Annual Report of the LaMer Project FY 2018

1. Title:

Utilization of Samples Stored in es-Bank for Assessment of Trace Elements in Fish Collected from Indian Ocean of Southern Java Waters, Indonesia.

2. Members' Names and Affiliations:

a. Prof. Tatsuya Kunisue, Division of Environmental Chemistry and Ecotoxicology, CMES, Ehime University, Japan
b. Dr. Muhammad Ilyas, Laboratory for Marine Survey Technology, BPPT, Indonesia
c. Ir. Thomas Hidayat, Ministry for Marine and Fisheries, Indonesia

3. Aim:

Utilize samples stored at es-Bank to characterize the level of trace elements in fish and human risk assessment through fish consumption from Indian Ocean of Southern Java Waters, Indonesia

4. Procedure:

- Use of fish samples stored at es-Bank for retrospective study of 21 trace elements.
- Samples are specimen of fish collected from Indian Ocean of Southern Java Waters, Indonesia during 2015 with 97 total number of sample (*n*= 97), stored in es-Bank at Box Number 01-641 and Sample ID number as follows: INA-F15-001 (No. 001) to INA-F15-097 (No. 097). Sample can be accessed through http://esbank-ehime.com/dnn/.

5. Result:

- *Samples and Sampling*

  A total of 97 number of fish samples belong to 30 species and 21 genus of fish from Indian Ocean of Southern Java Waters, Indonesia during 2015 and stored in es-Bank of CMES, Ehime University, Japan were used in this study for analyzing of 21 trace elements.

  These fish samples were collected by trawl from 5 station trawls using RV. Baruna Jaya IV belong to Agency for the Assessment and Application of Technology (BPPT), Indonesia at fisheries management area of Indian Ocean of Southern Java Waters, Indonesia during 2015 through fish stock assessment project by Ministry of Marine and Fisheries, Republic of Indonesia.

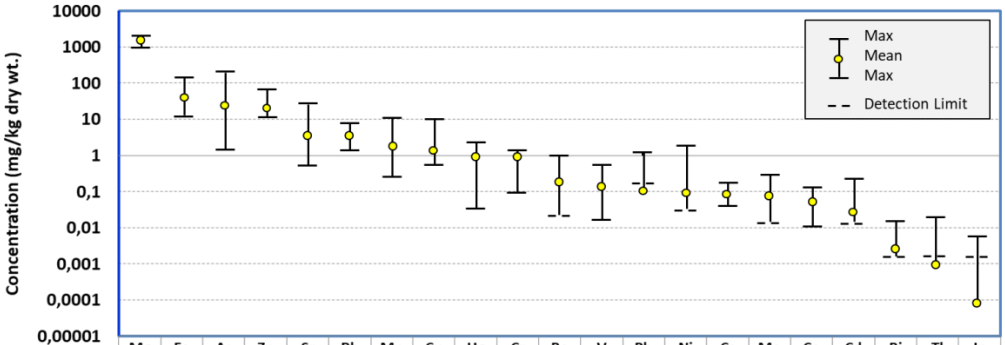
- *Chemical Analysis*

  Twenty one elements (V, Cr, Mn, Co, Cu, Zn, Se, Rb, Sr, Mo, Ag, Cd, In, Sn, Sb, Cs, Ba, Tl, Hg, Pb and Bi) were analyzed according to the method conducted by Ikemoto et al. (2004) with slight modification. Briefly, samples were dried at 80°C for 16 h and then uniformly homogenized into fine powder. Approximately 0.1 g of the dried powder sample was pre-digested with 3.5 ml of HNO3 at room temperature for overnight and then digested in a microwave oven with 1.5 ml of concentrated nitric acid. Twenty one targeted trace elements were then analyzed by using inductively coupled plasma-mass spectrometer (ICP-MS) and atomic absorption spectrometer (AAS). For elements of V, Cr, Mn, Co, Cu, Zn, Se, Rb, Sr, Mo, Ag, Cd, In, Sn, Sb, Cs, Ba, Tl, Pb and Bi were measured by ICP-MS (ICP-MS; HP4500, Hewlett-Packard, Avondale, PA, USA). Whereas, concentrations of Hg and Se were determined using an AAS (AA680, Shimadzu, Kyoto, Japan) equipped with a cold vapor system (Model HG-3000, Sanso, Tsukuba, Japan) and a hydride generation system (Model HFS-3, Hitachi, Tokyo, Japan), respectively.

  Accuracy of the elemental analysis was assessed using a certified standard reference material (Oyster tissue, SRM 1566b) provided by the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA). The results were in good agreement with the certified values with recoveries of all the elements ranged from 81–124%.

- *Results*

  All trace elements analyzed in this study were determined in the samples (*n*=97) belong to 30 species and 21 genus of fish collected from Indian Ocean of Southern Java Waters, Indonesia during 2015. Figure 1 shows concentration of trace elements (mg/kg dry wt.) detected in fish of the present study. Nutritional essential metals such as Mg, Fe, Zn, Mn, Cu were the predominant, whereas metals with no known benefial effects and considered toxics were also abundance such as As, Hg, and Sr.



	Mg	Fe	As	Zn	Sr	Rb	Mn	Cu	Hg	Cr	Ba	V	Pb	Ni	Cs	Mo	Co	Cd	Bi	Tl	In
Average	1500	41	22	20	3,9	3,6	1,6	1,2	0,56	0,31	0,16	0,13	0,091	0,091	0,077	0,063	0,042	0,025	0,002	0,000	8E-05
Minimum	850	11	1,3	11	0,49	1,4	0,25	0,49	0,039	0,085	0,00	0,016	nd	nd	0,034	nd	0,010	nd	nd	nd	nd
Maximum	1800	140	210	66	29	7,8	13	12	2,6	1,3	0,83	0,45	1,1	2,0	0,15	0,29	0,11	0,20	0,013	0,016	0,004

Figure 1. Concentrations of trace elements in fish (*n*=97) collected from Indian Ocean of Southern Java Waters, Indonesia during 2015.

Table 1. Concentrations of trace elements (mg/kg wet wt.) in fish of the present study compared to available for guideline standrads in Indonesia. Grey highlighted values exceed recommendations.

Station Trawl	Species	*n*	Moisture (%)	Concentration (mg/kg wet wt.)					
				Cd	Pb	Hg	As	Cu	Zn
FSA15_ST7	Longfin mojarra	10*	77	0,015	nd	1,2	8,4	0,38	15
	Goldband goatfish	12*	79	0,034	nd	0,98	9,1	0,77	11
	Yellowtail scad	5	74	0,032	0,051	0,21	1,5	0,55	9,3
	Shortfin lizardfish	3	78	0,0035	nd	0,42	2,0	0,46	15
FSA15_ST9	Shrimp	30*	72	0,11	nd	0,82	73	2,0	24
	Slender Conger	12*	75	0,081	nd	0,14	15	8,7	49
	Red bigeye	14*	76	0,010	nd	0,080	8,0	0,83	12
	Common hairfin anchovy	15*	67	0,064	0,418	0,27	2,1	0,96	16
	Yellowbelly Goatfish	13*	77	0,020	nd	1,3	7,3	0,91	11
	Bali sardinella	8*	77	0,11	nd	0,17	1,6	1,2	22
	Largescale tonguesole	3	79	0,0070	nd	0,27	29	0,49	13
	Black pomfret	3	77	0,016	nd	0,032	8,1	0,57	15
	Large-scale croaker	3	78	nd	0,070	0,34	13	0,58	12
FSA15_ST11	Shrimp	40*	75	0,023	nd	0,10	18	7,6	45
	Slender Grouper	1	71	nd	nd	1,3	0,90	0,40	19
	Oranyefin Ponyfish	40*	68	0,14	nd	0,028	1,9	1,0	18
	Smalleye Tonguesole	5*	75	nd	0,55	0,15	32	0,54	14
	Slender Conger	2*	80	0,031	nd	0,60	84	0,97	19
	Yellowbelly Goatfish	8*	75	0,011	nd	1,9	10	0,87	12
	Indian Halibut	1	81	nd	nd	0,62	5,6	0,44	12
	Japanese Threadfin Bream	4	77	nd	0,13	0,40	13	0,62	13
	Roughback sea catfish	3	77	nd	nd	0,84	134	0,58	20
	Bigeye Hairtail	3	74	0,0036	0,53	0,29	2,6	0,91	18
	Largefin Croaker	5	75	0,005	0,038	0,47	18	0,66	11
FSA15_ST12	Common hairfin anchovy	20*	66	0,059	nd	0,28	2,2	0,90	12
	Tardoore	1	72	0,022	0,24	0,37	1,5	0,70	18
	Smalleye Tonguesole	4	76	nd	0,13	0,48	28	0,60	16
	Shortfin lizardfish	3	76	0,0086	0,14	0,54	3,0	0,47	14
	Coitor croaker	1	72	0,021	0,36	0,44	5,7	0,63	14
	Donkey croaker	3	77	0,0060	0,16	0,092	13,7	0,72	15
	Big-head pennah croaker	3	78	0,015	0,078	0,23	5,8	1,0	14
FSA15_ST14	Bigeye Hairtail	4*	75	0,025	nd	0,13	2,3	2,1	25
	Yellowbelly Goatfish	5*	75	0,037	nd	0,86	7,9	0,98	12
	Coitor croaker	8*	76	0,037	nd	0,49	5,1	0,69	15
	Common hairfin anchovy	12*	61	0,082	nd	0,12	2,7	0,73	6,9
	Oranyefin Ponyfish	30*	76	0,045	nd	0,29	3,6	0,88	29
	Kawakawa	4	72	nd	nd	0,44	3,5	1,4	13
	Large-scale croaker	3	75	0,0041	nd	0,83	18	0,54	12
	Concertina fish	3	76	0,014	nd	0,57	2,1	0,66	13
	Smalleye Tonguesole	4	79	0,027	0,16	0,31	33	0,59	14
	Shortfin lizardfish	2	76	0,0074	nd	0,12	1,5	0,51	12
	Brushtooth lizardfish	3	77	0,0072	nd	0,12	13	0,55	14
	Roughback sea catfish	3	76	0,010	0,10	0,30	56	0,96	19
	Bludger	4	73	0,018	nd	0,44	7,4	1,1	13
	Blackspotted threadfin	3	76	0,026	nd	0,54	2,1	0,69	11
Guideline*		-	-	**0,10**	**0,30**	**0,50**	**1,0**	**20**	**100**

*Note: *Guideline standards proposed by National Standardize Agency and National of Food and Drug Agency of Indonesia.*

In this study, as shown in Table 1, fish were variably suffered of excesses of As, Hg, Cd and Pb with respect to reference guidelines. The concentration of As (0.90–130 mg/kg wet wt.) and Hg (0,028-1,9 mg/kg wet wt.) in most cases higher than thresholds values for maximum limit of metals in fish proposed by National Standardize Agency and National of Food and Drug Agency of Indonesia. Furthermore, some few fish also excedeed guideline for Cd and Pb (Table 1).

Table 2. Values of target hazard quotient (THQ), total target hazard quotient (TTHQ) and lifetime cancer risk (TR) of trace elements calculated for fish of the present study. Grey highlighted values exceed recommendations.

Site	Spesies	THQ (Non Carcinogen Risk)												TTHQ	TRs (Carcinogen Risk)		
		V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Hg	Pb	TTEs	As	Cd	Pb
ST7	Goldband goatfish	0,0083	0,00034	0,0082	0,046	0,013	-	0,0033	1,1	1,3	0,074	20	-	23	5,7E-04	4,5E-04	-
	Longfin mojarra	0,013	0,00034	0,011	0,061	0,017	0,0084	0,0016	1,6	1,2	0,031	24	-	27	5,3E-04	1,9E-04	-
	Shortfin lizardfish	0,0071	0,00023	0,0082	0,035	0,0058	0,0035	0,0020	1,6	0,28	0,0075	8,5	-	10	1,2E-04	4,6E-05	-
	Yellowtail scad	0,0087	0,00027	0,0098	0,035	0,0080	0,0036	0,0024	1,0	0,21	0,069	4,3	0,028	5,6	1,1E-03	2,4E-04	1,3E-06
ST9	Large-scale croaker	0,012	0,00041	0,0089	0,062	0,019	0,0046	0,0025	1,3	1,9	-	7,0	0,038	10	8,4E-04	-	1,2E-06
	Black pomfret	0,011	0,00023	0,0077	0,053	0,0087	0,0064	0,0025	1,6	1,2	0,034	0,70	-	3,5	5,1E-04	2,0E-04	-
	Largescale tonguesole	0,012	0,00024	0,0090	0,043	0,016	0,0016	0,0021	1,4	4,1	0,015	5,6	-	11	1,8E-03	9,1E-05	-
	Bali sardinella	0,019	0,00012	0,0105	0,067	0,014	-	0,0051	2,4	0,22	0,24	3,5	-	6,5	1,0E-04	1,5E-03	-
	Yellowbelly Goatfish	0,012	0,00012	0,0090	0,14	0,016	-	0,0039	1,2	1,0	0,043	26	-	29	4,6E-04	2,6E-04	-
	Common hairfin anchovy	0,026	0,00026	0,034	0,060	0,032	0,0057	0,0041	1,7	0,30	0,14	5,6	0,23	8,2	1,3E-04	8,4E-04	7,4E-06
	Red bigeye	0,013	0,00014	0,069	0,060	0,015	-	0,0036	1,3	1,2	0,023	1,6	-	4,2	5,0E-04	1,4E-04	-
	Slender Conger	0,021	0,00039	0,015	0,030	0,017	0,0057	0,038	5,3	2,1	0,17	2,9	-	11	9,3E-04	1,1E-03	-
	Shrimp	0,028	0,00060	0,146	0,054	0,031	0,011	0,0088	2,6	10	0,25	16,9	-	30	4,6E-03	1,5E-03	-
ST11	Shrimp	0,054	0,00088	0,016	0,058	0,013	0,0000	0,0329	4,9	2,6	0,050	2,0	-	10	1,2E-03	3,0E-04	-
	Largefin Croaker	0,016	0,00021	0,0091	0,070	0,014	0,0023	0,0029	1,2	2,6	0,011	9,6	0,021	14	1,1E-03	7,0E-05	6,8E-07
	Bigeye Hairtail	0,013	0,00028	0,018	0,094	0,017	0,0043	0,0039	1,9	0,38	0,0077	6,0	0,29	8,7	1,6E-04	4,7E-05	9,4E-06
	Roughback sea catfish	0,0078	0,00011	0,0073	0,0445	0,0133±	0,0079	0,0025	2,1	19	-	17	-	39	8,4E-03	-	-
	Japanese Threadfin Bream	0,015	0,00021	0,0105	0,0867	0,0173	0,0039	0,0027	1,4	1,9	-	8,2	0,073	12	8,1E-04	-	2,4E-06
	Indian Halibut	0,017	0,00025	0,0098	0,0563	0,0060	0,0046	0,0019	1,3	0,80	-	13	-	15	3,5E-04	-	-
	Yellowbelly Goatfish	0,0094	0,00015	0,0079	0,0787	0,0149	0,0000	0,0037	1,3	1,5	0,023	39	-	42	6,4E-04	1,4E-04	-
	Slender Conger	0,018	0,00022	0,0501	0,0626	0,0286	0,0071	0,0042	2,1	12	0,068	12	-	27	5,2E-03	4,1E-04	-
	Smalleye Tonguesole	0,021	0,00037	0,0156	0,0876	0,0146	0,0115	0,0023	1,5	4,6	-	3,0	0,30	10	2,0E-03	-	9,8E-06
	Oranyefin Ponyfish	0,021	0,00034	0,0239	0,0563	0,0140	0,0000	0,0043	1,9	0,27	0,29	0,60	-	3,2	1,2E-04	1,8E-03	-
	Slender Grouper	0,0081	0,00028	0,0080	0,0630	0,0090	0,0000	0,0017	2,1	0,13	-	27	-	29	5,6E-05	-	-
ST12	Big-head pennah croaker	0,018	0,00042	0,011	0,072	0,0193	0,0034	0,0045	1,5	0,84	0,032	4,7	0,042	7,3	3,7E-04	1,9E-04	1,4E-06
	Donkey croaker	0,013	0,00018	0,012	0,078	0,0188	-	0,0031	1,6	2,0	0,013	1,9	0,086	5,7	8,6E-04	7,9E-05	2,8E-06
	Coitor croaker	0,014	0,00014	0,0086	0,050	0,0428	0,0055	0,0027	1,5	0,82	0,045	9,0	0,19	12	3,5E-04	2,7E-04	6,3E-06
	Shortfin lizardfish	0,015	0,00028	0,0072	0,065	0,0085	0,0126	0,0020	1,5	0,43	0,019	11	0,074	13	1,9E-04	1,1E-04	2,4E-06
	Smalleye Tonguesole	0,030	0,00041	0,017	0,049	0,0170	0,0056	0,0026	1,8	4,0	-	9,8	0,068	16	1,8E-03	-	2,2E-06
	Common hairfin anchovy	0,035	0,00055	0,029	0,075	0,0224	0,0075	0,0039	1,3	0,31	0,13	5,8	-	7,7	1,4E-04	7,8E-04	-
	Tardoore	0,040	0,00057	0,017	0,043	0,0131	0,0062	0,0030	2,0	0,22	0,048	7,6	0,13	10	1,0E-04	2,9E-04	4,3E-06
ST14	Oranyefin Ponyfish	0,060	0,00033	0,023	0,11	0,0170	0,0076	0,0038	3,1	0,52	0,098	5,9	-	10	2,3E-04	5,9E-04	-
	Common hairfin anchovy	0,057	0,00055	0,029	0,15	0,0249	0,0090	0,0032	0,75	0,39	0,18	2,5	-	4,1	1,7E-04	1,1E-03	-
	Coitor croaker	0,063	0,00056	0,037	0,19	0,0440	0,0251	0,0030	1,7	0,73	0,080	10	-	13	3,2E-04	4,9E-04	-
	Yellowbelly Goatfish	0,041	0,00026	0,014	0,11	0,0251	0,0079	0,0042	1,3	1,1	0,080	18	-	20	5,0E-04	4,9E-04	-
	Bigeye Hairtail	0,054	0,00070	0,15	0,056	0,0241	0,0069	0,0089	2,7	0,32	0,054	2,7	-	6,0	1,4E-04	3,3E-04	-
	Blackspotted threadfin	0,043	0,00029	0,023	0,15	0,0215	0,0048	0,0030	1,2	0,30	0,056	11	-	13	1,3E-04	3,4E-04	-
	Bludger	0,044	0,00054	0,024	0,16	0,0200	0,0074	0,0046	1,4	1,1	0,039	9,0	-	12	4,6E-04	2,4E-04	-
	Roughback sea catfish	0,035	0,00075	0,022	0,14	0,0189	0,0287	0,0042	2,0	8,0	0,022	6,2	0,052	17	3,5E-03	1,4E-04	1,7E-06
	Shortfin lizardfish	0,017	0,00045	0,041	0,14	0,0161	0,0048	0,0022	1,3	0,21	0,016	2,5	-	4,2	9,1E-05	1,0E-04	-
	Smalleye Tonguesole	0,060	0,00043	0,022	0,16	0,0273	0,050	0,0025	1,5	4,7	0,058	6,4	0,087	13	2,0E-03	3,5E-04	2,8E-06
	Concertina fish	0,029	0,00043	0,015	0,078	0,0192	0,0073	0,0028	1,4	0,30	0,030	12	-	14	1,3E-04	1,8E-04	-
	Kawakawa	0,0055	0,00016	0,0056	0,071	0,0050	-	0,0061	1,4	0,50	-	9,1	-	11	2,2E-04	-	-

Note: THQ= Target Hazard Quation for Non Cancer Risk; TTHQ= Total Target Hazard Quation for Non Cancer Risk; TTEs= Total Trace Elements; TR= The Life Time Cancer Risk

Furthermore, target hazard quotient (THQ) and lifetime cancer risk (TR) indexes were calculated to assess cancer and non-cancer risk due to oral exposure based on formula by USEPA (1989). As shown in Table 2, calculation of target hazard quotient highlighted potential non-cancer risks associated with Hg, As and Zn exposure derived from consumption of fish from Indian Ocean Southern Java. The highest THQ values referred to Zn, Hg and As, with values ≥1 in 98%, 95% and 51% of cases, respectively. On the otherhand, cancer risk was mainly associated with As and Cd exposure, where the calculated TR index resulted in excess in more than 90% and in 80% of cases, respectively.

- *References*

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6. Publication/Conference presentation:

a. Currently preparing for a scientific paper. A tentative tittle and authorship are as follow: **Sudaryanto, A**., Ilyas, M., Itai, T., Tanabe, S., Kunisue, T. Level and Risk Assessment of Trace Elements in Fish from Indian Ocean of Southern Java Waters, Indonesia, In Preparation (Scientific Pubication).

b. The result finding has been presented as oral presentation in a national scientific conference in Indonesia. **Sudaryanto, A.**, Ilyas, M., Hidayat, T., Satria, F., Itai, T., Kunisue, T. 2018. Heavy Metals and Trace Elements in Fish from Indian Ocean of Southern Java Waters, Indonesia. 15th Annual Meeting of Indonesian Association for Oceanology (ISOI) - Pertemuan Ilmiah Nasional Tahunan (PIT) XV ISOI 2018, 1-3 November 2018, Eastparc Hotel, Yogyakarta, Indonesia (Oral Presentation).

7. **Perspectives in Future:**
   a. Elaborate more comprehensive on other contaminants including persistent organic pollutants (POPs)

(Abstract presented on 15th Annual Meeting of Indonesian Association for Oceanology (ISOI) 2018 - *Pertemuan Ilmiah Nasional Tahunan* (PIT) XV ISOI 2018, 1-3 November 2018, Eastparc Hotel, Yogyakarta, Indonesia. Oral Presentation, LaMer Project 2018).

Heavy Metals and Trace Elements in Fish from Indian Ocean Southern Java Waters

Agus Sudaryanto[1]*, Muhammad Ilyas[1], Thomas Hidayat[2], Fayakun Satriya[2], Takaaki Itai[3] and Tatsuya Kunisue[3]

[1]Balai Teknologi Survei Kelautan, Badan Pengkajian dan Penerapan Teknologi (BPPT), Jl. MH. Thamrin 8, Jakarta, Indonesia
[2]Balai Riset Perikanan Laut, Kementerian Kelautan dan Perikanan (KKP), Jl. Muara Baru Ujung, Komp. Pelabuhan Perikanan, Jakarta, Indonesia
[3]Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matsuyama 790-8577, Japan

*E-mail: agus.sudaryanto@bppt.go.id

Abstract

Heavy metals and trace elements have been released and distibuted widely on the earth both from natural and anthropogenic sources. The anthropogenic activities have contributed to significantly increase the amount of these elements into marine ecosystem which is thus of concern for environmental health risk. Fish may represent risks for human health since they are often contaminated by toxic elements from their environment. This study aims to determine and evaluate heavy metals and trace elements in marine fish from Indian Ocean, south of Java waters. Various fish samples were taken in September 2015 using bottom trawls by RV. Baruna Jaya IV. A total of 20 trace elements were analyzed from muscle fish using inductively coupled plasma-mass spectrometer (ICP-MS) and atomic absorption spectrometer (AAS) at the Center for Marine Environmental Studies (CMES), Ehime University, Japan. The levels of elements were following the order of Fe; As; Zn; Sr; Rb; Mn; Cu; Hg; Pb; Cr; Ba; Ni; V; Cs; Mo; Co; Cd; Tl; Bi and In, with average and range concentration (in ppm dry weight) of 41(11-144); 22(1.3-210); 20 (11-66); 3,9 (0.49-29); 3.6 (1.4-7.8); 1.6 (0.26-13); 1.2 (0.49-12); 0.56 (0.039-2.6); 0.44 (<0.25-1.05); 0.31(0.085-1.3); 0.17(<0.025-0.83); 0.14(<0.050-2.03); 0.13(0.016-0.45); 0,077 (0.034-0.15); 0.064(<0.013-0.29); 0.042(0.10-0.11); 0.041(<0.013-0.20); 0.007(<0.003-0.016); and 0,006(<0.003-0.013), respectively. In particular, some toxic metals were exceeded guideline for maximum limit such as As (1.0 ppm), Hg (0.50 ppm) and Pb (0.30) in fish proposed by National Standardize Agency and National of Food and Drug Agency. As for Cd, Cu and Zn were still bellow the guideline, while other elements are non-toxic.

Keywords: Contamination, heavy metals, trace elements, fish, Indian Ocean, health risk

Oral Presentation of the LaMer Project result at 15th Annual Meeting of Indonesian Association for Oceanology (ISOI) 2018 - *Pertemuan Ilmiah Nasional Tahunan* (PIT) XV ISOI 2018, 1-3 November 2018, Eastparc Hotel, Yogyakarta, Indonesia.

Collaboration by using storage samples in environmental specimen Bank (es-Bank)

Kind of samples used

Samples are fish samples collected from Indian Ocean of Southern Java Waters, Indonesia during 2015 with total of 97 number of sample (n= 97). The samples are stored in es-Bank at Box Number 01-641 (http://esbank-ehime.com/dnn/) and Sample ID number as follows:

Fish sample used and their Sample ID stored in es-Bank

No	Sample ID	No	Sample ID	No	Sample ID
1	INA-F15-001	34	INA-F15-034	66	INA-F15-066
2	INA-F15-002	35	INA-F15-035	67	INA-F15-067
3	INA-F15-003	36	INA-F15-036	68	INA-F15-068
4	INA-F15-004	37	INA-F15-037	69	INA-F15-069
5	INA-F15-005	38	INA-F15-038	70	INA-F15-070
6	INA-F15-006	39	INA-F15-039	71	INA-F15-071
7	INA-F15-007	40	INA-F15-040	72	INA-F15-072
8	INA-F15-008	41	INA-F15-041	73	INA-F15-073
9	INA-F15-009	42	INA-F15-042	74	INA-F15-074
10	INA-F15-010	43	INA-F15-043	75	INA-F15-075
11	INA-F15-011	44	INA-F15-044	76	INA-F15-076
12	INA-F15-012	45	INA-F15-045	77	INA-F15-077
13	INA-F15-013	46	INA-F15-046	78	INA-F15-078
14	INA-F15-014	47	INA-F15-047	79	INA-F15-079
15	INA-F15-015	48	INA-F15-048	80	INA-F15-080
16	INA-F15-016	49	INA-F15-049	81	INA-F15-081
17	INA-F15-017	50	INA-F15-050	82	INA-F15-082
18	INA-F15-018	51	INA-F15-051	83	INA-F15-083
19	INA-F15-019	52	INA-F15-052	84	INA-F15-084
20	INA-F15-020	53	INA-F15-053	85	INA-F15-085
21	INA-F15-021	54	INA-F15-054	86	INA-F15-086
22	INA-F15-022	55	INA-F15-055	87	INA-F15-087
23	INA-F15-023	56	INA-F15-056	88	INA-F15-088
24	INA-F15-024	57	INA-F15-057	89	INA-F15-089
25	INA-F15-025	58	INA-F15-058	90	INA-F15-090
26	INA-F15-026	59	INA-F15-059	91	INA-F15-091
27	INA-F15-027	60	INA-F15-060	92	INA-F15-092
28	INA-F15-028	61	INA-F15-061	93	INA-F15-093
29	INA-F15-029	62	INA-F15-062	94	INA-F15-094
30	INA-F15-030	63	INA-F15-063	95	INA-F15-095
31	INA-F15-031	64	INA-F15-064	96	INA-F15-096
32	INA-F15-032	65	INA-F15-065	97	INA-F15-097
33	INA-F15-033	*Detail samples can be seen at http://esbank-ehime.com/dnn/			

Date of Received the samples from *es*-BANK.

Samples of fish used in this study were received from es-Bank between the period of 31 May 2017 and 1 January 2018. Detail date of received the samples from es-Bank as below.

Date of received the samples from es-Bank.

No	Sample ID	Sample	Date of Sample Received from es-BANK
1	INA-F15-001	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
2	INA-F15-002	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
3	INA-F15-003	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
4	INA-F15-004	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
5	INA-F15-005	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
6	INA-F15-006	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
7	INA-F15-007	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
8	INA-F15-008	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
9	INA-F15-009	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
10	INA-F15-010	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
11	INA-F15-011	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
12	INA-F15-012	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
13	INA-F15-013	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
14	INA-F15-014	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
15	INA-F15-015	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
16	INA-F15-016	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
17	INA-F15-017	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
18	INA-F15-018	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
19	INA-F15-019	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
20	INA-F15-020	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
21	INA-F15-021	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
22	INA-F15-022	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
23	INA-F15-023	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
24	INA-F15-024	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
25	INA-F15-025	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
26	INA-F15-026	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
27	INA-F15-027	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
28	INA-F15-028	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
29	INA-F15-029	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
30	INA-F15-030	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
31	INA-F15-031	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
32	INA-F15-032	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
33	INA-F15-033	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
34	INA-F15-034	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
35	INA-F15-035	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
36	INA-F15-036	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
37	INA-F15-037	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
38	INA-F15-038	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
39	INA-F15-039	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
40	INA-F15-040	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
41	INA-F15-041	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23

42	INA-F15-042	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
43	INA-F15-043	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
44	INA-F15-044	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
45	INA-F15-045	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
46	INA-F15-046	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
47	INA-F15-047	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
48	INA-F15-048	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
49	INA-F15-049	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
50	INA-F15-050	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
51	INA-F15-051	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
52	INA-F15-052	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
53	INA-F15-053	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
54	INA-F15-054	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
55	INA-F15-055	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
56	INA-F15-056	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
57	INA-F15-057	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
58	INA-F15-058	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
59	INA-F15-059	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
60	INA-F15-060	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
61	INA-F15-061	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
62	INA-F15-062	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
63	INA-F15-063	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
64	INA-F15-064	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
65	INA-F15-065	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
66	INA-F15-066	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
67	INA-F15-067	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
68	INA-F15-068	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
69	INA-F15-069	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
70	INA-F15-070	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
71	INA-F15-071	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
72	INA-F15-072	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
73	INA-F15-073	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
74	INA-F15-074	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
75	INA-F15-075	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
76	INA-F15-076	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
77	INA-F15-077	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
78	INA-F15-078	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
79	INA-F15-079	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
80	INA-F15-080	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
81	INA-F15-081	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
82	INA-F15-082	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
83	INA-F15-083	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
84	INA-F15-084	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23

85	INA-F15-085	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
86	INA-F15-086	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
87	INA-F15-087	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
88	INA-F15-088	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
89	INA-F15-089	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
90	INA-F15-090	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
91	INA-F15-091	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
92	INA-F15-092	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
93	INA-F15-093	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
94	INA-F15-094	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
95	INA-F15-095	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
96	INA-F15-096	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23
97	INA-F15-097	Fish	2017/5/31, 2017/7/4, 2017/11/30, 2018/1/23