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**Research activities for biology on reproduction, ageing,
growth and recruitment monitoring of Pacific bluefin
tuna by NRIFSF, Fisheries Research Agency of Japan**

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Introduction

National Research Institute of Far Seas Fisheries (NRIFSF) has been carrying out biological researches in order to provide biological information useful for the stock assessment and management of Pacific bluefin tuna (PBF). The researches, in progress or in planning at NRIFSF in cooperation with institutes of Fisheries Research Agency (FRA), prefectural fisheries research laboratories and fisheries colleges of Japan, provide biological knowledge of reproduction, ageing, growth and recruitment of PBF.

The first category is related to reproduction. The estimated major nursery areas of PBF are in the subtropical waters off the Nansei Islands and in the Sea of Japan, and the spawning season in these areas are estimated to be from April to June and from June to August, respectively. However, details of locations and environmental and/or biological factors of spawning and nursery areas have not been specified yet. In addition, adequate method to identify the nursery area of the PBF is not yet established, though it is one of the most important factors to improve the accuracy and precision of the stock assessment. The second one is related to ageing and growth. The new growth parameters were estimated in recent years (Shimose et al, 2009), which are to be more practical because of wider coverage of ages and lengths than those reported in previous studies. On the other hand, the growth of the age-0 to 1 remain less accurate (Ichinokawa, 2008). Therefore the daily ageing study is needed to improve the information of the growth of young fish. Besides the information necessary to improve the stock assessment, identification of the timing when the first year ring is formed is of interest. The daily age information may also be useful to distinguish the fish derived from different nursery areas (Itoh, 2009). The last one is related to recruitment. The estimation of recruitment level at earlier stage either from fishery independent or dependent survey may be of particular benefit for better stock assessment and management of the stock.

1. Localization of nursery area

1-1. PBF larva and juvenile sampling survey

The NRIFSF has conducted studies the early life history of PBF in order to understand the mechanisms controlling the recruitment. Recent studies by NRIFSF clarified the biological attributes of the larvae; back-calculated standard lengths by the biological intercept method showed that only larger and faster growing larvae were able to survive to the postflexion phase (Tanaka *et al.* 2006). RNA/DNA ratio analyses suggested that the nutritional condition of the wild larvae was influenced by the ambient prey density, and starvation itself and starvation-induced predation could greatly affect to increase the mortality (Tanaka *et al.* 2008). From larval patches trajectory studies, the larval instantaneous mortality rates were estimated to be between 0.06 and 2.75 day⁻¹ (Satoh *et al.* 2008) and larval patches (within an approximate 10 km range) were entrained in mesoscale eddies (~100 to 500 km diameter) which propagated westward around the Nansei Islands (Satoh 2010). In contrast, wild PBF juveniles have rarely been studied since collection of specimens are very few.

However in the research cruise of the RV *Shunyo-Maru* of NRIFSF in 2009, the sampling gear for the juveniles was changed from the pelagic trawl (NST-99-K1, Nichimo Co., Tokyo Japan) to the LC net (LC-100m2R3, Nichimo Co., Tokyo Japan). As a result, 224 *Thunnus* spp. juveniles with 13-35 mm SL were collected and one third of them were identified genetically as *Thunnus orientalis* (Tanabe 2009). The LC net appeared to be more effective fishing gear for collecting smaller juveniles from 10 to 30 mm SL. In future study, the life history of juveniles is expected to be clarified as well as the larvae.

Those studies mentioned above were conducted around the Nansei Islands, NW Pacific. In 2010, the research cruise of the RV *Shunyo-Maru* will be carried out not only in NW Pacific but also in the Sea of Japan, another spawning area of the PBF. Moreover, the NRIFSF will collaborate with fisheries research institutes in Ishikawa, Tottori and Shimane prefecture for the larva and juvenile sampling survey.

1-2. Gonad sampling and observation of adult PBF

Since 1994, gonad samples of adult PBF were collected at several fishing ports in Japan by cooperative research activities of NRIFSF, Ishigaki Tropical Station of Seikai National Fisheries Research Institute (ITS, SNFRI), which is one of the branch of Fisheries Research Agency, and School of Marine Science and Technology of Tokai University (SMST, TU) under the financial support of Japan Fishery Agency. These investigations were usually accompanied with measurement of gonad weight at landing port for estimation of the GSI and histological analysis for determination of maturity stages in detail. Okochi et al. (2008) reported maturity and spawning of PBF based on 2680 ovary specimens collected by longline boats from the Nansei Islands and by purse seine boats from the Sea of Japan and offshore of east Honshu from 1994 to 2003. Their sampling had been carried out until 2006. They are preparing manuscripts of the reproductive biology of PBF in the subtropical waters off the Nansei Islands and in the Sea of Japan. Aonuma et al. (2008) reported maturity of PBF based on specimens collected by small longline boats at Ishigaki fishing port during 2008 fishing season. Their sampling activities have been continued in the years of 2009 and 2010. Histological analyses of these gonad samples are carried out in cooperative study by ITS, SNFRI and NRIFSF. Detailed histological analyses are carrying out by National Research Institute of Fisheries Science (NRIFS) since 2009, in order to determine individual experience of spawning activity after the spawning period. These reports indicated that most of adult PBF caught in the Nansei Islands during May-June and those caught in the Sea of Japan during June-July were consisted of matured individuals and the estimated spawning period started from early May and from early June, respectively. A new research activity by Japanese scientists is in planning stage for collection of fishing data, measurement data and gonad samples of adult PBF at main landing port of Japan.

1-3. Association between results of gonad observation and fisheries logbook data to specify the nursery area location

The subtropical waters around the Nansei Islands and the Sea of Japan are the most important fishing grounds for longline and purse-seine boats, respectively, targeting matured PBF which is gathering for reproduction. Both fisheries are supposed to start their operation from the beginning of the spawning season. Basic biological measurements such as fork length and body weight are carried out at main landing ports during the fishing season, and gonad samples are collected from some of fish to examine gonad somatic index (GSI) and histological observation described in above section.

Association of the logbook information and the result of gonad examination expected to

provide some findings to clarify the reproductive behavior of PBF. Because the logbook submitted by those fisheries contain detail information of every operation such as date, location, surface-water temperature, and depth of gear; the analysis of these data are very valuable to examine reproductive behavior of PBF. Actually, for the products of survey on longline fishery, the relationship among the gonad maturity and the records of logbook, such as fishing location and water temperature, were considered in the previous study (Okochi et al., 2008). The previous study also showed the evidence that the gonad maturity stage among individuals caught in the same purse-seine operation were almost synchronized, but associating with the logbook is not conducted (Okochi et al., 2008).

NRIFSF started attempts to figure out spatial-temporal distribution of matured fish by associating logbook information and biological data from this year.

1-4. Natal origin determination for age-0 PBF by otolith microchemistry analysis

Otolith microchemistry of the age-0 fish was measured to assess differences in composition among two spawning areas in the NW Pacific and Sea of Japan. Our preliminary results showed higher Zn concentration of the fishes caught in the Pacific side, inferred to be hatched in NW Pacific. In 2010, we have been developing analysis technique, and had prospect to select appropriate elements and determine the concentration at each daily age. In Atlantic Bluefin tuna stable isotope analyses of otolith core was demonstrated to be very useful to determine natal origin of the fish. Similar technique has been tested as joint study project of National Taiwan University and NRIFSF. Preliminary results are very promising.

2. Age determination and growth

2-1. Large fish

Otoliths of large PBF caught from Japanese waters have been collected since 1990's by NRIFSF. Shimose et al. (2009) carried out age determination of 808 individuals of lengths from 47 to 260 cm FL that were collected from 1992 to 2008 (Table 1). The estimated age of these specimens ranged from 1 to 26 years. They reported the von Bertalanffy growth function with parameters of 249.6 cm FL, 0.173 and -0.254 years for the asymptotic length, growth coefficient and theoretical age at length 0, respectively. Otoliths from 508 individual PBF were collected in 2009, and the sampling activities are intended to otolith with sex information from year 2010. Enough number of specimens is expected be sampled to examine the sex difference of growth in coming few years.

2-2. Age-0 fish

Daily age determination for age-0 fish based on otolith microstructure have been undergoing from year 2008. The 0-age PBF tuna caught in the Japanese coasts showed several modes in the size, especially in winter. The estimated date of fertilization could be divided to before and after July and they were corresponded to the fish hatched in NW Pacific and Sea of Japan (Tanaka *et al.* 2007; Itoh 2009). To elucidate more detail growth pattern (i.e. comparison of growth rate among differential spawning/nursery area, annual and seasonal fluctuation in growth rate, daily age composition of the catch, and validation of the timing at first yearly ring formation). Collecting otolith specimens of 0-age and young (1-2 age) fish from various locations over 12 months have been undergoing, 502 and 785 specimens are obtained in year 2008 and 2009, respectively. Some of specimens had already been analyzed.

3. Fishery independent survey on age-0 PBF by troll gear

Troll fishery targeting age-0 PBF for farming is operated in Tosa Bay during summer. Troll survey, a fishery-independent survey, started in the summer of 2008 as collaborative research project of NRIFSF and Kochi Prefectural Fisheries Experimental Station under financial support of Japan Fishery Agency and deployed two vessels in the western part of Tosa Bay (Fig. 3). This survey aims to gain abundance index of age-0 PBF that migrates to Tosa Bay during summer from the subtropical water off Nansei Islands. In this survey, fixed transect lines are set in research area where water depth is shallower than 200 m. Two chartered fishing boats track the lines with trolling (Fig. 4). This survey provides number of fishing per unit distance (e.g. nautical mile), which is expected to be utilized as an abundance index, and knowledge on distributional pattern of age-0 PBF in coastal area. Further development of protocol of this survey and expansion of the survey to other area where age-0 PBF migrates are proposed in order to develop a recruitment abundance index through this survey.

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Table 1
 Sampling details for the otolith of Pacific bluefin tuna, *Thunnus orientalis*, referred from Shimose *et al.* (2009)

No.	Fishing port	Fishing gear	Year	Month	<i>n</i>	Fork length (cm)
1	Bikuni, Hokkaido	Set net	2000	Oct.	10	62–75
2	Yoichi, Hokkaido	Set net	2002, 2003	Oct.	7	61–66
3	Sawara, Hokkaido	Longline, set net	2006	Aug.	2	229–234
4	Toi, Hokkaido	Longline+?	2006, 2007	Aug.–Dec.	30	142–260
5	Matsumae, Hokkaido	Pole and line	2001, 2003	Nov.	7	69–78
6	Ohma, Aomori	Longline, Hand line	2006, 2007	Aug., Sep., Dec.	126	119–259
7	Ishinomaki, Miyagi	Purse seine, set net	1998–2000, 2003, 2004	Aug.–Nov.	50	54–175
8	Shiogama, Miyagi	Purse seine	1997, 2000, 2002, 2006	June, July	44	60–190
9	Ryotsu, Niigata	Set net	2004	June	1	131
10	Himi, Toyama	Set net	2001	Jan.	1	103
11	Sakaiminato, Tottori	Purse seine	1992, 1998–2007	Mar., June–Aug, Dec.	64	100–223
12	Hagi, Yamaguchi	Troll	2003	Nov.	3	85–87
13*	Fukuoka	Purse seine	2003	July	12	46–55
14	Katsumoto, Nagasaki	Troll?	2007, 2008	Feb., Nov., Dec.	15	95–160
15	Tsushima, Nagasaki	Pole and line	1998	Nov.	1	71
16	Katsuura, Wakayama	Longline	2007, 2008	Mar.–June	53	158–245
17	Abratsu, Miyazaki	Longline	2007, 2008	May, June	11	175–224
18	Naha, Okinawa	Longline	2007	May, June	96	183–240
19	Itoman, Okinawa	Longline	2007, 2008	Apr.–July	11	203–240
20	Tungkang, Taiwan	Longline	2006, 2007	May, June	258	183–255
	R.V. Kurosaki	Longline	2002	May	1	177
	R.V. Taikei-Marui	Longline	2000	May, June	5	129–206
Total					808	46–260
*: Landing port						

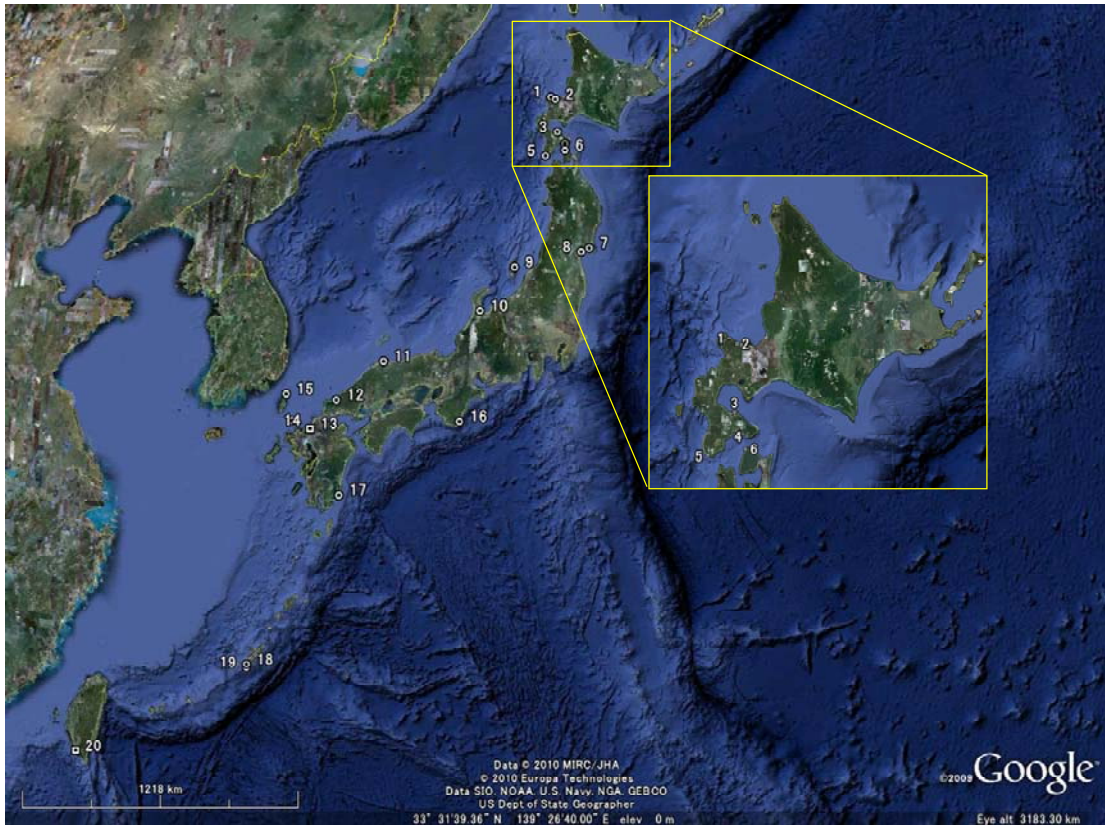


Fig. 1 Map of fishing ports for Pacific bluefin tuna, *Thunnus orientalis*. The numbers correspond to the numbers in Table 1.

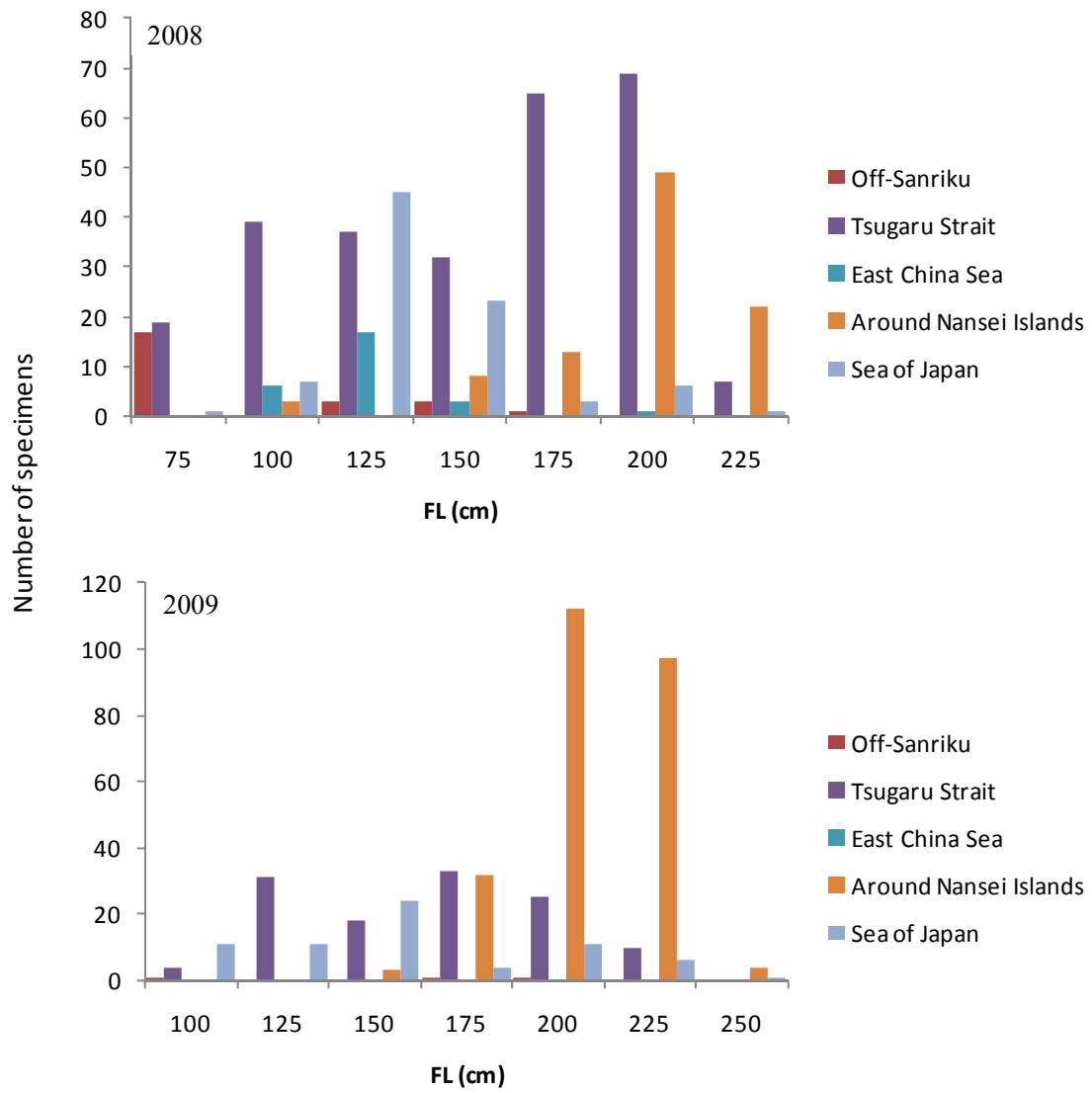


Fig. 2 Size frequencies and fishing area of Pacific bluefin tuna, *Thunnus orientalis* collected from 2008 to 2009 (> 75 cm Fork length).

Table 2

Sampling details for the otolith of Pacific bluefin tuna *Thunnus orientalis* from 2008 to 2009
(< 75 cm Fork length).

Year	Sampling area	FL(cm)	Month												Total number
			J	F	M	A	M	J	J	A	S	O	N	D	
2008															
Sea of Japan side	20-30									10	48	8	2	68	
	30-40									1	12	24	1	38	
	40-50						16	3				35	18	72	
	50-60							9				2	32	43	
	60-70							3						3	
Pacific side	10-20							4	1					5	
	20-30							27	14			30		71	
	30-40											100		100	
	40-50											71	6	77	
	50-60											4	23	27	
2009															
Sea of Japan side	10-20										12			12	
	20-30									67	2	39	2	110	
	30-40									31		6	9	46	
	40-50	10				16	4					68	15	113	
	50-60	14		16		11	14					32	38	125	
Pacific side	10-20							45	11					56	
	20-30							96	119					215	
	30-40								4	13	6			23	
	40-50						2			5	33	27		67	
	50-60		3		4									7	
2010															
Sea of Japan side	30-40	2												2	
	40-50	10												10	
	50-60	46												46	
Total number			82	3	16	4	27	36	187	247	43	144	412	135	1336

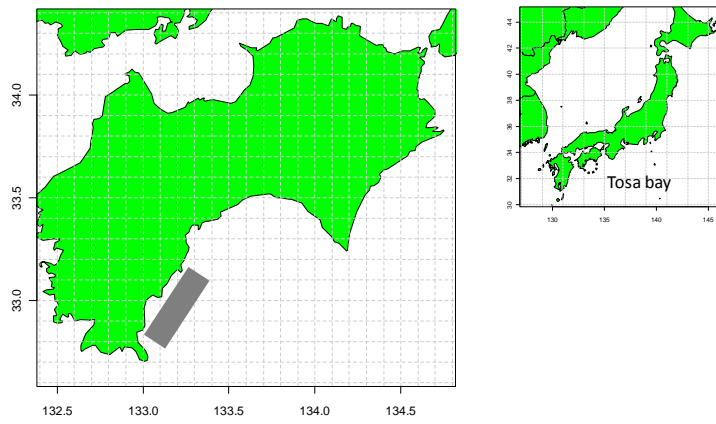


Fig. 3 Location of research area for troll survey in Tosa Bay. Area colored in grey indicates the research area.

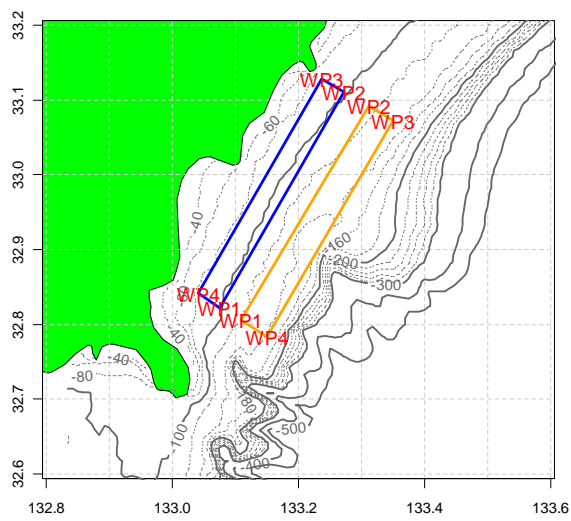


Fig. 4 Fixed transect lines of troll survey set in research area.