Vol. 607: 207–220, 2018
https://doi.org/10.3354/meps12807

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published December 6

Comparative analysis of the early growth history of Pacific bluefin tuna *Thunnus orientalis* from different spawning grounds

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ABSTRACT: This study compared the growth history of young-of-the-year (YOY) Pacific bluefin tuna (PBF) *Thunnus orientalis* from 3 cohorts (those presumably born in the Nansei Islands area and collected in the Pacific coast of Japan, those presumably born in the Nansei Islands areas and collected in the Sea of Japan, and those presumably born and collected in the Sea of Japan) in 5 year-classes (2011–2015). Growth rates during the larval stage in the Sea of Japan were highly variable compared to those in the Nansei Islands areas, and were not necessarily higher in the former, as expected from the inherent higher primary and secondary productivity of the Sea of Japan. The uncoupling of larval growth rates and food abundance in the Sea of Japan appears to be related to thermal instability and the proximity of winter in relation to the spawning season in this area. Unlike in the larval stage, growth rates during the juvenile and YOY stages were higher in the Sea of Japan compared to those in the Pacific regardless of the natal origin of the fish. This finding is consistent with the higher productivity in this area and also suggests 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.

KEY WORDS: Early life history · Otolith microstructure · *Thunnus orientalis*

INTRODUCTION

Pacific bluefin tuna (PBF) *Thunnus orientalis* is a highly migratory species distributed from the Gulf of Alaska to Baja California in the eastern Pacific, and from the Sea of Okhotsk to the Philippines in the western Pacific, and is also occasionally recorded in the Southern Hemisphere (Collette & Nauen 1983, Muhling et al. 2017). The spawning stock size of PBF is now at a historically low level 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 (ISC 2016). Successful management of fish resources requires knowledge of all biological processes, from spawning and early larval life until successful recruitment to the fisheries and spawning stocks. At present, 2 distinct spawning grounds of PBF have been identified (see Fig. 1), each with a characteristic spawning period. The first occurs between April and June in the Nansei Islands area from close to the Philippines to the Ryukyu

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Islands, and the second, between July and August in the Sea of Japan (Ashida et al. 2015, Okochi et al. 2016, Ohshimo et al. 2017, 2018). Fish born in the Nansei Islands area drift northward starting from 2–3 wk 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 larvae born in the Sea of Japan grow in that area until reaching the young-of-the-year (YOY) stage, and then start migrating to the East China Sea (Itoh et al. 2003). Hence, the Nansei Islands area, the Pacific (East) coast of mainland Japan, and the Sea of Japan are important grounds for developing young PBF (Tanaka et al. 2006, 2007). However, these areas show substantially different environmental conditions, such as thermohaline structure and biological productivity, with the Sea of Japan being colder, less saline, and more productive than the Pacific (including the Nansei Islands area) (Kodama et al. 2015).

The analysis of growth and survival of each developmental stage can help identify critical life stages in the pre-recruitment period of fish (Bradford & Cabana 1997). Natural mortality of larval PBF in the Nansei Islands area 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 survival of PBF born in the Nansei Islands area depends largely on their growth rates during the early stages of life (6–13 d after hatching), before the onset of piscivory. Moreover, their analysis of past larval growth rates of PBF born between 2011–2015 revealed significant inter-annual differences in growth rates, which likely reflect yearly differences in environmental conditions within the Nansei Islands area. The 2 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 and their contribution to the PBF stocks remain largely unknown. In the present study, we applied otolith microstructure analysis to study the past growth rates of YOY PBF captured between 2011 and 2015 in the Pacific (East) coast of Japan and in the Sea of Japan, in relation to their place of growth through the larval, juvenile, and YOY stages. Thus, the YOY PBF in this study were classified in 3 cohorts following criteria described by Itoh (2009); namely, those (1) presumably born in the Nansei Islands area in the northwestern Pacific and collected in the Pacific (East) coast of Japan (hereafter referred to as P/P), (2) presumably born in the Nansei Islands area and collected in the Sea of Japan and (P/J), and (3) presumably born and collected in the Sea of Japan (J/J). This study was designed to compare the growth patterns of these 3 cohorts from their birth until the YOY stage during a 5 yr period.

MATERIALS AND METHODS

Sampling and body size measurement

A total of 4653 age-0 PBF were caught by the commercial fisheries using trolling lines, purse seines, or set nets off the Pacific (East) coast of Japan (Kochi) and in the Sea of Japan (Nagasaki, Yamaguchi, Shimane, Ishikawa, and Toyama) between 2011 and 2015 (Fig. 1). Fish were sent to the laboratory either fresh or frozen, and their fork lengths (FLs) were measured with a caliper to the nearest 0.1 mm. The FL values were converted to standard length (SL) with the equation: SL = 0.976 FL − 1.926 (r^2 = 0.99,

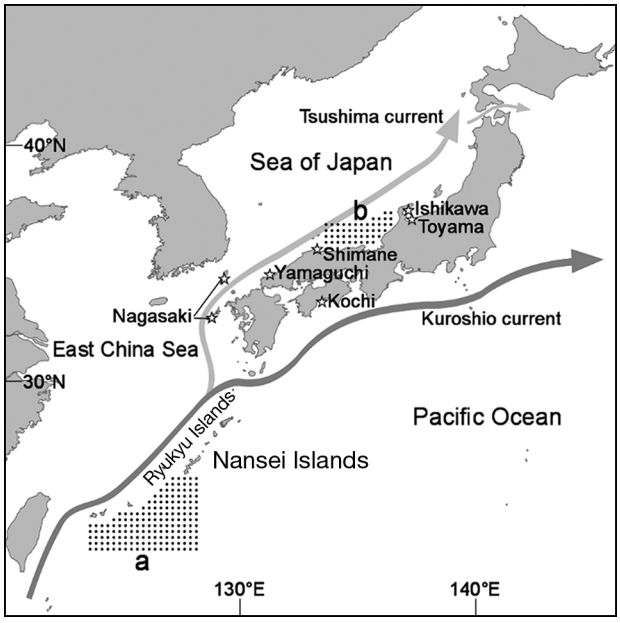

Fig. 1. Approximate location of the capture sites (stars) of age-0 Pacific bluefin tuna for this study. Capture sites are represented by the names of the prefectures in whose fishing port the fish were landed. The 2 currently known spawning grounds in the Nansei Islands (a) and Sea of Japan (b) are indicated and overlaid with dots representing the grids of 0.25° latitude × longitude cells used to estimate sea surface temperatures from National Oceanic and Atmospheric Administration Advanced Very High Resolution Radiometer (NOAA/AVHRR) data

$p < 0.01$), that was developed using SL and FL values from 514 specimens chosen randomly from the 4653. About 10% (n = 429) of the fish >150 mm SL, that are defined as YOY (Watai et al. 2017), were then randomly chosen for otolith analysis. See Table 1 for details of the capture date, estimated hatch date (details below), and body size of the fish that were analyzed.

Otolith analysis and hatch date estimation

The sagittal otoliths were removed from each fish in the laboratory, washed in distilled water, dried, and preserved in individually labelled tubes for subsequent analysis. Different analytical protocols, i.e. scanning electron microscope (SEM) and optical microscope, were used depending on whether the samples were collected in periods of slow (winter) or fast (summer to fall) growth, respectively, because otolith increments formed in winter are very thin and are only discernible by SEM.

The otoliths of samples collected during winter were mounted in epoxy resin and sectioned with a low-speed saw (Isomet; Buehler). The cross-section blocks were then polished with a series of 400–4000 grit abrasive paper (Reflex NAC M; Meiwafosis) to expose the core region, and etched with 0.5 or 2 N HCl until all increments and the otolith nucleus could be easily distinguished. Specimens were then examined and photographed in an SEM (Hitachi TM 3030; Hitachi). The otoliths of samples collected in summer and fall were processed in the same way until the core region was exposed. 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) 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) 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 100–500×.

The photographs and live video images in the case of SEM and optical microscope observation, respectively, were then analyzed using the RATOC otolith measurement system (ARP/W+RI; Ratoc System Engineering). Measurements of increment widths were performed along the direction of maximum otolith growth connecting the otolith nucleus and the postrostrum edge and typically included 2 mild inflection points where the otolith growth axis shifted slightly (Fig. 2). These inflection points were not associated with sudden or significant changes in growth rates and therefore were not considered further in the analysis. Increment identification was done first using the software and then visually corroborated by an observer. Increments were enumerated and assigned the corresponding age in days (days after hatching; 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 and fishing ground-based cohort assignment

Cohort (P/P, P/J, or J/J) 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 5 yr using the package 'mclust', Version 3 (https://cran.r-project.org/web/packages/mclust/index.html) and the statistical computing program R (R Development Core Team 2016) 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 2 cohorts

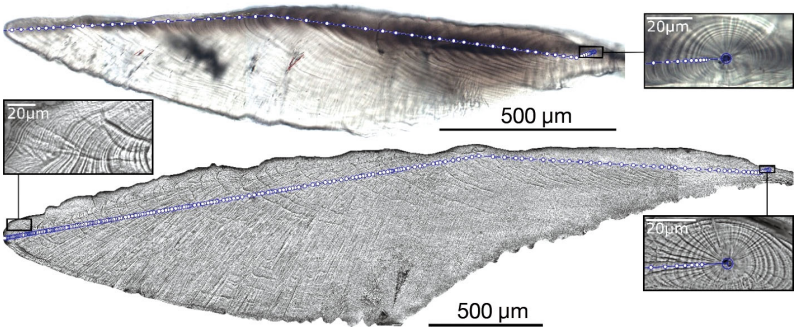

Fig. 2. Appearance of otolith preparations and growth increments in the sagittal otoliths of Pacific bluefin tuna under light microscopy (top: 57 d old, 171 mm standard length, SL) and scanning electron microscope (bottom: 237 d old, 499 mm SL) analysis. Dots on the surface of the otoliths: approximate position of each increment. Insets: higher magnifications of the otolith nuclei and the marginal area of the otolith

(see Fig. 3). To reduce the possibility of cohort misidentification, all fish born within 10 d of the reference date were excluded from the analysis. Based on the extensive information available on the spawning period and spawning grounds of PBF (Kitagawa et al. 1995, Tanaka et al. 1996, Itoh 2009, Ashida et al. 2015, Okochi et al. 2016, Ohshimo et al. 2017, 2018), the early- and late-born individuals were assumed to represent cohorts of fish hatched in the Nansei Islands area in the northwestern Pacific Ocean (hereinafter referred to as 'P/') and in the Sea of Japan ('J/'). To this, information on the fishing ground, i.e. '/P' for fish captured in the Pacific (East) coast of Japan (Kochi) and '/J' for fish captured in the Sea of Japan (all other sites) was combined to define the 3 cohorts for comparison as P/P, P/J, and J/J.

Otolith radius–SL relationship and growth back-calculation

The otolith radius (OR) of each individual was calculated as the sum of all increment widths from otolith nucleus to edge regardless of the presence of 2 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 3 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) to select the best model. Data on OR and SL for larval and early juvenile specimens from the Nansei Islands area and the Japan Sea from Watai et al. (2017; n = 598) and T. Ishihara et al. (unpubl. data; n = 166), respectively, were added to the corresponding data sets to compensate for the lack of younger individuals in the current study. 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 5 yr 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. The back-calculated body size for each day of life was then used to estimate the mean daily growth rates of each individual fish in 5 d periods between the ages of 5–54 and 110–139 DAH.

Water temperature profiles during the spawning season

The daily sea surface temperatures (SSTs) during the spawning seasons in the Nansei Islands area (April–June) and in the Sea of Japan (July–August) for 2011–2014 were estimated from high resolution (0.25° latitude × 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 Fig. 1 (data for 2015 was not yet available at the time of this study). SST in the Nansei Islands area was calculated as the average of 169 grid cells within 22.875–25.875° N latitude and 123.625–128.125° E longitude. For the Sea of Japan, the average SST included 48 grid cells located within 35.875–37.125° N latitude and 133.625–136.375° E longitude.

Statistical analysis

The back-calculated body sizes per daily age for fish assigned to the 3 cohorts (P/P, P/J, and J/J) were first checked for normality and homogeneity of variances by a Bartlett test. If normality was respected, back-calculated body sizes for the same daily age were compared using 1-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Else, values were analyzed by the Kruskal-Wallis test followed by the Steel-Dwass post hoc test. The *t*-test was used for comparisons with only 2 cohorts (e.g. P/J and J/J when P/P specimens were not available). Older specimens of P/P were not available, and in this case the *t*-test was used to compare J/J and P/J fish. Essentially, the same statistical procedures used in the comparison of back-calculated body sizes per daily age were used to compare the growth rates of the 3 cohorts in equivalent periods of 5 d of daily age within each year-class and of the same cohort in different years of observation. The ANOVA tests comparing back-calculated size and growth rates between cohorts had df_1 of 2 and df_2 of 39–88, depending on the daily age of the individuals. The ANOVA tests comparing growth rates per daily age between year-classes had df_1 of 4 and df_2 of 43–192. In all cases, statistical significance between groups was defined as

$p < 0.05$. Moreover, in the comparison of back-calculated size per daily age, only stretches of ≥3 consecutive days with exactly the same trend and statistical significance were considered as truly significant. This criterion was adopted to prevent taking into consideration significant results achieved just by chance. All statistical analyses were performed with R (R Development Core Team 2016).

RESULTS

Hatching date estimation and cohort assignment

The estimated hatch dates of the specimens ranged from April to September (Fig. 3, Table 1). 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 were, respectively, 31 May and 1 August in 2011, 15 June and 23 August in 2012, 6 June and 26 July in 2013, 4 June and 29 July in 2014, and 1 June and 30 July in 2015, 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 presumably born in the Nansei Islands area (cohort P/J) and locally (cohort J/J), whereas those captured in the Pacific Ocean were all presumed to be born in the Nansei Islands area (cohort P/P).

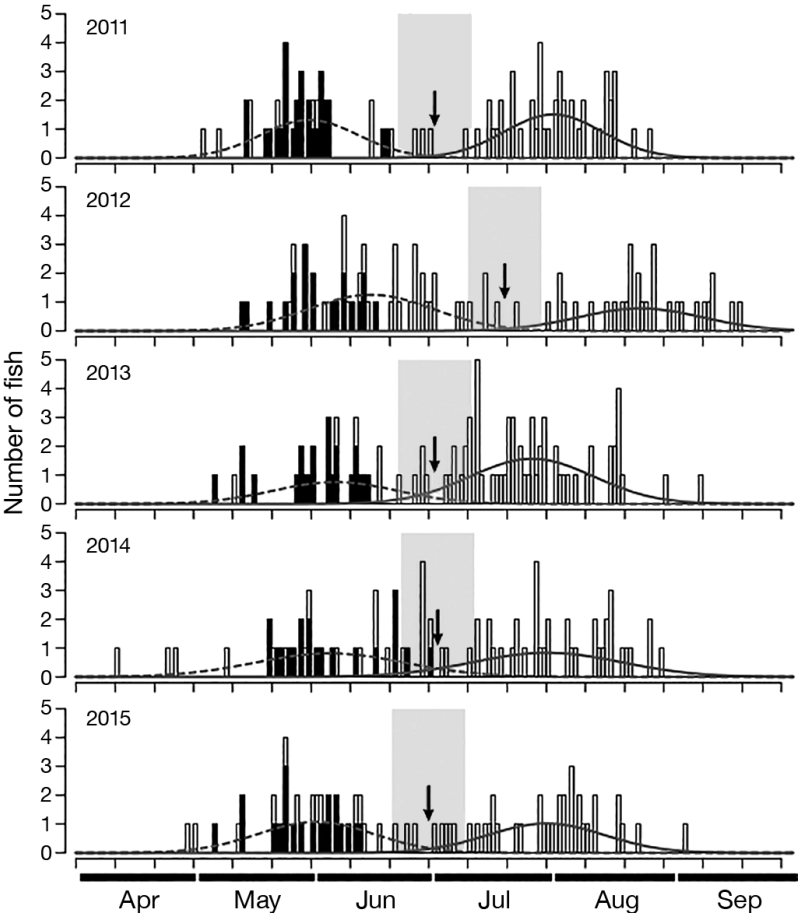

Fig. 3. Distribution of hatch dates of Pacific bluefin tuna collected for this study between 2011 and 2015 as estimated from otolith daily increment analysis. Black and white bars: fish collected in the Pacific (East) coast of Japan (Kochi) and Sea of Japan (Nagasaki, Yamaguchi, Shimane, Ishikawa, and Toyama) fishing grounds, respectively. Solid and dashed lines: distribution of hatch dates for the 'early-' and 'late-born' cohorts, respectively, that were discriminated with a Gaussian mixture model (see text for details). Arrows: reference date (middle point between the average hatch dates for the 2 cohorts); shaded areas: range of hatch dates that were excluded from subsequent analyses to reduce the possibility of cohort misidentification in each year-class

OR–SL relationship and back-calculated growth history

As expected from Watai et al. (2017), a quadratic function gave the best fit to the SL and OR data as follows: $SL = 0.00002OR^2 + 0.1OR + 2.1$ ($r^2 = 0.99$, $p < 0.01$, $n = 1259$; Fig. 4). Back calculation of the daily growth history of individual fish using this equation 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. 5). Between 5 and 20 DAH, individuals in the late-born (J/J) cohort were larger on average than same-aged counterparts in the 2 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 3 cohorts in 2011 and 2013. From 20 to about 75 DAH, the last point in time when the 3 cohorts could be compared, the mean SLs for each daily age 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 for each

Table 1. Fishing ground, year-class, collection date (yr/mo/d), estimated hatch date, and body size (standard length, SL) 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	N	Collection date	Estimated hatch date	SL (mm)		
					Mean ± SD	Min.	Max.
Early-born cohort							
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
	2015	5	2015/9/3–2015/12/6	2015/4/29–2015/6/17	461.8 ± 81.6	325.5	522.8
Yamaguchi	2011	19	2011/11/6–2012/1/18	2011/4/24–2011/6/29	483.4 ± 31.2	439.5	534.4
	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
	2014	6	2014/11/25–2015/1/14	2014/5/24–2014/6/28	489.4 ± 27.2	445.2	512.8
	2015	7	2015/11/7–2015/11/7	2015/5/12–2015/6/24	476.1 ± 5.3	468.5	481.2
Shimane	2013	4	2013/10/10–2013/12/17	2013/6/22–2013/6/29	431.0 ± 37.9	398.3	466.7
	2015	1	2015/10/3	2015/6/26	354.6		
Ishikawa	2012	8	2012/12/21–2013/1/30	2012/5/26–2012/7/17	521.9 ± 14.4	497.0	540.0
	2013	2	2013/12/9–2013/12/11	2013/6/26–2013/6/28	451.1	450.1	452.1
	2014	13	2014/11/21–2015/1/5	2014/4/11–2014/6/30	521.6 ± 8.8	507.8	535.1
	2015	3	2015/12/7–2016/1/14	2015/5/1–2015/6/13	532.2 ± 30.1	514.3	567.0
Toyama	2012	8	2012/12/28–2013/1/21	2012/6/8–2012/7/14	517.0 ± 11.9	497.0	536.9
	2015	2	2015/12/8–2015/12/8	2015/5/24–2015/6/1	520.5	509.4	531.6
Late-born cohort							
Nagasaki	2012	1	2013/1/9	2012/8/20	366.2		
	2015	10	2015/10/8–2016/1/16	2015/7/4–2015/8/17	428.9 ± 56.1	323.6	525.9
Yamaguchi	2011	17	2011/11/30–2012/1/29	2011/7/4–2011/8/29	415.5 ± 54.4	313.8	483.6
	2013	8	2013/11/25–2013/12/17	2013/7/6–2013/9/7	471.1 ± 17.2	457.1	511.3
	2014	19	2014/11/25–2015/1/14	2014/7/3–2014/8/28	454.5 ± 11.8	442.0	477.5
	2015	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	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	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	18	2012/11/8–2013/1/30	2012/7/22–2012/9/17	372.3 ± 55.5	341.5	524.4
	2013	16	2013/10/26–2013/12/11	2013/7/4–2013/8/25	386.9 ± 35.7	336.9	436.5
	2014	5	2014/12/15–2015/1/21	2014/7/16–2014/8/25	307.3 ± 27.1	244.0	338.0
	2015	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	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	2015/12/4–2015/12/4	2015/8/5–2015/8/11	354.4	352.3	356.4

daily age between 100 and 150 DAH for the P/J cohort were significant larger than those for the J/J cohort in 2011 and 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 2013–2015.

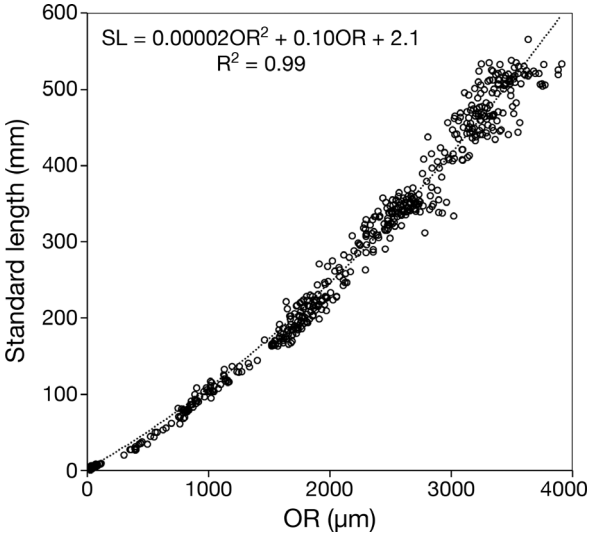

Fig. 4. Relationship between standard length and otolith radius (OR) of Pacific bluefin tuna used in this study

Developmental and inter-annual differences in growth rates

The results of daily growth rates between 5–54 and 110–139 DAH are shown in (Fig. 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 Fig. 5. For example, the late-born (J/J) cohort showed faster growth rates between 5 and 19 DAH than the early-born cohorts (P/P and P/J) in 2012, whereas the opposite pattern was found for 2014 and 2015. In contrast, in 2011 and 2013 there were no clear trends during this period for any of the 3 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 2014 and 2015 but not in 2011–2013. The P/J cohort consistently showed significantly faster growth rates after 110 DAH than the J/J cohort in all years except 2013 (Fig. 6).

Significant inter-annual differences in growth rates for equivalent 5 DAH segments were noted almost exclusively in the J/J cohort (Figs. 7 & 8). For example, in the P/P cohort they were limited to between 5 and 9 DAH, and were completely absent in the P/J cohort. In contrast, growth rates in the J/J cohort had significant inter-annual differences at all daily ages investigated until 124 DAH. Growth rates in this cohort were notably faster between 5 and 24 DAH in 2012 and slower between 5 and 19 DAH in both 2014 and 2015.

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 2014 are shown in Fig. 9. The range of temperatures during the spawning season in the 2 areas is similar, with a minimum of 22°C and a maximum of 29°C. However, while the temperature in the Nansei Islands area increases steadily throughout 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 northwestern Pacific Ocean and one in the Sea of Japan, but the spawning season in these 2 areas differs considerably in time to the point that it hardly overlaps (Ashida et al. 2015, Okochi et al. 2016, Ohshimo et al. 2017, 2018). Thus, spawning in the Nansei Islands area occurs earlier, usually from April to June, whereas in the Sea of Japan it occurs mainly between July and August (Ashida et al. 2015, Okochi et al. 2016, Ohshimo et al. 2017, 2018). Fish born around the Nansei Islands remain in the natal area for about 2–3 wk 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 (East) coast of Japan where they spend their first year (Kitagawa et al. 2010, Masujima et al. 2014, Furukawa et al. 2017, Fujioka et al. 2018), but some 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 2 spawning 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 those larvae with 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

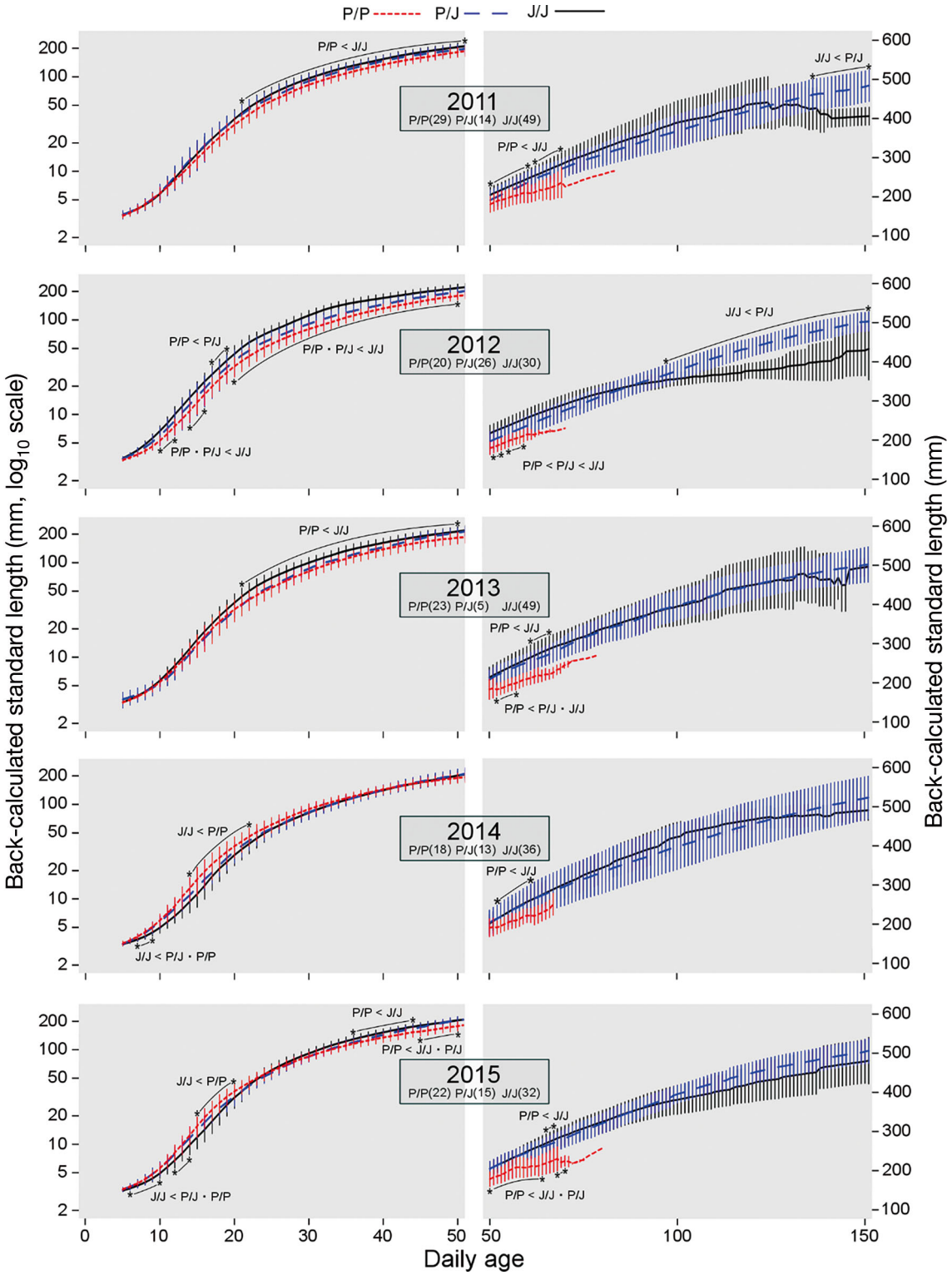

Fig. 5. Comparison of the back-calculated standard lengths (mean ± SD) of Pacific bluefin tuna from 5 year-classes (2011–2015) that were assigned to 3 cohorts; namely, those presumably born in the Nansei Islands area and collected either in the Pacific coast of Japan (P/P) or in the Sea of Japan (P/J) and those presumably born and collected in the Sea of Japan (J/J) between 0 and 150 d after hatching (DAH). Asterisks: statistically significant differences ($p < 0.05$) in 1-way ANOVA followed by the Tukey's multiple comparison test or the Kruskal-Wallis test followed by the Steel-Dwass post hoc test for comparisons between P/P, P/J, and J/J or the *t*-test for comparisons only between P/J and J/J. In all cases, only stretches of 3 or more consecutive days with exactly the same trend and statistical significance are indicated. Values for 0–50 DAH are shown in logarithmic scale to increase visibility of the differences. Values within parenthesis: number of samples analyzed for each year-class

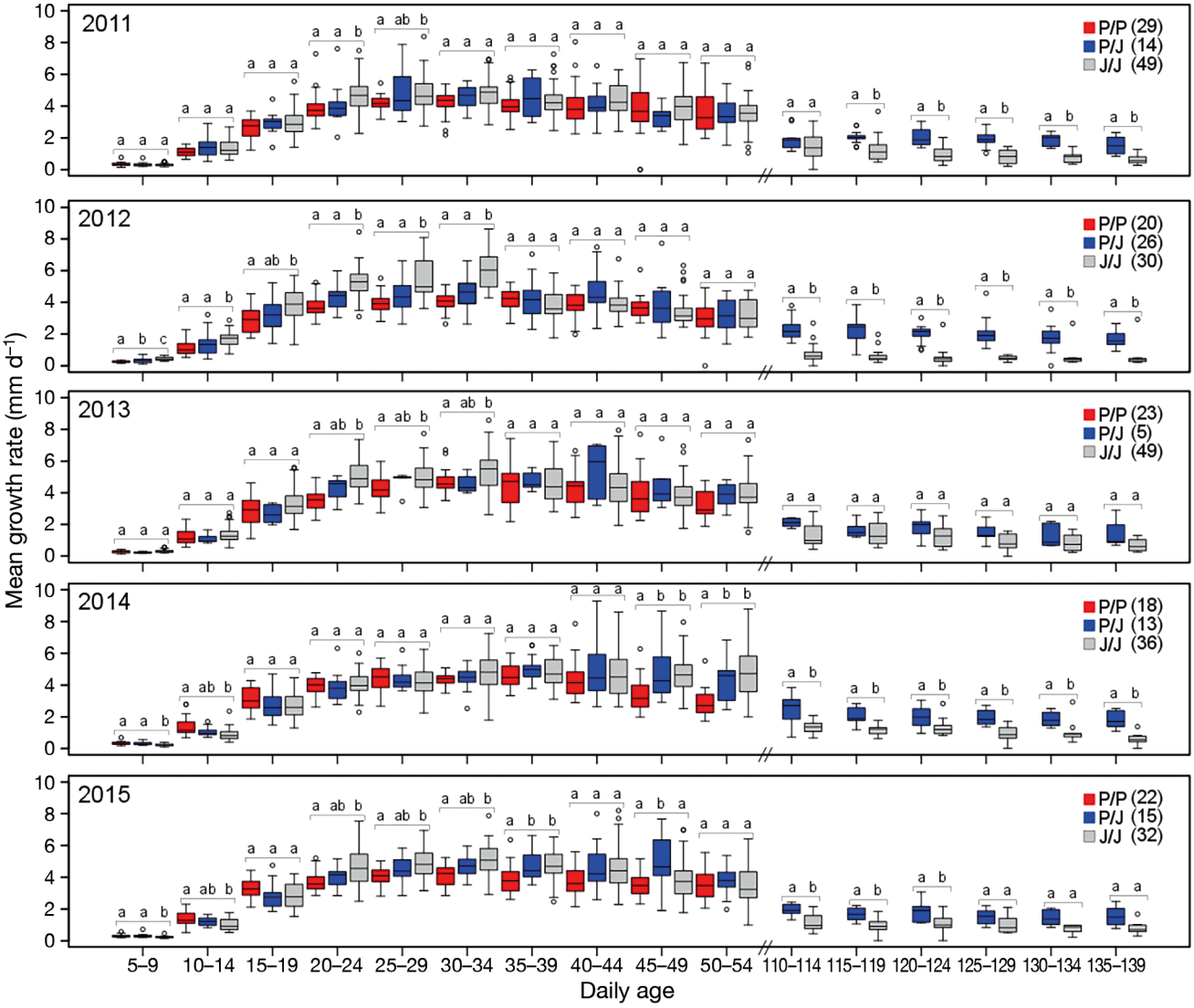

Fig. 6. Comparison of the mean growth rates (whisker plots with median, ±SD, maximum and minimum values for specified 5 d intervals; circles: outliers) of Pacific bluefin tuna from 5 year-classes (2011–2015) that were assigned to 3 cohorts; namely, those presumably born in the Nansei Islands area and collected either in the Pacific coast of Japan (P/P) or in the Sea of Japan (P/J) and those presumably born and collected in the Sea of Japan (J/J). Mean values for cohorts within each daily age interval with different lowercase letters differ significantly ($p < 0.05$; 1-way ANOVA followed by the Tukey's multiple comparison test or Kruskal-Wallis test followed by the Steel-Dwass post hoc test for comparisons of P/P, P/J, and J/J, or *t*-test for comparisons only between P/J and J/J). Values within parenthesis: number of samples analyzed for each year-class

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 2 spawning grounds and compare their past growth rates using otolith-based growth back-calculation analysis. Unlike in our previous study, which examined the growth history of larval, juvenile, and YOY specimens within the same cohort (Watai et al. 2017), in this study we back-calculated the past growth rates corresponding to the larval, juvenile, and YOY stages of young PBF captured by the fisheries that were (1) presumably born in the Nansei Islands area in the northwestern Pacific and collected in the Pacific (East) coast of Japan (cohort P/P), (2) presumably born in the Nansei Islands area and collected in the Sea of Japan (cohort P/J), and (3) presumably born and collected in the Sea of Japan (cohort J/J).

The first and most critical stage is from hatching to about 20 DAH, which corresponds to the larval and early juvenile stages (Kaji et al. 1996, Miyashita et al. 2001, Miyashita 2002). This is a period when mortality is usually high and when growth rates have the

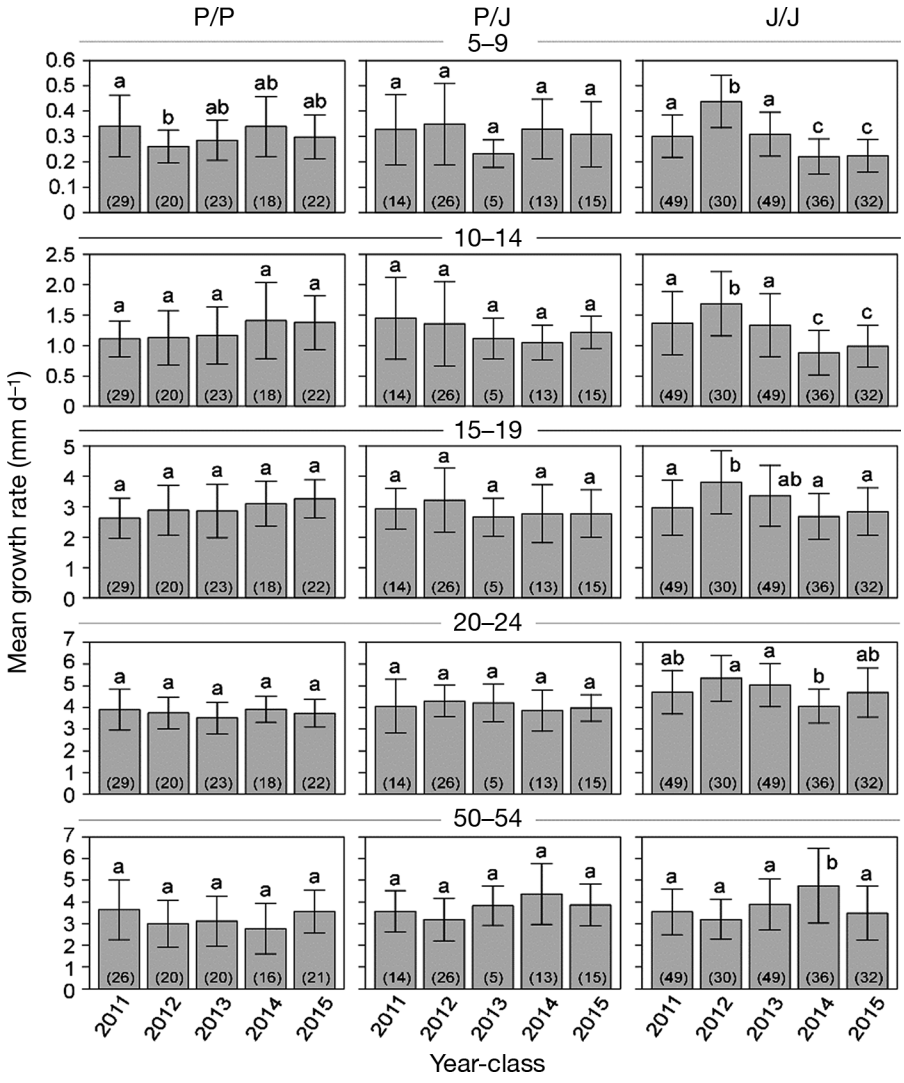

Fig. 7. Interannual comparison of the mean (±SD) larval and juvenile growth rates of Pacific bluefin tuna from 5 year-classes (2011–2015) that were assigned to 3 cohorts; namely, those presumably born in the Nansei Islands area and collected either in the Pacific coast of Japan (P/P) or in the Sea of Japan (P/J) and those presumably born and collected 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 d after hatching). Mean values for year-classes within each cohort and daily age interval with different letters differ significantly ($p < 0.05$, 1-way ANOVA followed by Tukey's multiple comparison test, or Kruskal-Wallis test followed by the Steel-Dwass post hoc test). Parentheses: number of samples analyzed for each daily age interval and year-class

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 during the larval stage were significantly higher than in the cohorts born in the Nansei Islands area (P/P and P/J) in 2012 but lower in 2014 and 2015. In the 2 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 fish (e.g. highest and lowest in 2012 and 2014–2015, respectively) rather than in fish born in the Nansei Islands area, which showed little or no variation during this period across the years. It is noteworthy that the Sea of Japan is considered to be more productive than the Nansei Islands area 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 2014. Moreover, PBF larval growth seems to be positively affected by in-

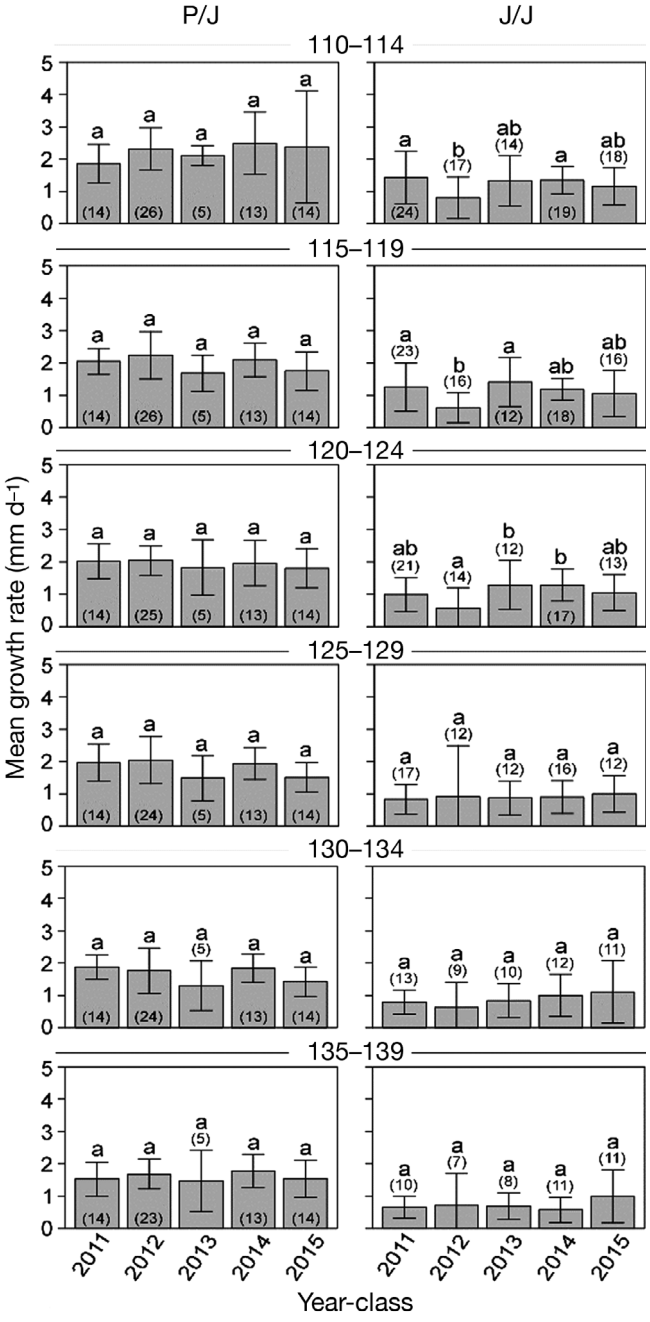

Fig. 8. Interannual comparison of the mean (±SD) young-of-the-year (YOY) growth rates of Pacific bluefin tuna from 5 year-classes (2011–2015) that were assigned to 2 cohorts; namely, those presumably born in the Nansei Islands area and collected in the Sea of Japan (P/J) and those presumably born and collected 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 d after hatching). Mean values for year-classes within each cohort and daily age interval with different letters differ significantly ($p < 0.05$, 1-way ANOVA followed by Tukey's multiple comparison test, or Kruskal-Wallis test followed by the Steel-Dwass post hoc test). Parentheses: number of samples analyzed for each daily age interval and year-class

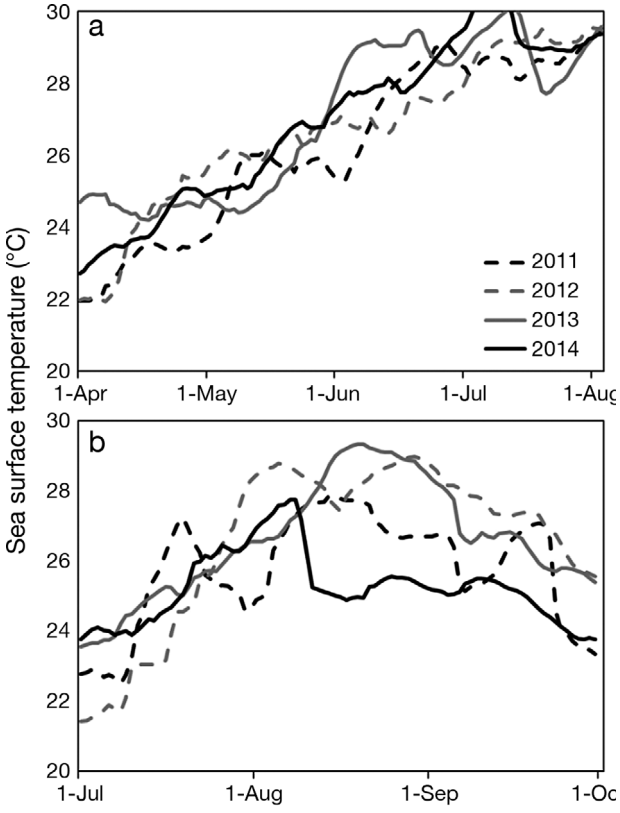

Fig. 9. Daily sea surface temperature in (a) the Nansei Islands and (b) Sea of Japan spawning grounds between 2011 and 2014. Temperatures were estimated from National Oceanic and Atmospheric Administration Advanced Very High Resolution Radiometer (NOAA/AVHRR) data (see 'Materials and methods: Water temperature profiles during the spawning season' for details)

creasing temperature within the range observed during the spawning season (Satoh et al. 2008), whereas a decrease of only 3°C causes a sharp drop in growth rates (Kimura et al. 2010, Tanaka et al. 2010, see also similar information for the Mediterranean bluefin tuna *Thunnus thynnus* in García et al. 2013). In this context, the variable growth rates during the first weeks of life in PBF presumably 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. This hypothesis and the importance of alternative factors such as the origin, age, and nutritional condition of spawners and eggs should be the focus of further studies.

The period between 20 and around 50 DAH corresponds to the juvenile stage (Kaji et al. 1996, Miyashita et al. 2001, Miyashita 2002). For Nansei Islands-born PBF, it 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 (East) 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 fish had higher growth rates than P/P at almost any time point during the juvenile stage (Fig. 6). The actual temperatures experienced by fish that took different northward routes are not known, but the higher growth rates of fish 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 fish may have experienced. Interestingly, the differences between P/J and P/P fish was already evident 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 and other possibilities should be scrutinized in further studies.

Individuals that successfully reach 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. 2018). Unfortunately, all YOY specimens collected in the Pacific (East) coast of Japan were less than 75 d old, so information on the growth rates of P/P fish after this period is not available. YOY from the 2 spawning grounds that mix in the Sea of Japan (P/J and J/J) produce a bimodal size distribution in which the immigrants, born 50–60 d earlier in the Nansei Islands area, 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 fish consistently had inferior growth rates than same daily aged P/J fish. However, it must be noted that the 2 cohorts differ in average daily age by about 2 mo when they coinhabit 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, more unfavorable thermal conditions for growth than P/J at comparable ages, and this ultimately causes the size difference 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 also confer them a competitive advantage over J/J fish when they coexist.

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, fish presumed to be born in the Sea of Japan do not necessarily benefit from the high primary and secondary productivity in this area during their larval stage, although this is occasionally observed, as in 2012. This uncoupling of growth rates and food abundance in the larval stage is probably related to the inherent thermal instability and proximity of winter in relation to the spawning season in this area, causing fish to experience suboptimal temperatures for growth. On the other hand, the high growth rates shown by fish living in the Sea of Japan during their juvenile and early YOY stages, regardless of their natal origin, 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 (see Boyce et al. 2008 and Kimura et al. 2010 for information on adults and larvae, respectively). Further studies should examine the effects of temperature and other abiotic factors on growth in relation to developmental stage of PBF, and examine the actual correlation of growth rates in different developmental stages with the strength of each year-class. Finally, it is important to keep in mind that the conclusions of this study are based on the current

knowledge about the presence of 2 discrete spawning grounds with characteristic spawning periods for PBF. It is not impossible, though, that future surveys could reveal the existence of hitherto unknown spawning ground(s) for this species, as recently discovered for the Atlantic bluefin tuna (Richardson et al. 2016).

Acknowledgements. This research was implemented as part of the Research Project on Japanese Bluefin Tuna conducted by the Fisheries Agency of Japan. The authors sincerely appreciate the support from the Kochi Prefectural Fisheries Experiment Station, Ishikawa Prefectural Fisheries Experimental Station, Shimane Prefectural Fisheries Experimental Station, Nagasaki Prefectural Fisheries Experimental Station, and Toyama Prefectural Fisheries Experimental Station for collecting PBF. The authors thank S. Konagaya, K. Koizumi, S. Yoshimura, Dr. A. Tawa, Dr. N. Suzuki, and Dr. A. Takasuka (The National Research and Development Agency, Japan Fisheries Research and Education Agency), and Dr. M. Yokota (Tokyo University of Marine Science and Technology) for help collecting samples, statistical advice, and helpful suggestions to improve the manuscript.

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Editorial responsibility: Stylianos Somarakis, Heraklion, Greece

Submitted: January 30, 2018; Accepted: October 10, 2018
Proofs received from author(s): November 20, 2018