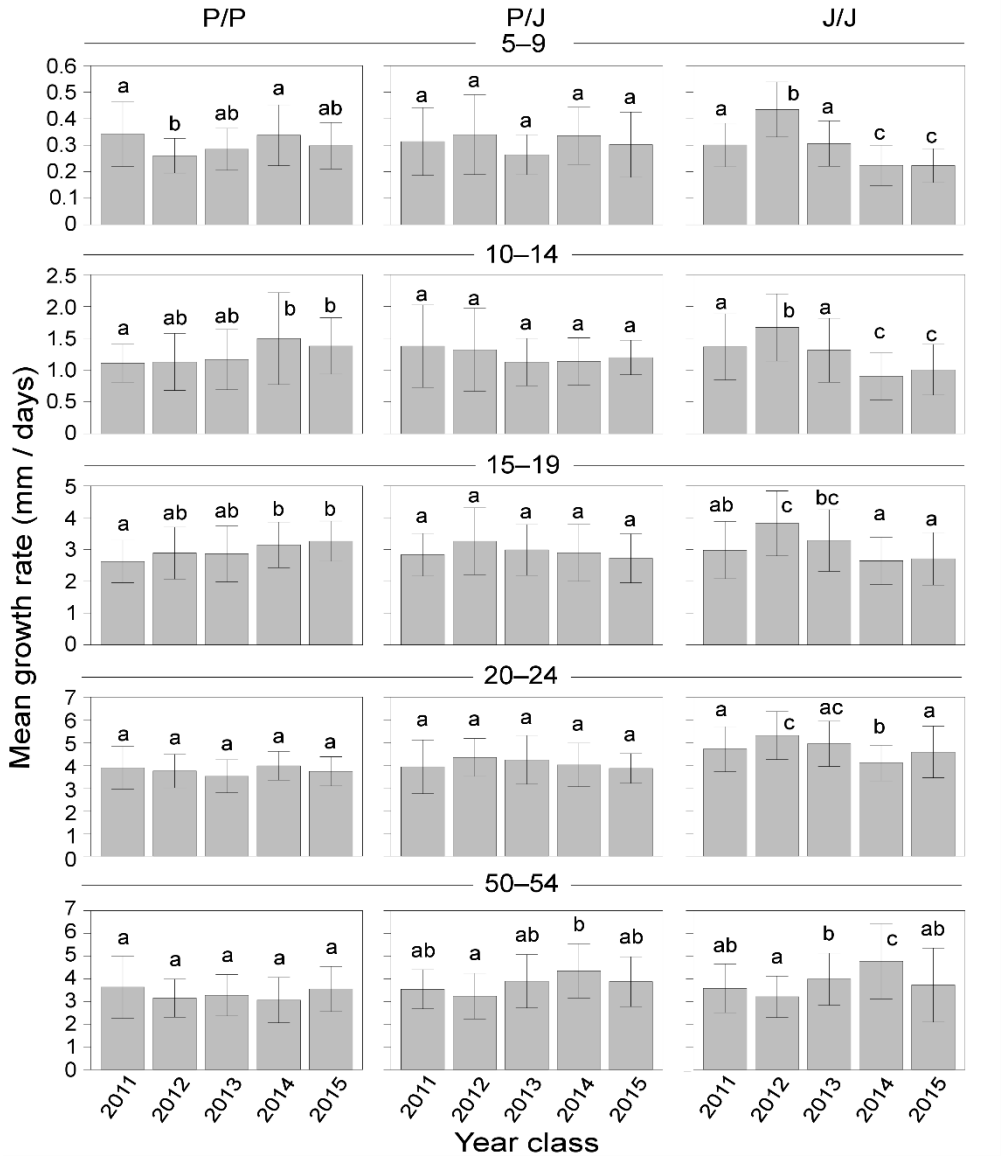

Fig. 2.7. Interannual comparison of the mean (± standard deviation) growth rates of Pacific bluefin tuna from five year-classes (2011–2015) that were assigned to three cohorts, namely those born in the Nansei Islands area and grown up either in the Pacific coast of Japan (P/P) or in the Sea of Japan (P/J) and those born and grown up in the Sea of Japan (J/J). Results are shown for specified daily age intervals (5–9, 10–14, 15–19, 20–24 and 50–54 days after hatching). Mean values for year-classes within each cohort and daily age interval with different letters differ significantly ($P < 0.05$, ANOVA followed by Tukey's multiple comparison test).

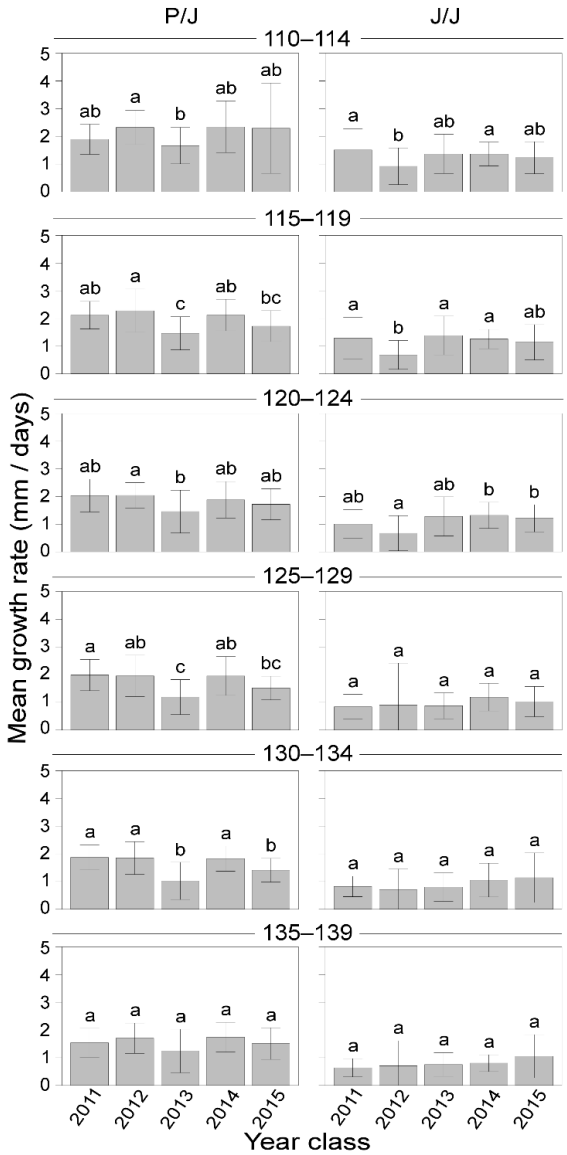

Fig. 2.8. Interannual comparison of the mean (± standard deviation) growth rates of Pacific bluefin tuna from five year-classes (2011–2015) that were assigned to three cohorts, namely those born in the Nansei Islands area and grown up either in the Pacific coast of Japan (P/P) or in the Sea of Japan (P/J) and those born and grown up in the Sea of Japan (J/J). Results are shown for specified daily age intervals (110–114, 115–119, 120–124, 125–129, 130–134 and 135–139 days after hatching). Mean values for year-classes within each cohort and daily age interval with different letters differ significantly ($P < 0.05$, ANOVA followed by Tukey's multiple comparison test).

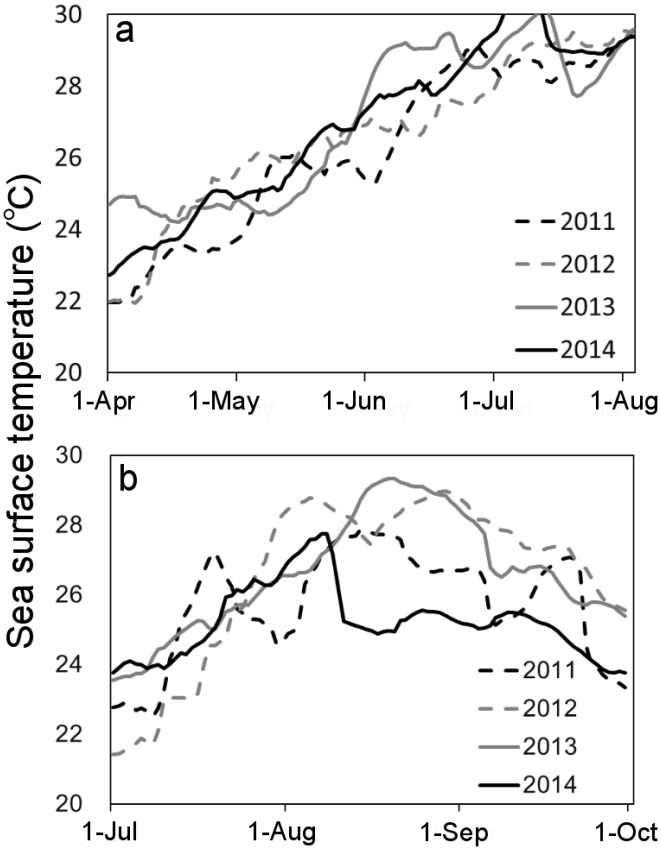

Fig. 2.9. Daily sea surface temperatures (SSTs) in the Nansei Islands (a) and Sea of Japan (b) spawning grounds between 2011 and 2014. Temperatures were estimated from NOAA/AVHRR data (see text for details).

Chapter 3. Effects of birth date and water temperature on larval growth rates

Introduction

Two main spawning grounds of PBF have been identified, one near the Nansei Islands in the north-western Pacific Ocean and one in the Sea of Japan (Ashida *et al.*, 2015; Ohshimo *et al.*, 2017 Okochi *et al.*, 2016). The two grounds differ in their biological and physical conditions during the time PBF are at the larval stages and this may potentially affect larval growth (Satoh *et al.*, 2008; Satoh *et al.*, 2010; Satoh *et al.*, 2013). For example, even slight decreases from the optimal temperatures have marked effects on the growth rates of PBF larvae (Kimura et al., 2010; Tanaka et al., 2010). Such effects may result in differential growth and survival and this in turn could impact the contribution of the larval cohorts to the adult and fisheries stocks.

It was shown in the second chapter that the water temperature in the Sea of Japan fluctuates greatly and decreases towards the end of the spawning season of PBF compared to that in the Nansei Islands area, which shows a gradual increase throughout the season that is essentially predictable across the years. Furthermore, the Sea of Japan has richer primary production than areas in the Pacific located east of Japan (Chiba *et al.*, 2005; Hsiao *et al.*, 2011; see Taniguchi 1977 and Satoh *et al.* 2013 for information on the Nansei Islands area spawning ground). While temperature is a direct and strong determinant of larval growth, under stable conditions of water temperature other factors such as primary production may set the limits for growth. This study was designed to compare the larval growth rates of fish born successively later during the spawning season in the two currently known spawning areas of PBF and to analyze the correlation of larval growth rates with water temperature.

Materials and methods

Sampling and body size measurement

This study used the same YOY specimens that were analyzed in chapter 2 for the year-classes 2011 to 2014; specimens from the year-class 2015 could not be used because at the time the study was conducted water temperature information for the area was not yet available. Hence, in order to obtain a five-year data series, YOY specimens collected in 2010 were processed and analyzed following the same methods described previously for the other year-classes. Briefly, age-0 PBF were caught by the commercial fisheries using trolling lines, purse seines or set nets off the coast of Kochi, Kagoshima, Nagasaki, Yamaguchi, Shimane, Ishikawa and Toyama Prefecture between July and December each year from 2010 to 2014 (Fig. 2.1, Table 3.1). The FL were measured with a caliper to the nearest 0.1 mm and the conversion from FL to SL was performed as described in the first and second chapters using the equation SL = 0.976FL-1.926 (R^2 = 0.99, n = 514, 11.9-543.0 mm FL). Details of the capture date, body size and daily age (see details below) of the 422 fish that were analyzed in this chapter are given in Table 3.1.

Otolith analysis and hatch date estimation

All procedures were as described in chapter 2.

Hatch date/fishing ground-based cohort assignment

All procedures were as described in chapter 2, and the hatching date compositions in each cohort were divided into the equivalent 15-day periods in the two specific five-day periods.

OR-SL relationship and growth back-calculation

All procedures were as described in chapter 2.

Water temperature profiles during the spawning season and relation with larval growth rates

All procedures were as described in chapter 2, and the mean SSTs during specific periods of the larval stage (5-9 DAH and 10-14 DAH) were calculated.

Statistical analysis

The statistical significance of the differences in larval growth rates of fish born in different periods during the spawning season was assessed by analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The relationship between sea surface temperatures during specific periods of the larval stage (5-9 DAH and 10-14 DAH) and corresponding back-calculated growth rates was examined using the Spearman's correlation coefficient.

Results

Hatching date estimation and cohort assignment

The estimated hatch dates ranged from April to September in 2010~2014 (Fig. 2.3 and 3.2, Table 3.1). As described in chapter 2, I assumed that the early- and late-born groups of specimens separated by the GMM model in each year represented individuals born in the Nansei Islands area and the Sea of Japan, respectively. The average hatching dates for PBF from the Nansei Islands area and the Sea of Japan and the reference date separating the two cohorts in 2010were May 28, August 11, and July 5, respectively.

Relation between growth rate and hatch date

The back-calculated body size for each day of life was then used to estimate the mean instantaneous growth rates of individual fish in two periods after hatching (5-9 DAH and 10-14

DAH) for five fortnight periods during the spawning season in each ground (Fig. 3.3). There were no noticeable hatch date-dependent differences in growth rates of Nansei Islands-born larvae with the progression of the spawning season. In contrast, the growth rates of Sea of Japan-born larvae increased with hatch date from July to August and then decreased in September.

Relation of growth rate and water temperature

The water temperature profiles in the Nansei Islands area and the Sea of Japan during the spawning season between 2010 and 2014 are shown in Figure 3.1. The water temperature in 2010 showed essentially the same pattern as in the other years (2011-2014) in both spawning grounds, that is, it increased steadily in the Nansei Islands area whereas in the Sea of Japan it increased first and then decreased towards the end of the spawning season. There was no clear relationship between growth rates and water temperature during the corresponding period in the larval life of the YOY born in the Nansei Islands. In contrast, the larval growth rates of the Sea of Japan-born YOY were positively correlated with water temperature (Fig. 3.4).

Discussion

In the second chapter, larval growth rates of YOY PBF in the Sea of Japan were highly variable compared to those in the Nansei Islands areas and were not necessarily higher as expected from the inherent higher primary and secondary productivity of the Sea of Japan. Given this fact, in this chapter, the relation of growth rate with hatch date and water temperature was evaluated for both the Nansei Islands area and the Sea of Japan. Using similar methods as described in the second chapter, I assigned the early- and late-born specimens identified within each year as individuals born in the Nansei Islands area and the Sea of Japan, respectively. Next, the back-calculated body size for each day of life was used to estimate the mean instantaneous larval growth

rates of individual fish that hatched in different two-week periods during the spawning season in each area.

This study revealed different growth trends in the early larval period (5-14 DAH) during the spawning season for Nansei area- and Sea of Japan-born YOY. Thus, YOY born in the Sea of Japan showed first an increase and then a decrease in larval growth rates from the beginning towards the end of the spawning season. Moreover, these fish showed a positive relationship between growth rates and the water temperature during the spawning season, whereby the maximum growth rates coincided with the highest water temperatures observed in August. In contrast, there were no clear temporal changes in larval growth rates with the progression of the spawning season in YOY born in the Nansei Islands area even though the temperature in this area increased steadily during the season. This is surprising considering that Nansei Islands-born larvae have also been shown to respond to food, water temperature, and stratification parameter (Satoh et al., 2013). This suggests that factors other than water temperature might be more influential on growth rate than water temperature in this area. It is hypothesized that lower availability of food per larvae in the Nansei Islands area, either because of the inherent low primary production in this area (Taniguchi 1977; Satoh *et al.* 2013) or because the higher larval densities lead to increased competition for food, present a more immediate and crucial limiting factor for growth and survival in this area than temperature. This possibility must be evaluated in further studies to clarify the effects of growth on subsequent survival and recruitment into the adult and fishery populations.

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Table 3.1. Fishing ground, year-class, collection date, estimated hatch date, body size, and estimated daily age of Pacific bluefin tuna young-of-the-year that were analyzed in this study. Results are shown separately for "early-" and "late-born" cohorts that were discriminated with a Gaussian mixture model (see text for details).

Fishing ground	Year-class	Early-born cohort						Late-born cohort					
		N	Collection date	Estimated hatch date	SL (mm)			N	Collection date	Estimated hatch date	SL (mm)		
					Mean ± SD	Min	Max				Mean ± SD	Min	Max
Kagoshima	2010	15	2010/8/26 - 2010/9/20	2010/5/21 - 2010/6/14	280.2 ± 32.9	230.1	320.2						
Kochi	2010	6	2010/7/14 - 2010/8/13	2010/4/29 - 2010/5/23	213.3 ± 6.9	173.8	263.2						
	2011	29	2011/7/15 - 2011/8/11	2011/5/14 - 2011/6/18	194.8 ± 20.0	165.7	229.9						
	2012	20	2012/7/13 - 2012/8/15	2012/5/13 - 2012/6/16	194.0 ± 19.3	164.6	231.6						
	2013	23	2013/7/25 - 2013/8/9	2013/5/6 - 2013/6/14	203.2 ± 19.6	165.0	229.3						
	2014	20	2014/7/18 - 2014/8/22	2014/5/20 - 2014/6/30	200.6 ± 23.1	165.9	232.8						
Nagasaki	2010	22	2010/10/12 - 2010/11/30	2010/5/16 - 2010/6/18	422.5 ± 28.7	371.1	472.2						
	2012	3	2012/10/22 - 2012/10/22	2012/6/14 - 2012/6/28	467.1 ± 3.4	463.9	470.6	1	2013/1/9	2012/8/20	366.2		
Yamaguchi	2010	8	2010/11/17 - 2010/11/25	2010/5/31 - 2010/6/20	478.5 ± 12.4	456.0	495.5	2	2010/11/8 - 2010/11/14	2010/8/7 - 2010/8/18	360.9 ± 51.7	324.4	397.4
	2011	19	2011/11/6 - 2012/1/18	2011/4/24 - 2011/6/29	483.4 ± 31.2	439.5	534.4	17	2011/11/30 - 2012/1/29	2011/7/4 - 2011/8/29	415.5 ± 54.4	313.8	483.6
	2012	11	2012/11/18 - 2012/12/4	2012/5/25 - 2012/7/14	501.4 ± 7.7	490.3	514.3						
	2013	5	2013/11/25 - 2013/12/17	2013/5/11 - 2013/6/17	483.3 ± 28.2	452.9	522.1	8	2013/11/25 - 2013/12/17	2013/7/6 - 2013/9/7	471.1 ± 17.2	457.1	511.3
	2014	6	2014/11/25 - 2015/1/14	2014/5/24 - 2014/6/28	489.4 ± 27.2	445.2	512.8	19	2014/11/25 - 2015/1/14	2014/7/3 - 2014/8/28	454.5 ± 11.8	442.0	477.5
Shimane	2010							18	2010/9/30 - 2010/11/24	2010/8/8 - 2010/8/23	285.0 ± 60.3	195.4	371.5
	2011							11	2011/11/1 - 2011/11/14	2011/7/13 - 2011/8/20	334.2 ± 10.1	323.6	352.5
	2012							8	2012/11/15 - 2012/11/30	2012/8/6 - 2012/8/26	342.0 ± 5.8	329.1	346.6
	2013	4	2013/10/10 - 2013/12/17	2013/6/22 - 2013/6/29	431.0 ± 37.9	398.3	466.7	30	2013/10/2 - 2013/12/17	2013/7/6 - 2013/8/17	331.4 ± 45.2	264.5	466.7
	2014							17	2014/10/26 - 2014/11/19	2014/7/22 - 2014/8/25	307.3 ± 27.1	244.0	338.0
Ishikawa	2011							6	2011/12/21 - 2011/12/21	2011/7/16 - 2011/8/20	344.8 ± 5.6	339.3	352.4
	2012	8	2012/12/21 - 2013/1/30	2012/5/26 - 2012/7/17	521.9 ± 14.4	497.0	540.0	18	2012/11/8 - 2013/1/30	2012/7/22 - 2012/9/17	372.3 ± 55.5	341.5	524.4
	2013	2	2013/12/9 - 2013/12/11	2013/6/26 - 2013/6/28	451.1	450.1	452.1	16	2013/10/26 - 2013/12/11	2013/7/4 - 2013/8/25	386.9 ± 35.7	336.9	436.5
	2014	13	2014/11/21 - 2015/1/5	2014/4/11 - 2014/6/30	521.6 ± 8.8	507.8	535.1	5	2014/12/15 - 2015/1/21	2014/7/16 - 2014/8/25	307.3 ± 27.1	244.0	338.0
Toyama	2010							15	2010/10/4 - 2010/10/8	2010/7/31 - 2010/8/15	219.9 ± 32.3	181.5	263.3
	2011							16	2011/9/29 - 2011/10/28	2011/7/23 - 2011/8/18	287.2 ± 29.9	245.8	330.8
	2012	8	2012/12/28 - 2013/1/21	2012/6/8 - 2012/7/14	517.0 ± 11.9	497.0	536.9	4	2012/12/19 - 2012/12/19	2012/8/20 - 2012/8/30	360.8 ± 1.0	359.3	361.3
	2013							4	2013/12/19 - 2013/12/19	2013/7/26 - 2013/8/15	350.1 ± 7.1	340.1	356.6

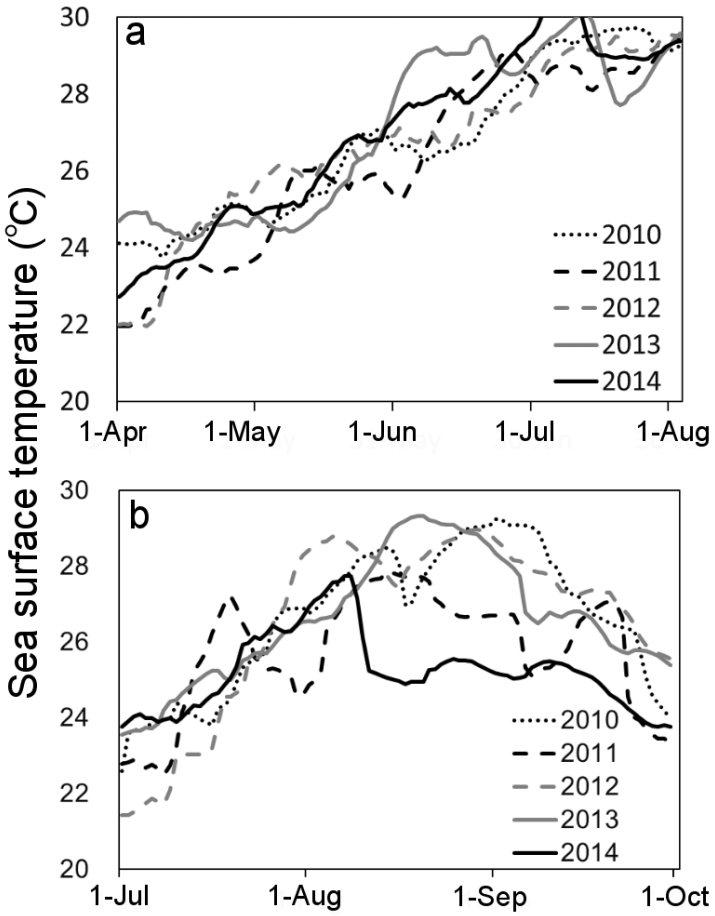

Fig. 3.1. Daily sea surface temperatures (SSTs) in the Nansei Islands (a) and Sea of Japan (b) spawning grounds between 2010 and 2014. Temperatures were estimated from NOAA/AVHRR data (see text for details).

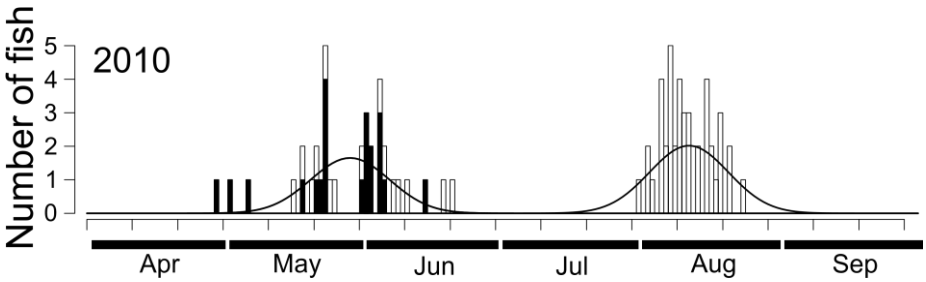

Fig. 3.2. Distribution of hatch dates of Pacific bluefin tuna collected for this study in 2010 as estimated from otolith daily increment analysis. Black and white bars represent fish collected in the north-western Pacific Ocean and Sea of Japan fishing grounds, respectively. The smooth lines represent the distribution of hatch dates for the "early-" and "late-born" cohorts that were discriminated with a Gaussian mixture model (see text for details).

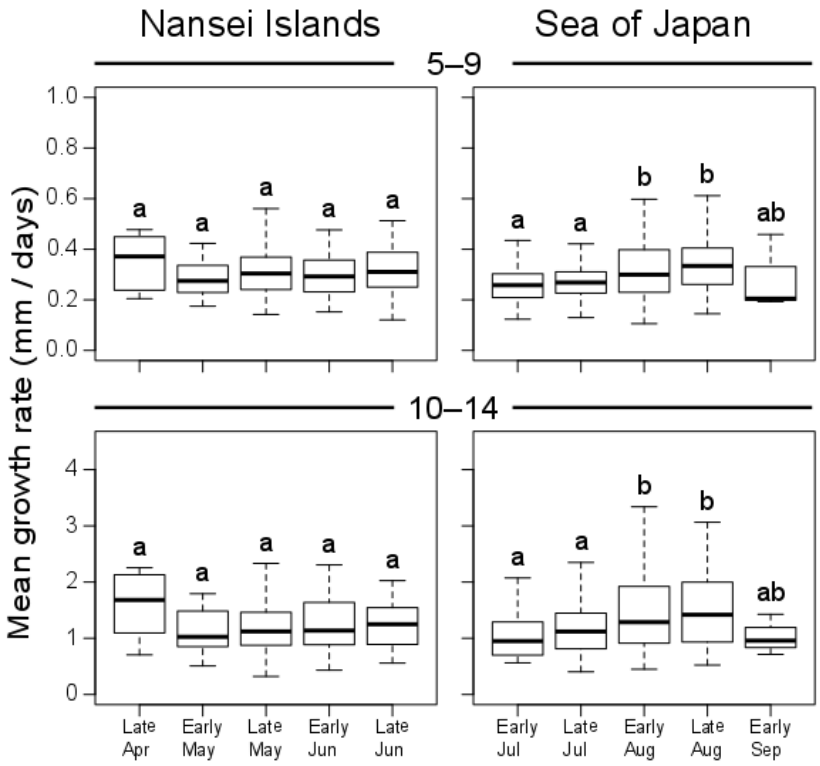

Fig. 3.3. Larval growth rates of Pacific bluefin tuna YOY born in the Nansei Islands and the Sea of Japan between 2010 and 2014. Results shown as whisker plots with median, ± standard deviation, maximum and minimum values for two specific five-day periods during early life (upper panels:5-9 DAH; lower panels: 10-14DAH). Boxes within each cohort and daily age interval with different letters differ significantly ($P < 0.05$, ANOVA followed by Tukey's multiple comparison test).

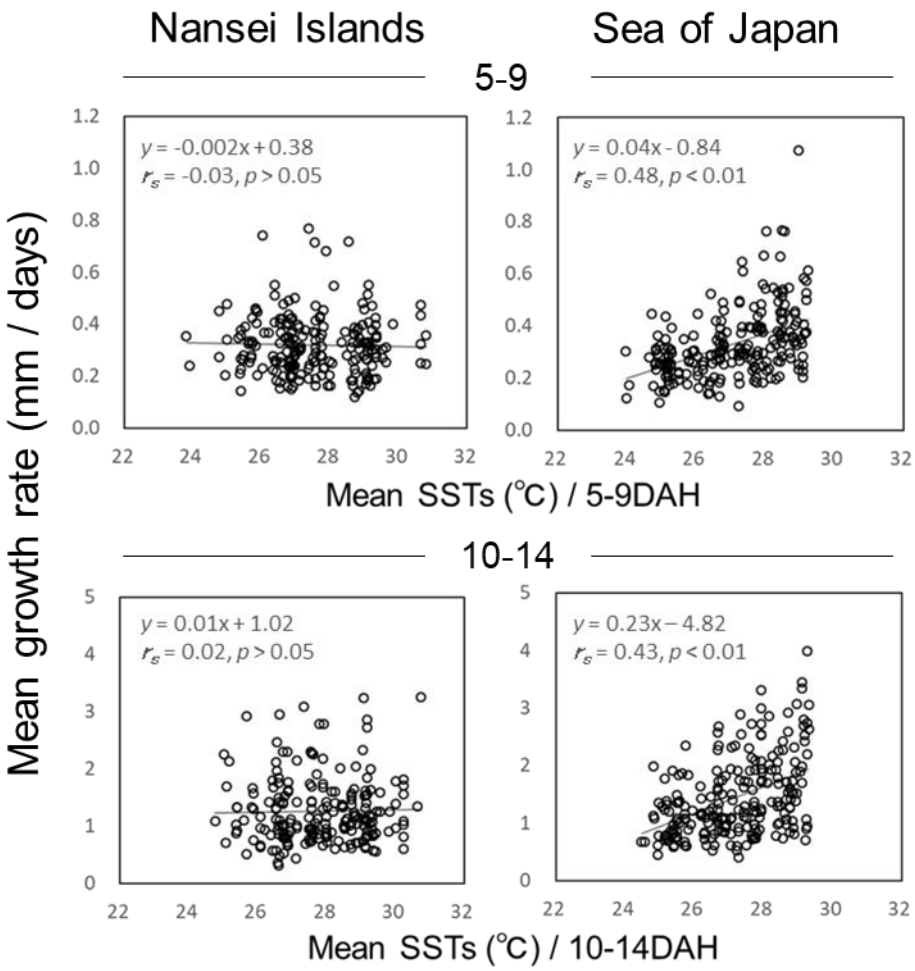

Fig. 3.4. Relationship between mean daily growth rates and water temperature in two periods of the larval stage (5-9 and 10-14 DAH) of Pacific bluefin tuna YOY born in the Nansei Islands area and Sea of Japan between 2010 and 2014.

General Discussion

The spawning stock size of PBF is now at a historically low level and the fraction of the PBF fisheries catch represented by immature fish has become dominant (ISC, 2016). Fluctuation in the recruitment of Scombrid fish are strongly correlated with initial growth and survival, and differences in the early growth of each individual ultimately lead to variations in recruitment (Tanaka *et al.*, 2006; Robert *et al.*, 2007; Watai *et al.*, 2017). PBF recruitment varies from 6 to 10 times depending on year (Yamada *et al.*, 2006). In these circumstances, it is imperative to gather knowledge on the early life history before recruitment in order to devise sound fisher resource management, the knowledge about early life history before recruitment need to be accumulated.

In this study, body size back-calculation showed that only larvae with fast, steady growth successfully transitioned to the juvenile stage. Moreover, larval growth rates of PBF in the Sea of Japan were highly variable compared to those in the Nansei Islands areas and were not necessarily higher as expected from the inherent higher primary and secondary productivity of the Sea of Japan. The effect on the growth by the water temperature was evaluated, and there is different response of growth rate by water temperature between Nansei area and Sea of Japan. On the other hands, the higher productivity in Nansei Islands area affects stronger influences than water temperature to the somatic growth. Therefore, it is assumed that there is no clear effect against survival from water temperature in Nansei Islands area.

Further studies should examine the effects of environmental factor (e.g. temperature) on growth in relation to developmental stage of PBF. Larval and juveniles PBF migrate in a varied sea area (Satoh *et al*., 2008; Kitagawa *et al*., 2010; Masujima *et al*., 2014), and larvae have also been shown to react to biological and physical conditions during their early life stages (Satoh *et*

al., 2008; Satoh *et al*., 2010; Satoh *et al*., 2013). The environment experienced during larval stage is predicted to be an important factor for early growth and survival. The history of the environment experienced by fish can be inferred from otoliths (e.g. Radtke, 1989; Radtke *et al*., 1990; Secor, 1992; Townsend *et al*., 1992; Secor *et al*., 1995; Townsend *et al*., 1995). Furthermore, feeding ecology of larvae and juveniles is important for growth and survival (Tanaka *et al*., 2014). In rearing experiment, larval feed between 14 and 21 DAH shifts from zooplankton to larvae, and longer larvae of 14 DAH when feeding larvae begin to eat larvae are faster growth, thereby making a difference in growth among individual (Tanaka *et al*., 2014). By field sampling, larvae predominantly prey on copepods and their nauplii, thereafter the growth of individuals shifted to fish feeder earlier may be improved. As described above, timing of change of feeding trophic stage is also important in growth and survival of this species. It is considered necessary for future research to compare the timing and growth of transition of feeding trophic stage. Moreover, the ultimate goal will clarify relationship the early growth and recruitment. We should examine the actual correlation of growth rates in different developmental stages with the strength of each year-class.

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