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Prey Availability for Larval and Juvenile Pacific Bluefin Tuna *Thunnus orientalis* Estimated from the Mouth Gape Size in Relation to Their Piscivory

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Abstract

In this study, we estimated prey availability in the early life stages of Pacific bluefin tuna (PBF) based on their mouth gape size, collected in two spawning grounds, the Pacific Ocean around the Nansei Islands and the Sea of Japan. The increase in mouth gape sizes was investigated using laboratory-reared PBF larvae and juveniles. Thereafter, we compared the mouth gape size of PBF with the body size of prey larvae, including PBF, collected in field surveys in the spawning grounds of PBF conducted in 2017 and 2018. We defined the collected larvae smaller than the mouth gape, mouth width, and 1/3rd the body length (BL) of PBF as potential prey larvae (PPL), which have the potential of being captured and ingested by PBF. The number of PPL in each sampling station was estimated for PBF of 8, 10, 15, 20, and 30 mm BL size classes as predatory PBF. The density of PPL increased for PBF larger than 20 mm in BL, suggesting that prey availability could change drastically for PBF larvae and juveniles larger than 20 mm BL.

Discipline: Fisheries
Additional keywords: cannibalism, feeding, Scombridae

Introduction

Pacific bluefin tuna (PBF) *Thunnus orientalis* is distributed widely in the North Pacific Ocean and is a highly migratory species between the western and eastern Pacific Ocean (Bayliff 1994). PBF are one of the most important fishery resources for several countries managed by several regional fisheries management organs. Interannual variability of the recruitment of PBF is very high (Yamada et al. 2006, Ishida et al. 2018), which was 10 times at maximum (Ishida et al. 2018), and there is no clear relationship between the spawning stock biomass and recruitment of PBF (ISC 2016). In general, survival during the egg, larval, and juvenile stages is considered the main cause of interannual variability in the recruitment of marine fishes (Bailey & Houde 1989).

Growth in the early life stages of fish is one of the important factors elucidating the recruitment variability. Growth of PBF during the early life period influences their consequent survival and recruitment success (Tanaka et al. 2006, Watai et al. 2017, Ishihara et al. 2019). It has been reported that food availability and ambient water temperature influence growth performance in early life history (e.g., Houde 1989). To understand the recruitment process and its annual variation in PBF, information about biotic and abiotic factors influencing the early growth of PBF is necessary.

Several studies have reported the feeding habits of wild PBF larvae smaller than 10 mm in BL (Uotani et al. 1990, Kodama et al. 2017, Kodama et al. 2020a). The main prey of PBF larvae smaller than 10 mm in BL was zooplankton. However, no study has reported the feeding habits of PBF larvae and juveniles around 10 mm-20 mm BL in the field because field collection of PBF larvae is

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rare, mainly due to net avoidance.

Several research institutes and private companies in PBF aquaculture have recently developed mass-production techniques for PBF larvae and juveniles to produce fingerlings for PBF aquaculture. Laboratory-reared PBF larvae have been reported to show piscivory from 8 mm-9 mm in body length (BL). They show a much higher growth performance than PBF larvae fed only zooplankton (Tanaka et al. 2014a). The fast-growing larvae at the onset of piscivory had a high possibility of survival in the mass culture tank of PBF, which was characterized as growth-selective mortality (Tanaka et al. 2018). Accordingly, the importance of piscivory during the early life stages of PBF in the field should be evaluated to understand the recruitment process. However, no study has evaluated the feeding habits of PBF larvae in relation to piscivory in the field mentioned above. So, utilizing laboratory-reared fish can help to elucidate the early life ecology of PBF in the field (Tanaka & Suzuki 2016).

This study aims to examine the potential prey availability for PBF larvae in the field in relation to their piscivory. Firstly, we investigated the development of mouth gape size of laboratory-reared PBF larvae. Secondly, the larval body size of fish species as potential prey collected during larval surveys were compared to the mouth gape size and width of PBF. Thereafter, we calculated the density and percentage of prey larvae that could be consumed by PBF larvae and juveniles which are smaller than the mouth of PBF and preliminarily evaluated the ambient prey availability for PBF larvae after the onset of piscivory in the field, and also discussed the possibility of cannibalistic prey-predator relationship of PBF.

Materials and methods

1. Sampling of laboratory-reared larval and juvenile PBF

We used PBF larvae and juveniles reared in the Amami Field Station, Aquaculture Research Department, Fisheries Technology Institute, FRA. PBF larvae hatched on 23 June 2018 were transferred into a 50 kL mass-culture tank and reared until 30 days after hatching (DAH). The feeding regime for PBF larvae and juveniles was as follows: rotifers from 2 to 17 DAH; yolk-sac larvae of spangled emperor *Lethrinus nebulosus* from 14 to 30 DAH; artificial diets from 17 to 30 DAH. Fifteen fish were sampled on 3, 10, 15, 18, 21, 24, 27, and 30 DAH and preserved in 99.5% ethanol.

2. Measurements of laboratory-reared PBF

We measured the body length (BL), body height (BH), and body width (BW) of the laboratory-reared larval and juvenile PBF. In the larval stage, the length from the tip of the snout to the tip of the notochord was measured as BL. In this study, we measured the highest and widest parts of the larval bodies as BH and BW, respectively. The relationships between BL and BH and between BL and BW for PBF larvae were used to estimate BH and BW from the BL of field-captured scombrid larvae (see "Estimation of body sizes of scombrids larvae").

We measured the upper jaw length and mouth width of these PBF larvae and juveniles, and we calculated the mouth gape size at a 45° mouth opening according to the method of Shirota (1970).

3. Field collection of fish larvae

Larval surveys were conducted by surface trawls in two spawning grounds, the Nansei Islands area and the Sea of Japan, in 2017 and 2018 aboard RV "Shunyo-Maru" (Fig. 1). We used the samples collected from 32 stations

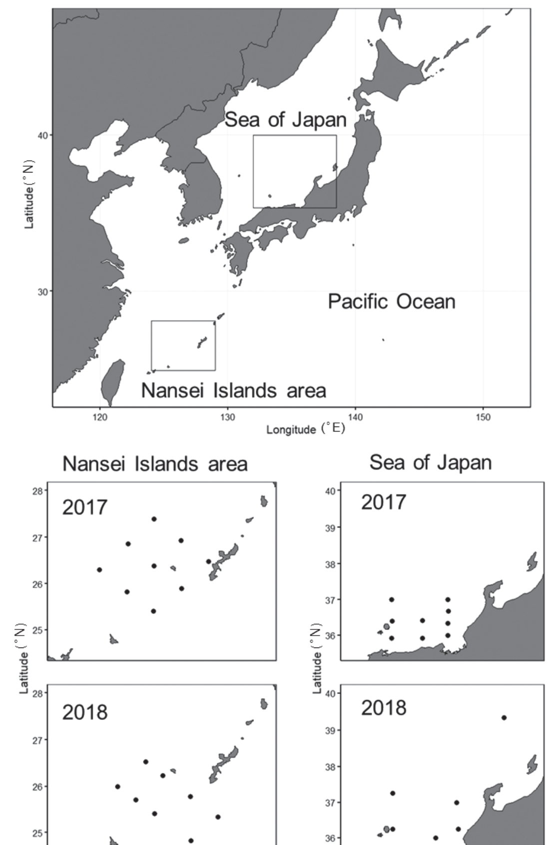

Fig. 1. Map showing the survey area in the Nansei Islands area of the Pacific Ocean and in the Sea of Japan
Black dots indicate the sampling stations.

in the Nansei Islands area and the Sea of Japan (Table 1). These stations were distributed according to the occurrence of PBF larvae reported by Ohshimo et al. (2017) and Tawa et al. (2020). A 2 m diameter ring net with 334 µm mesh was used for larval collection. The net was towed at the sea surface beside the hull for 10 min at a speed of approximately 1.5 knots. The volume of water filtered was calculated from the mouth area of the net multiplied by the distance traveled, which was measured by a calibrated flowmeter (5571-B; Rigo Co., Ltd, Tokyo, Japan) attached to the net mouth. Scombrid larvae were sorted from the net samples onboard as soon as possible and preserved in 99.5% ethanol immediately after being sorted. After removing scombrid larvae onboard, the net samples were fixed in 99.5% ethanol and sorted again in the laboratory.

4. Estimation of body sizes of scombrid larvae

Photographs of scombrid larvae were taken individually from the lateral side. Photos of the larvae of other fish species were also taken individually from the lateral and ventral sides. We took photos of scombrid larvae only from the lateral side because the body tissue of the samples was used for other analyses immediately after taking the photos.

The BL of scombrid larvae was measured using

Table 1. Date, location, sea surface temperature (SST), and the number of Pacific bluefin tuna (PBF) larvae collected at sampling stations used in this study in the Nansei Islands area and the Sea of Japan

	Sampling year	St. ID	Sampling date	Latitude	Longitude	SST (°C)	No. of PBF collected
Nansei Islands area	2017	01	18 June	25°24′ N	126°18′ E	26.9	3
		02	20 June	25°50′ N	125°40′ E	27.9	7
		03	21 June	26°18′ N	124°60′ E	27.5	0
		04	21 June	26°51′ N	125°41′ E	27.9	17
		05	22 June	26°23′ N	126°19′ E	28.4	2
		06	23 June	25°54′ N	126°58′ E	28.0	0
		07	25 June	26°29′ N	127°38′ E	28.0	3
		08	26 June	26°56′ N	126°58′ E	28.6	163
		09	28 June	27°23′ N	126°19′ E	29.3	0
Nansei Islands area	2018	10	22 June	25°45′ N	127°11′ E	28.0	40
		11	23 June	25°20′ N	127°51′ E	28.8	2
		12	24 June	24°49′ N	127°12′ E	29.5	0
		13	25 June	25°24′ N	126°20′ E	28.5	12
		14	26 June	25°43′ N	125°52′ E	29.0	17
		15	27 June	25°60′ N	125°26′ E	28.9	0
		16	27 June	26°14′ N	126°31′ E	29.2	16
		17	28 June	26°32′ N	125°06′ E	28.9	40
Sea of Japan	2017	18	24 July	35°55′ N	133°28′ E	27.2	24
		19	25 July	36°24′ N	133°28′ E	26.8	1
		20	25 July	37°00′ N	133°27′ E	25.7	28
		21	29 July	35°55′ N	134°33′ E	27.4	0
		22	29 July	36°25′ N	134°33′ E	27.2	1
		23	2 August	36°00′ N	135°26′ E	27.5	0
		24	2 August	36°20′ N	135°26′ E	26.9	123
		25	3 August	36°41′ N	135°27′ E	26.7	60
		26	3 August	37°00′ N	135°26′ E	26.6	3
Sea of Japan	2018	27	25 July	37°15′ N	133°30′ E	27.0	2307
		28	25 July	36°15′ N	133°30′ E	28.8	1
		29	27 July	36°14′ N	135°48′ E	28.1	39
		30	27 July	36°60′ N	135°45′ E	29.0	67
		31	26 July	36°00′ N	135°00′ E	28.2	110
		32	1 August	39°20′ N	137°25′ E	26.9	9

image J 1.47v software (Abramoff et al. 2004). Thereafter, the BH and BW of scombrid larvae were calculated using the relationships made from laboratory-reared PBF. In the present study, we applied the BL-BH and BL-BW relationships of laboratory-reared PBF to the body size estimations of other scombrid larvae because it was impossible to use laboratory-reared samples of each scombrid species (Fig. 2).

5. Comparison between mouth gape size of PBF and body size of potential prey larvae

Scombrid larvae were identified by the pigmentation of larvae according to Okiyama (2014). Additionally, species identification by DNA was carried out for individuals which were difficult to identify morphologically according to the method by Suzuki et al. (2014). However, larvae of the bullet tuna *Auxis rochei* and frigate tuna *Auxis thazard* were combined and expressed as *Auxis* spp. in the present study. Larvae other than scombrids were morphologically identified at the family level based on Okiyama (2014).

The BL, BH, and BW of larvae of other species were measured individually using image J 1.47v software by two photos from the lateral and ventral sides. When more than 50 larvae of the same taxon were collected, we measured the 50 larvae, and the length data were extended to the total number of larvae in the sample. The BH and BW of the collected larvae were compared to the mouth gape size and mouth width of the laboratory-reared PBF larvae and juveniles (see results and Table 3-6).

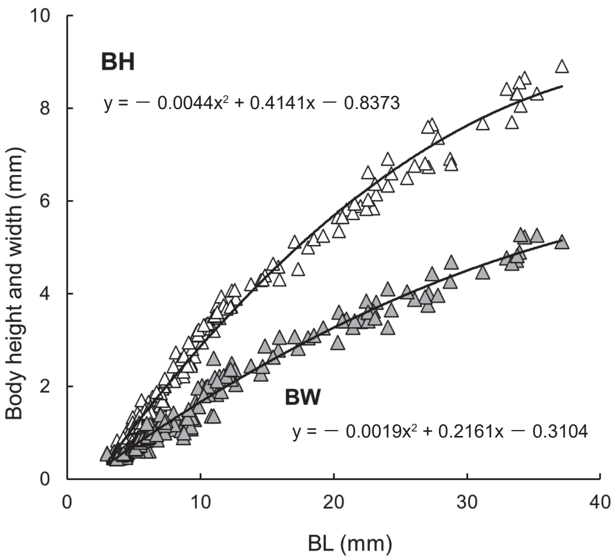

Fig. 2. Relationship between body length (BL), body height (BH), and body width (BW) of laboratory-reared Pacific bluefin tuna larvae and juveniles

Open and grey triangles indicate BH and BW of laboratory-reared PBF larvae and juveniles, respectively.

In terms of BL, we assumed that PBF larvae and juveniles can ingest prey organisms that have a length one-third of their length based on Scharf et al. (1998) and Tanaka et al. (2014a). Tanaka et al. (2014a) showed that an 8 mm BL laboratory-reared PBF could ingest newly hatched larvae of spangled emperor *Lethrinus nebulosus* as prey, of which the BL was 2.8 mm (Tanaka unpublished data). Scharf et al. (1998) demonstrated that the feeding success of piscivorous juvenile bluefish *Pomatomus saltatrix* increased when the length of prey fish was smaller than one-third of the BL of predatory bluefish. Based on the comparison, we regarded the BH, BW, and BL of larvae smaller than the mouth gape, mouth width, and 1/3rd the BL of body length of PBF, respectively, as the potential prey larvae (PPL).

It has been reported that PBF juveniles with a fork length (FL) larger than 50 mm were collected by surface trawl (Tanaka et al. 2020). However, there are few records of PBF juveniles being collected with a FL smaller than 50 mm. The piscivory for laboratory-reared PBF occurred at a BL larger than 8 mm (Tanaka et al. 2014a). Accordingly, in this study, we focused on PBF with sizes between 8 and 50 mm, and prey availabilities of the five size classes of PBF larvae and juveniles, 8, 10, 15, 20, and 30 mm BL, were examined. Thus, the number of PPL in each sampling station that could be ingested (able to be swallowed) was calculated for PBF of 8, 10, 15, 20, and 30 mm BL size classes. The densities and percentages in the number of PPL in each sampling station were calculated for the five size classes of PBF using the following equations.

Density of PPL$_{i\ \mathrm{mm}}$ = number of PPL$_{i\ \mathrm{mm}}$ / the volume of water filtered (m^3)

Percentage of PPL$_{i\ \mathrm{mm}}$ = 100 × number of PPL$_{i\ \mathrm{mm}}$ / number of all larvae collected

where PPL$_{i\ \mathrm{mm}}$ indicates potential prey larvae for PBF of the 5 size classes (i = 8, 10, 15, 20 and 30).

In this study, we assumed that the rate of shrinkage of samples by ethanol fixation was constant among the BL, BH, and BW, as well as among fish species.

A U-test was used to test for significant differences in the densities and percentages of PPL between the Nansei Islands area and the Sea of Japan using R version 3.5.0 (R Development Core Team: https://cran.rproject.org/bin/windows/base/).

Results

1. Development of mouth gape size of laboratory-reared PBF larvae and juveniles

The mouth gape of laboratory-reared PBF estimated from the fitted curve was 1.21 mm for 8 mm BL, 1.61 mm for 10 mm BL, 2.3 mm for 15 mm BL, 2.87 mm for 20 mm BL, and 3.60 mm for 30 mm BL (Fig. 3, Table 2). For these, the mouth width was 0.70 mm for 8 mm BL, 0.90 mm for 10 mm BL, 1.26 mm for 15 mm BL, 1.52 mm for 20 mm BL, and 1.88 mm for 30 mm BL (Fig. 3, Table 2).

2. Larvae collected in the Nansei Islands area and the Sea of Japan

The list of the larvae collected in each station of the Nansei Islands area and the Sea of Japan in 2017 and 2018 is shown in Tables 3-6. In total, 56 families of larvae were collected in 2017 and 2018. For scombrid larvae, PBF, yellowfin tuna *T. albacares*, skipjack tuna *Katsuwonus pelamis*, and two *Auxis* species, dogtooth tuna *Gymnosarda unicolor* and wahoo *Acanthocybium solandri*, were collected.

We observed very high densities of PBF, Engraulidae and Carangidae in both areas. The density of Carangidae in St. 10, Engraulidae in St. 20, and PBF in St. 27 was 1,504.1, 1,769.5, and 1,743.6 per 1,000 m^3, respectively (Tables 4, 5, 6). Of these collected larvae, we measured the body sizes of 3,077 and 4,721 fish in the Nansei Islands area and the Sea of Japan collected in 2017, and 4,055 and 1,780 fish in 2018.

The BL, BH, and BW of the larvae collected in the Nansei Islands area ranged from 1.47 to 23.90 mm, 0.36 to 4.89 mm, and 0.21 to 2.71 mm in 2017, and from 1.17 to 16.43 mm, 0.14 to 6.7 mm, and 0.15 to 3.56 mm in 2018. Those of the larvae collected in the Sea of Japan ranged from 1.28 to 15.18 mm, 0.19 to 3.29 mm, and 0.14 to 1.90 mm, and from 1.90 to 12.92 mm, 0.21 to 1.72 mm, and 0.22 to 1.12 mm in 2018.

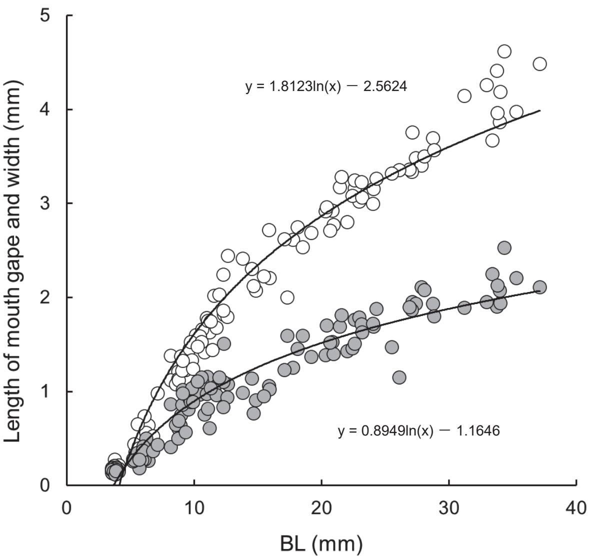

Fig. 3. Relationship between body length (BL), mouth gape size, and mouth width of laboratory-reared Pacific bluefin tuna larvae and juveniles
Open and grey circles indicate the length of the mouth gape and mouth width of laboratory-reared PBF larvae and juveniles, respectively.

3. Density and ratio of potential prey larvae in Nansei Islands and the Sea of Japan

The density of PPL in both areas increased with the growth of predatory PBF (Fig. 4). Significant differences in the density of PPL between the two areas were not detected in both years. The densities of PPL in both areas were very low for PBF smaller than 10 mm BL. However, the density of PPL gradually increased to ca. 200 fish / 1,000 m^3 for PBF with a BL larger than 20 mm. Extremely high densities of Carangidae, Engraulidae, and PBF were observed in St. 10 in the Nansei Islands area in 2018, St. 20 in the Sea of Japan in 2017, and St. 27 in 2018, respectively. The outlier values in Figure 4 were due to such high densities for these sampling stations.

The percentage of PPL in both areas was very low for PBF with a BL smaller than 10 mm, as well as the density of PPL (Fig. 5). The percentage of PPL were relatively higher for PBF larger than 15 mm BL. Almost 90% of the collected larvae were potential prey for PBF with a BL of 30 mm. Significant differences in the percentage of PPL between the two areas were detected only in the 30 mm size class of predatory PBF in 2017 ($U = 16.5$, $P < 0.05$).

Table 2. Mouth gape and width of predatory Pacific bluefin tuna (PBF) larvae and juveniles in five size classes, 8, 10, 15, 20, and 30 mm BL

	Body length of predatory PBF				
	8 mm	10 mm	15 mm	20 mm	30 mm
Mouth gape (mm)	1.21	1.61	2.35	2.87	3.60
Mouth width (mm)	0.70	0.90	1.26	1.52	1.88

Table 3. List of the larvae collected in stations of the Nansei Islands area in 2017

Family	Species	St. ID 01	02	03	04	05	06	07	08	09
Engraulidae		2	245	90	126	114	2	60	8	867
Chanidae										
Gonostomatidae								14		
Phosichthyidae								2		
Stomiidae										
Chlorophthalmidae										
Paralepididae		3								
Neoscopelidae										
Gempylidae		27	3	2	2	4		24	38	
Bregmacerotidae					1					
Ophidiidae				1						
Carapidae										
Holocentridae					2			20	4	
Caproidae									4	
Hemiramphidae		1					1		10	
Exocoetidae					1				7	
Scorpaenidae						1				
Triglidae				1						
Platycephalidae				1						
Dactylopteridae									1	
Serranidae				5	2	4		34	15	
Priacanthidae										
Apogonidae										
Coryphaenidae						1				
Carangidae		88	4	11	11	9		17	29	
Bramidae										
Emmelichthyidae								1		
Lutjanidae				2	5			34	8	
Gerreidae								2		
Lethrinidae						1				
Sillaginidae										
Mullidae				5	1				3	
Pempheridae										
Chaetodontidae						1				
Pomacentridae				1	7			4	2	
Teraponidae										
Oplegnathidae			2							
Kyphosidae										
Nomeidae										
Polynemidae										
Labridae				1						
Uranoscopidae										
Blenniidae										
Gobiidae				1		1		10	1	
Siganidae										
Acanthuridae				1						
Sphyraenidae										
Gempylidae		1								
Trichiuridae										
Scombridae										
	Katsuwonus pelamis	16	3	0		1	3			1
	Thunnus albacares	15	4	1	5	1		7	161	9
	Thunnus orientalis	3	7		17	2		3	163	
	Thunnus sp.								1	
	Auxis sp.	7	4	132	187	7	0	13	2	121
	Gymnosarda unicolor					1				
	Acanthocybium solandri		1			1				
	Scombridae sp.									
Bothidae						1				
Soleidae									1	
Cynoglossidae										
Monacanthidae									1	
Ostraciidae										
Tetraodontidae										
Unidentified		8	3	13	36	7	1	24	51	18
Total		171	276	268	403	157	7	269	510	1016

Table 4. List of the larvae collected in stations of the Nansei Islands area in 2018

Family	Species	St. ID							
		10	11	12	13	14	15	16	17
Engraulidae					4		1	1	2
Chanidae									1
Gonostomatidae		7							
Phosichthyidae			12			1			1
Stomiidae			2						
Chlorophthalmidae			1						
Paralepididae			1					1	
Neoscopelidae						3			1
Gempylidae		4	11	2		9		9	4
Bregmacerotidae									
Ophidiidae							1		
Carapidae						1			
Holocentridae				1		2		5	20
Caproidae						2			1
Hemiramphidae		3	7	2	5	15	6	4	7
Exocoetidae		1	3		2	5		1	4
Scorpaenidae						1			
Triglidae									
Platycephalidae									
Dactylopteridae						2			3
Serranidae			1		1			3	4
Priacanthidae					4		1	1	6
Apogonidae									
Coryphaenidae		1				20	3		8
Carangidae		2244	1		21	10	25	118	95
Bramidae							1	1	
Emmelichthyidae			1					1	
Lutjanidae		9			13	1	21	4	19
Gerreidae									
Lethrinidae						1			
Sillaginidae									
Mullidae					3			1	50
Pempheridae					1		1		2
Chaetodontidae									2
Pomacentridae		1				5	4	3	1
Teraponidae					7	14			5
Oplegnathidae							7		
Kyphosidae									2
Nomeidae						1		2	
Polynemidae							1		
Labridae									
Uranoscopidae									1
Blenniidae									
Gobiidae					1		1		6
Siganidae						1			
Acanthuridae									1
Sphyraenidae			1			1		4	6
Gempylidae			2						1
Trichiuridae			1						
Scombridae									
	Katsuwonus pelamis	20	7					19	
	Thunnus albacares	42	117	2	4		18	172	16
	Thunnus orientalis	40	2		12	17		16	40
	Thunnus sp.								
	Auxis spp.	14			6	2	17	16	5
	Acanthocybium solandri		2						
	Gymnosarda unicolor								
	Scombridae sp.		2			1		3	
Bothidae									
Soleidae									
Cynoglossidae									1
Monacanthidae						1		2	3
Ostraciidae									2
Tetraodontidae			1		12	19	2	5	4
Unidentified		17	75	3	43	17	118	44	134
Total		2403	250	10	139	152	228	436	458

Table 5. List of the larvae collected in stations of the Sea of Japan in 2017

Family	Species	St. ID								
		18	19	20	21	22	23	24	25	26
Engraulidae		321	569	2305	153	964	49	119	14	10
Chanidae										
Gonostomatidae										
Phosichthyidae										1
Stomiidae										
Chlorophthalmidae										
Paralepididae										
Neoscopelidae										
Gempylidae										
Bregmacerotidae										
Ophidiidae										
Carapidae										
Holocentridae										
Caproidae										
Hemiramphidae							1	1		
Exocoetidae		3	2		0					
Scorpaenidae										
Triglidae					1					
Platycephalidae										
Dactylopteridae										
Serranidae										
Priacanthidae										
Apogonidae			1							
Coryphaenidae										
Carangidae					2		1			
Bramidae										
Emmelichthyidae										
Lutjanidae		2								
Gerreidae										
Lethrinidae										
Sillaginidae							2			
Mullidae		4			0	1		8		5
Pempheridae										
Chaetodontidae										
Pomacentridae					3		4			
Teraponidae										
Oplegnathidae										
Kyphosidae										
Nomeidae										
Polynemidae										
Labridae										
Uranoscopidae										
Blenniidae			2							
Gobiidae										
Siganidae										
Acanthuridae										
Sphyraenidae			6		4	17	26	10		
Gempylidae										
Trichiuridae										
Scombridae										
	Katsuwonus pelamis									
	Thunnus albacares									
	Thunnus orientalis	24	1	28	0	1	0	133	60	3
	Thunnus sp.									
	Auxis spp.	1	2	22	0	3	1	6	0	0
	Gymnosarda unicolor									
	Acanthocybium solandri									
	Scombridae sp.	12	1	39				1		
Bothidae										
Soleidae										
Cynoglossidae										
Monacanthidae							2			
Ostraciidae										
Tetraodontidae			2				3			
Unidentified		31	66	5	30	42	8	11		3
Total		398	652	2399	193	1028	97	289	74	22

Table 6. List of the larvae collected in stations of the Sea of Japan in 2018

Family	Species	St. ID					
		27	28	29	30	31	32
Engraulidae		4	1	5	3	103	1
Chanidae							
Gonostomatidae							
Phosichthyidae							
Stomiidae							
Chlorophthalmidae							
Paralepididae							
Neoscopelidae							
Gempylidae							
Bregmacerotidae							
Ophidiidae							
Carapidae							
Holocentridae							
Caproidae							
Hemiramphidae							
Exocoetidae							
Scorpaenidae							
Triglidae							
Platycephalidae							
Dactylopteridae							
Serranidae							
Priacanthidae							
Apogonidae					1		
Coryphaenidae					6		1
Carangidae					1		
Bramidae							
Emmelichthyidae							
Lutjanidae							
Gerreidae							
Lethrinidae							
Sillaginidae							
Mullidae							
Pempheridae							
Chaetodontidae							
Pomacentridae							
Teraponidae							
Oplegnathidae							
Kyphosidae							
Nomeidae							
Polynemidae							
Labridae						1	
Uranoscopidae							
Blenniidae			1				
Gobiidae							
Siganidae							
Acanthuridae							
Sphyraenidae				2		5	
Gempylidae							
Trichiuridae							
Scombridae							
	Katsuwonus pelamis						
	Thunnus albacares	1					
	Thunnus orientalis	2307	1	39	67	110	9
	Thunnus sp.	1					
	Auxis spp.	10	1	1	158	13	19
	Gymnosarda unicolor						
	Acanthocybium solandri						
	Scombridae sp.						
Bothidae							
Soleidae							
Cynoglossidae							
Monacanthidae							
Ostraciidae							
Tetraodontidae			1				
Unidentified		3	3	5	2	15	1
Total		2326	8	52	238	247	31

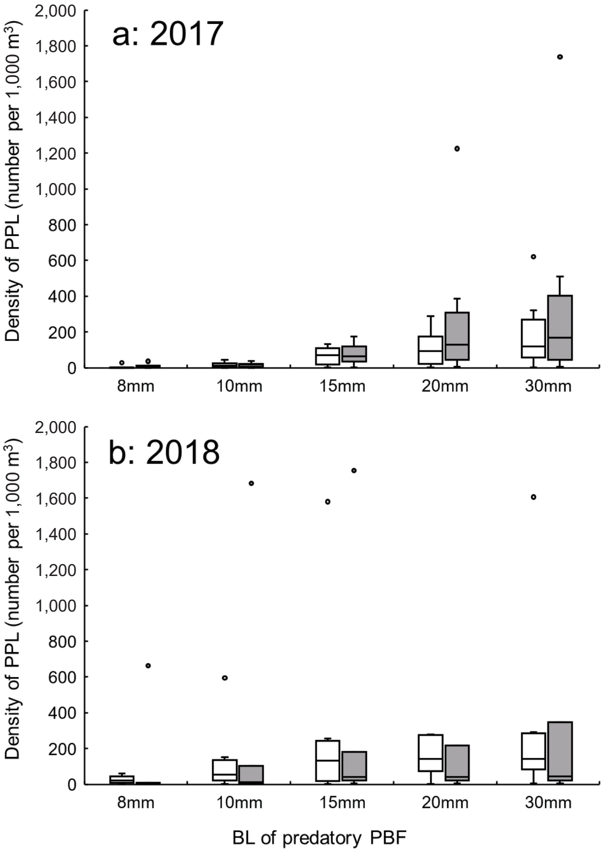

Fig. 4. Box plots of the densities (number / 1,000 m^3) of the potential prey larvae (PPL) collected in the Nansei Islands area (open box) and the Sea of Japan (grey box) for predatory Pacific bluefin tuna (PBF) of five size groups

The upper and lower panels show the data collected in 2017 (a) and 2018 (b), respectively.

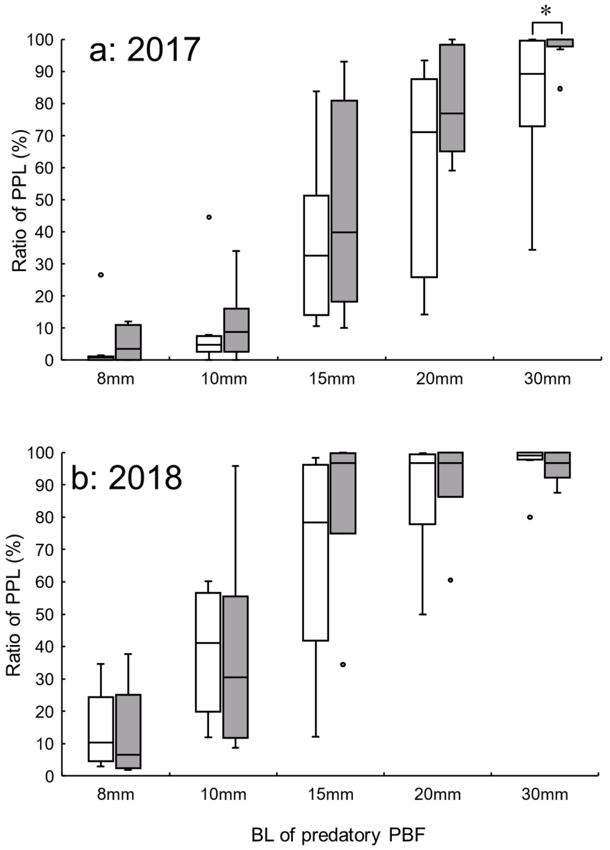

Fig. 5. Box plots of the percentages of the potential prey larvae (PPL) collected in the Nansei Islands area (open box) and the Sea of Japan (grey box) for predatory Pacific bluefin tuna (PBF) of 5 size groups

The upper and lower panels show the data collected in 2017 (a) and 2018 (b), respectively.

* indicates significantly different at $P < 0.05$ (U-test).

4. Density and ratio of larval PBF as potential prey larvae

The density and percentage of potential prey PBF for predatory PBF of the five size classes are shown in Figures 6 and 7, respectively. The density of potential prey PBF in the Nansei Islands area was lower than 20 fish/1,000 m^3 in both years. On the other hand, in the Sea of Japan, the density of potential prey PBF increased in size classes larger than a BL of 15 mm of predatory PBF. Significant differences in the density of potential prey PBF between the two areas were detected in the 10 and 15 mm size classes of predatory PBF in 2018 ($U = 11$, $P < 0.05$ for 10 mm size class, $U = 7$, $P < 0.05$ for 15 mm size class).

The percentage of potential prey PBF in the Nansei Islands area was very low through all size classes of predatory PBF in both years, as well as the density. In the Sea of Japan, the percentage of potential prey PBF increased for larger size classes than 15 mm BL of predatory PBF, particularly in 2018, which were induced by extremely high densities of PBF in St. 27. Significant differences in the density of potential prey PBF between the two areas were detected in the 10 to 30 mm size class of predatory PBF in 2018 ($U = 9$, $P < 0.05$ for 10 mm size class, $U = 0$, $P < 0.01$ for 15 mm size class, $U = 0$, $P < 0.01$ for 20 mm size class, $U = 1$, $P < 0.01$ for 30 mm size class).

Discussion

The densities and percentages of PPL for PBF larvae smaller than 15 mm BL were very low. However, those for PBF larvae and juveniles with a BL larger than 20 mm increased with their growth. Particularly, our results indicated that predatory PBF with a BL of 30 mm could potentially ingest more than 90% of the cohabiting larvae. These results suggest that prey availability could change drastically for PBF larvae and juveniles with a BL larger

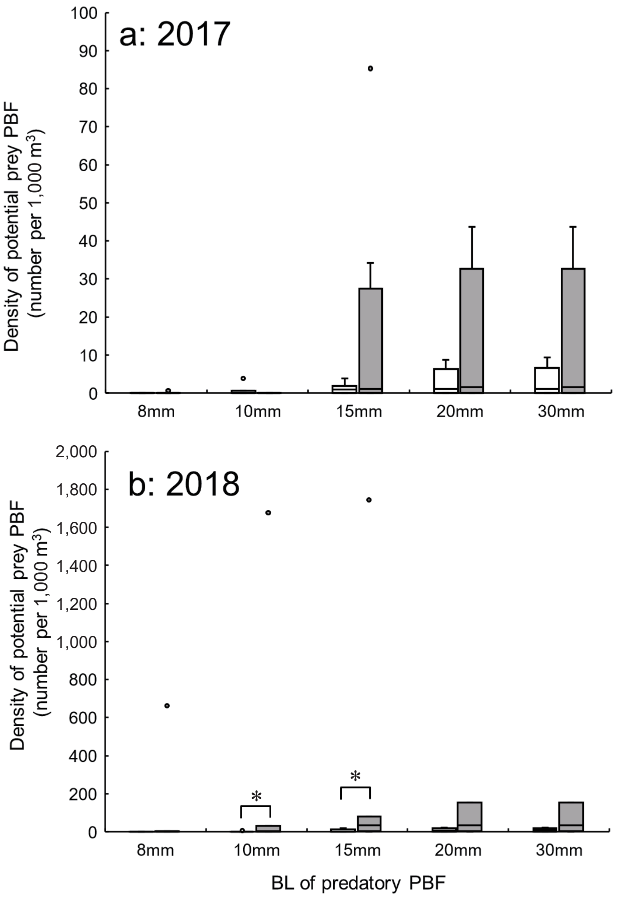

Fig. 6. Box plots of the densities (number / 1,000 m³) of the potential prey Pacific bluefin tuna (PBF) collected in the Nansei Islands area (open box) and the Sea of Japan (grey box) for predatory PBF of 5 size groups
The upper and lower panels show the data collected in 2017 (a) and 2018 (b), respectively.
* indicates significantly different at $P < 0.05$ (U-test).

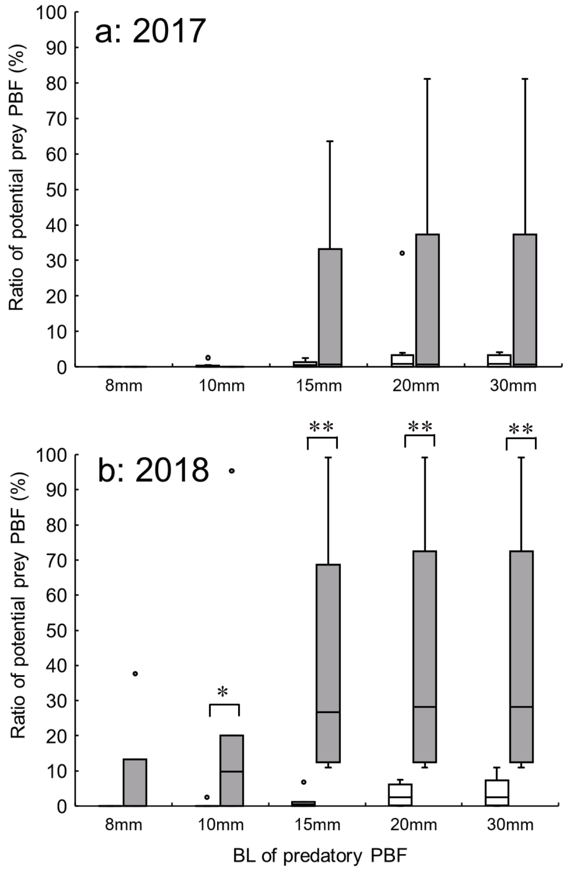

Fig. 7. Box plots of the percentages of the potential prey Pacific bluefin tuna (PBF) collected in the Nansei Islands area (open box) and the Sea of Japan (grey box) for predatory PBF of 5 size groups
The upper and lower panels show the data collected in 2017 (a) and 2018 (b), respectively.
* indicates significantly different at $P < 0.05$ (U-test).
** indicates significantly different at $P < 0.01$ (U-test).

than 20 mm. For laboratory-reared PBF larvae, the digestive system developed eco-physiologically in the early larval stage, and PBF larvae have switched their prey organisms from zooplankton to fish larvae (Tanaka et al. 1996, Miyashita et al. 1998, Tanaka et al. 2014a). Our findings indicate that the prey availability in the field for PBF larvae smaller than 15 mm BL could be low up to 20 mm BL. Accordingly, PBF larvae in the field may have to utilize zooplankton as prey even after sufficient development of the digestive system to enable piscivory.

PBF larvae show a high growth performance after the shift of feeding habits from zooplanktivory to piscivory (Tanaka et al. 2014a, Tanaka et al. 2014b). Furthermore, it has been reported that the high growth performance in the early life period contributes to their consequent survival and recruitment success (Tanaka et al. 2006, Watai et al. 2017, Ishihara et al. 2019). Accordingly, prey availability for PBF with a BL around 20 mm in relation to piscivory could play an important role in their recruitment processes, which induce interannual variability in recruitment. We observed the high density of Engraulidae in the Sea of Japan in 2017, Carangidae in the Nansei Islands area in 2018, and PBF in the Sea of Japan in 2018 (Tables 4, 5, 6). In such environments, the density of PPL greatly increased for predatory PBF with a BL larger than 15 mm, suggesting that the high density of larvae provided suitable prey availability.

In the present study, we estimated the prey availability for PBF from the larval assemblage collected using a ring net with 0.334 mm mesh aperture by sea surface tow. The following two criteria should be considered when estimating the availability of prey. One is the vertical distribution of PBF larvae and juveniles. PBF larvae were vertically distributed mainly in the surface layer from 0 to 10 m depth in the Nansei Islands area and in the surface layer in the Sea of Japan (Kodama et al. 2020b). Juvenile PBF ranging from 100 mm-200 mm in FL are also distributed in the surface layer (Mohri et al. 2005, Tanaka et al. 2020). Since PBF larvae and juveniles with a BL from 8 to 30 mm examined in this study would also be distributed in the surface layer, the prey organisms collected by sea surface tows can be indices of prey availability for PBF larvae and juveniles. The other criterion is newly hatched larvae as prey. Usually, it is very difficult to collect newly hatched larvae because they can pass through this mesh size, or the bodies of newly hatched are destroyed due to attrition in the net. The density of PPL for PBF larvae and juveniles could be underestimated since laboratory-reared PBF larvae could feed on newly hatched larvae with a BL from 8 mm (Tanaka et al. 2014a). Accordingly, the density of PPL for PBF of all size classes examined may be higher than we estimated.

It has been reported that cannibalism was observed in larval Atlantic bluefin tuna *Thunnus thynnus* (Uriarte et al. 2019) and southern bluefin tuna *Thunnus maccoyi* (Young & Davis 1990) and that cannibalism in Atlantic bluefin tuna may influence the size structure and dynamics of the larval cohort based on a mathematical model (Reglero et al. 2011). However, there is no field evidence of cannibalism for PBF larvae and juveniles, although cannibalistic behavior has frequently been observed in hatchery tanks (Masuma et al. 2011, Ishibashi et al. 2013). In the case of PBF, our results indicate that the density of PBF larvae was much lower than the densities of other species. The importance of PBF larvae as prey for predatory PBF may be low relative to the PPL of other species. However, when predatory PBF encounter high-density areas of PBF larvae, cannibalism may occur due to the higher ratio of prey PBF larvae than other PPL, as observed at St. 27 in the Sea of Japan in 2018. Although the impact of cannibalism on the dynamics of larval PBF cohorts remains unclear, the degree of density may be an important factor for cannibalism in PBF.

In the present study, we estimated the potential prey availability for piscivorous PBF larvae and juveniles from an analysis of the size of their mouth. Feeding success could be influenced not only by mouth size but also by behavioral traits of the prey larvae related to the vulnerability and increase of catch ability of PBF with growth. We need field evidence of their feeding habits in relation to piscivory by collecting PBF larvae and juveniles around 10 mm - 30 mm in order to reveal the survival process of PBF in their early life stages, which can induce interannual variability in the recruitment of PBF.

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