Thesis for the Degree of Master of Fisheries Science

Molecular Biological Analysis of Ichthyoplankton Collected from Ulleung and Dokdo Islands

by

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(울릉도와독도에서채집한어류플랑크톤의분자생물학적분석)

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A dissertation

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Abstract

The ichthyoplankton survey was conducted in the waters around Ulleung and Dokdo Islands in the months of March, May, August and November, 2016. Eleven stations were established along the area and samples were collected using a bongo net with 550µm mesh size that was double obliquely towed from surface to 100m. Fish larvae and eggs samples were sorted and counted using stereomicroscope and molecular approach was used for identification. Species name was assigned in samples with sequence identity of more than 98%. A total of 14 species were identified for fish eggs and among them, *Cololabis saira* (Pacific saury) and *Seriola quinqueradiata* (yellow tail) were commercially important species in Korea. Deep sea egg species including *Coelorinchus* sp. (grenadiers or rattails) and *Trachipterus trachypterus* (ribbonfish) were identified for the first time in this study and further studies are needed to verify their existence in the survey area. For fish larvae results, a total of 28 species were identified and *Scomber japonicus* (chub mackerel), *Scomber australasicus* (spotted mackerel), *Cololabis saira* (Pacific saury) and *Auxis rochei* (bullet tuna) were commercially important species. The occurrence of ichthyoplankton species during different months of survey suggests spawning period. However, further studies are still needed to verify and confirm this result.

Key words: molecular approach, ichthyoplankton, Ulleung Island, Dokdo Island.

Introduction

Ulleung and Dokdo Islands are located in the middle of the East/Japan Sea and the waters around them has been considered as an important fishing ground where in North Korea Cold Current (NKCC) from the north and East Korea Warm Current (EKWC) from the south collides, shedding eddies along the way (Ashjian et al., 2005; Talley et al., 2006). Collision of currents influences the mixing of waters causing high primary productivity in the area and making it ideal for some fisheries resources to thrive (Lim et al., 2012), therefore, the area has always been one of the main sources for the common squid, Pacific saury, anchovy, herring, and flatfishes. Moreover, catch in East/Japan Sea accounts for the 16% (range 10-23%) of the total Korean coastal and offshore fisheries in terms of annual production (Kim and Zhang, 2016). However, recent report showed that East/Japan Sea is considered to be one of the most highly affected area by the climate change in the world (Belkin, 2009). Winter sea surface temperatures in the area have increased by 1.6–2.4° C over the last century (Yamano et al., 2011). This rise in water temperature changed the ecosystem in East/Japan Sea in various ways including the occurrence of subtropical species (Kim and Zhang, 2016), extended area of coral reefs (Hoegh-Guldberg et al., 2017; Kim et al., 2010), and changes of major catch (Zhang et al., 2004) . In addition, climate change may affect the reproduction season as well as the change in the spawning and nursery grounds (Gucinski et al., 1990). Thus, it is required that a regular ichthyoplankton survey should be made for the effective management or conservation of the fisheries resources in East/Japan Sea.

Several ichthyoplankton surveys have been made to monitor the change of marine ecosystem mostly by the typical morphological identification (Kim and Choi, 2015; Lee et al., 2010b). However, morphological analysis of fish larvae and eggs by the stereomicroscopic observation requires trained experts in identification which needs considerable amount of time and efforts to verify results particularly in identification of the samples up to species level (Ahlstrom and Moser, 1996; Chamchang and Chayakul, 2000). Furthermore, morphological characteristics are often indistinguishable either by the damage during sampling and storage or by the similarity during early developmental stages (Chamchang and Chayakul, 2000; Ko et al., 2013; Strauss and Bond, 1990). Identification of fish eggs to the species level is almost impossible (Kawakami et al., 2010). Those have been a major obstacle for researchers to perform a large scale ichthyoplankton survey and molecular identification is now being paid attention as an alternative approach (Ward, 2000; Zhang and Hanner, 2012).

Although a regular ichthyoplankton surveys have been made in the waters around Ulleung and Dokdo Islands to monitor the ecological effects by the climate change, traditional morphological analysis requires a lot of time and effort, which is not suitable for the long-term surveys. The ultimate goal of this is to establish the strategy for the fast and accurate identification of fish larvae and eggs collected in the waters around Ulleung and Dokdo Islands by the combination of simple morphological sorting and counting and following molecular identification. These methods can provide the both qualitative and quantitative data about the ichthyoplankton, which would be useful information for the long-term changes in the marine ecosystem of East/Japan Sea.

Materials and Methods

1. Plankton sample collection

The fish larvae and eggs survey was supported by a grant from the Dokdo Center, National Institute of Fisheries Science (R2017029). A total of 11 stations were established in the waters around Ulleung and Dokdo Islands between coordinates $37^{o}00'$ - $37^{o}60'$N to $130^{o}00'$ - $132^{o}15'$ E (Fig. 1). Samples from 11 sampling sites were collected four times (March, May, August and November) in 2016. Plankton samples were collected using a Bongo net with a mouth diameter of 80 cm and mesh size of 550 μm, equipped with a flow meter. The net was double obliquely towed from surface to layer100 m depth and each tow was done with a speed of approximately 1-3 knots depending on the current for 15 minutes and wire position kept at 45^{o}angle. Collected samples were immediately placed in 5% formalin for an hour, washed and then transferred 1L polyethylene bottle with 99% ethanol.

1.1 Study Site

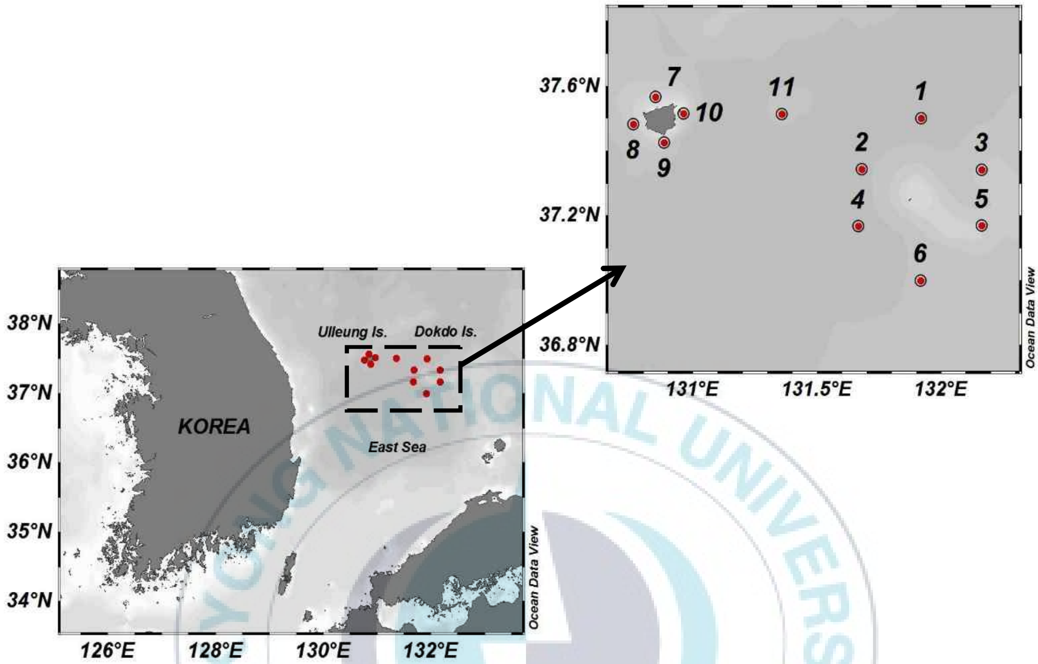


Fig. 1. Sampling stations around Ulleung and Dokdo Islands

Eleven sampling sites located between coordinates 37^{o}00' - 37^{o}60'N to 130^{o}00' - 132^{o}15' E around Ulleung and Dokdo Islands.

2. Morphological sorting and counting of fish larvae and eggs

Samples from each station were sorted to fish larvae and eggs and counted manually using a stereomicroscope (Olympus, SZX16). Sorted and counted fish larvae and eggs from each station were photographed individually (except during March period) for reference and then placed into a vial separately with 99% ethanol ready for molecular identification procedure. The collection of samples from study site, morphological analysis of samples and computation of densities per periods of surveys were based on the protocol on “Standard Techniques for Pelagic Fish Eggs and Larva Surveys” (Smith and Richardson, 1977) and Ocean Data View Version 4.10 was used for data interpretation (Schlitzer, 2015).

3. Molecular Identification of ichthyoplankton

Sorted samples from each station were physically disrupted separately using a homogenizer and a tissue lyser. After homogenizing, the samples were transferred into a 2ml tube with proteinase K and incubated at $60^{o}C$ for 12-16 hours ready for DNA extraction. DNA of sample was extracted using genomic DNA purification kit (Bioneer, Korea). Extracted genomic DNA was quantified using ND-1000 spectrophotometer (Thermo Scientific, USA) and stored at $-70^{o}C$ for later analysis.

Universal primers were used for the samples and PCR were performed using TP-600 (Takara, Japan). Thermal cycles used were 1 minute initial denaturation at $94^{o}C$, then 30 cycles with 30 sec $94^{o}C$, 30 sec $50^{o}C$, 30 sec $72^{o}C$ and final extension at $72^{o}C$ for 3 minutes were conducted. PCR products were separated by 1.5% agarose gel electrophoresis and stained with loading star (Dynebio, Korea). Amplicons of expected size were purified using the AccuPrep® Gel Purification Kit (Bioneer, Korea). All purified amplicons were sent to sequencing company (Macrogen, Korea) and BLASTn search assigned top matched description to samples for accurate identification. Species names were assigned for samples with more than 98% sequence identities and genus names were assigned for those with more than 77% sequence identity. Common name for each species was referred from fish base; www.fishbase.org (Froese and Pauly, 2012)

Results

1. Fish eggs abundance, distribution and composition

The results of the four surveys gave a total of 337 fish eggs collected from the waters around Ulleung and Dokdo Islands (Table 1).The highest numbers offish eggs (145) were collected in August which is equivalent to 1,263 inds./1000m^3 in density. Smaller numbers of eggs were identified in the other three sample periods including March, May, November whose ranges were between 59 and 73 (Table 1). There was no significant pattern in the spatial distribution of fish eggs which were observed to be unevenly distributed in all the stations during the samplingperiods (Fig. 2).

A total 14 different species of fish eggs were analyzed with 98 % nucleotide sequence identity (Table 2). Among 14 species identified by COI region, only one sequence showed 100 % nucleotide sequence identity to *Cololabis saira* (GenBank Accession Number: KF386407). Eight species including *Ditrema temminckii, Trachipterus trachypterus,* and *Upeneus japonicus* showed 99 % identity to GenBank database, whereas 5 species showed lower than

98 % identity (Table 2). Interestingly, one fish egg belonging to Genus Sebastes exhibited 99 % sequence identity to five species including *Sebastes pachycephalus, Sebastes thompsoni, Sebastes trivittatus, Sebastes koreanus and Sebastes steindachneri* and the exact species name cannot be determined based on the obtained sequence. One sequence belonging to Family Gobiidae collected in November showed only 77 % sequence identity to GenBank database.

The highest variety of fish eggs (8 species) were identified in March including *Coelorinchus* sp., *Ditrema temminckii*, *Gymnocanthus herzensteini*, *Icelus* sp., *Sebastes* sp., *Trachipterus trachypterus*, and *Trachipterus* sp. (Fig. 3). Only 4, 2, and 3 species were identified in the other three sample seasons, May, August, and November, respectively. Although the highest egg numbers were identified in August, only two small pelagic fish *Maurolicus* sp. and *Engraulis japonicus* were identified (Table 2). In particular, eggs of *Maurolicus* sp. were dominated in August (122) and November (53). The fish egg *Etruemeus teres* were predominately identified in May (Table. 2). The study showed that most diverse fish species spawn in early spring and only a few small pelagic fish species including *Engraulis japonicus, Etrumeus micropus* and *Maurolicus* sp. spawn during summer and fall (Fig. 3).

Table 1. Summary of fish eggs collected from Ulleung and Dokdo islands in 2016

No.	Fish Eggs Species	March	May	August	November
1	*Coelorinchus* sp.	8	0	0	0
2	*Cololabis saira*	0	22	0	0
3	*Ditrema temminckii*	10	0	0	0
4	*Engraulis japonicus*	0	0	23	0
5	*Etruemeus teres*	0	49	0	0
6	Gobiidae	0	0	0	3
7	*Gymnocanthus herzensteini*	10	0	0	0
8	*Icelus* sp.	14	0	0	0
9	*Maurolicus* sp.	0	0	122	53
10	*Sebastes* sp.	10	0	0	0
11	*Seriola quinqueradiata*	0	1	0	0
12	*Trachipterus* sp.	1	0	0	0
13	*Trachypterus trachypterus*	2	1	0	3
14	*Upeneus japonicus*	5	0	0	0
	Total count per month	**60**	**73**	**145**	**59**
	Equivalent density per month (inds./1000 m^3)	**741**	**704**	**1263**	**636**

Table 2. Molecular identification of fish eggs collected from Ulleung and Dokdo islands in 2016

Period	Scientific Name	Numbers	NCBI Accession No.	Identity (%)
March	*Coelorinchus* sp.	8	AP002929	89%
March	*Ditrema temminckii*	10	AP009129	99%
March	*Gymnocanthus herzensteini*	10	KX148474	99%
March	*Icelus* sp.	14	KT004432	95%
March	*Sebastes* sp.	10	KF836442	99%
March	*Trachypterus* sp.	1	AP002925	97%
March	*Trachypterus trachypterus*	2	AP002925	99%
March	*Upeneus japonicus*	5	AB355921	99%
May	*Trachypterus rachypterus*	1	AP002925	99%
May	*Etruemeus teres*	49	KU199135	99%
May	*Seriola quinqueradiata*	1	KU199150	99%
August	*Engraulis japonicus*	23	KY223630	99%
August	*Maurolicus* sp.	122	JX121820	90%
November	*Maurolicus* sp.	53	JX121820	90%
November	*Trachipterus trachypterus*	3	AP002925	99%
November	Gobiidae	3	KF415353	77%

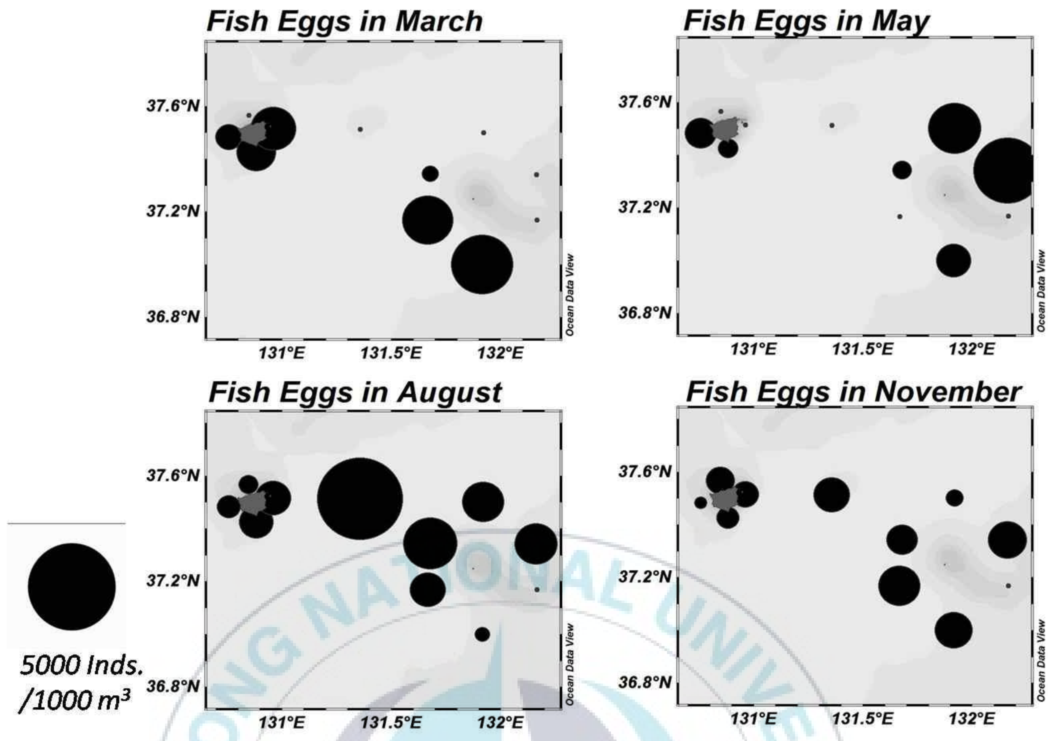


Fig. 2. Spatial and temporal distribution of fish eggs

Spatial and temporal distribution of fish eggs in March, May, August and November 2016.

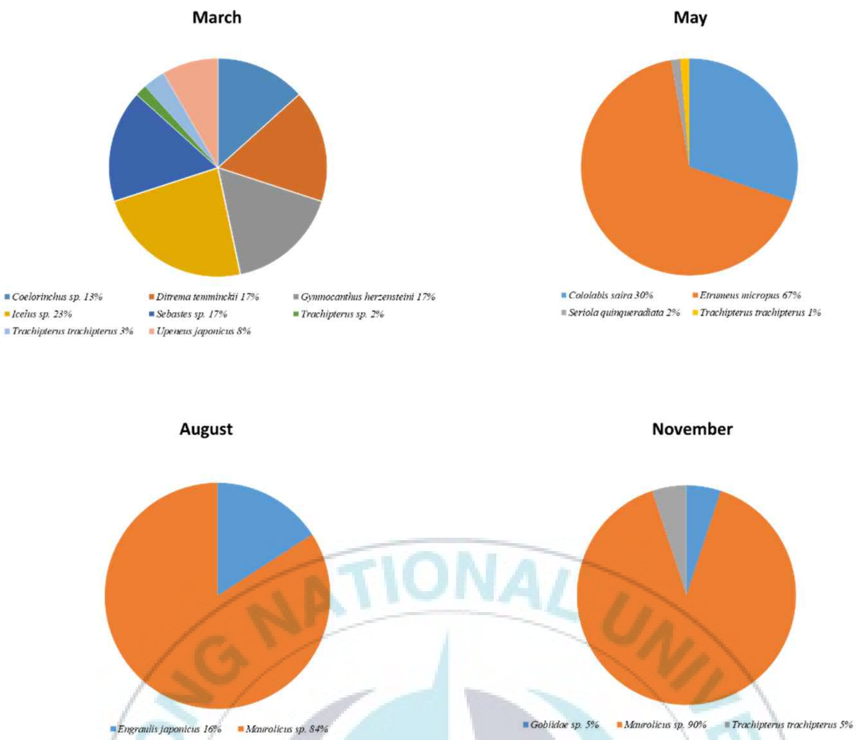


Fig. 3. Species proportions of fish eggs collected from Ulleung and Dokdo islands in 2016

2. Fish larvae abundance, distribution and composition

A totalof 2,937 fish larvae were collected from 11 sample sites in the waters around Ulleung and Dokdo Islands from March to November. More individuals of fish larvae were caught compared to fish eggs during the survey periods (Table 3). Although highest fish larval numbers were identified in August (2034) and May (852), most of them were only *Engraulis japonicus* and *Maurolicus* sp. The month of November, on the other hand, had 50 individuals while March had only one individual (Table 2).

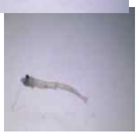
Twenty eight different species of fish larvae were identified from 11 sample sites during the survey periods. Among 28 species identified in this study, only 9 sequences showed 100 % identity to GenBank database which include *Cololabis saira*, *Parapercis descemfaciata*, *Thamnaconus modestus*, *Trachurus japonicus*, *Auxis rochei*, *Engraulis japonicus*, *Gerres oyena, Neobythites sivicola*, *Sillago japonica* (Table 4). Thirteen species showed 99 % sequence identity to the database, whereas genus names were assigned for fish larvae individuals with 84-97 % sequence identity which include *Bothus* sp., *Bregmaceros* sp., *Decapterus* sp., *Lutjanus* sp., *Maurolicus* sp., *Parapaglusia* sp., *Repomucenos* sp., and *Stethojulis* sp. (Table 4).

The highest variety of fish larvae was noted in August where in 17 species were identified, followed by May (12) and November (3) (Table 4). In March, only one larvae of *Maurolicus* sp. was identified .*Cololabis saira*, *Engraulis japonicus*, *Maurolicus sp*., and *Sebastes sp*. were some of the identified species present for both fish larvae and eggs during the survey periods (Table 2 and 4).

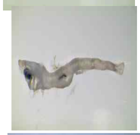
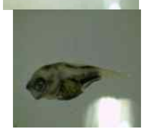
Table 3. Summary of fish larvae collected from Ulleung and Dokdo islands in 2016

No.	Fish Larvae Species	March	May	August	November
1	*Auxis rochei*	0	0	10	0
2	*Bothus* sp.	0	0	1	0
3	*Bregmaceros* sp.	0	0	3	0
4	*Callanthias japonicus*	0	4	0	0
5	*Callionymus enneactis*	0	2	0	0
6	*Cleisthenes herzensteini*	0	2	0	0
7	*Cololabis saira*	0	3	0	0
8	*Decapterus* sp.	0	0	1	0
9	*Engraulis japonicus*	0	713	1656	0
10	*Gerres oyena*	0	0	1	0
11	*Girella punctata*	0	1	0	0
12	*Glossanodon semifasciatus*	0	2	0	0
13	*Halichoeres tenuispinis*	0	0	3	0
14	*Lutjanus* sp.	0	0	1	0
15	*Maurolicus* sp.	1	105	340	44
16	*Neobythites sivicola*	0	0	1	0
17	*Parapercis descemfaciata*	0	3	0	0
18	*Paraplagusia* sp.	0	0	1	0
19	*Repomucenus* sp.	0	0	5	5
20	*Scomber australasicus*	0	0	1	0
21	*Scomber japonicus*	0	14	0	0
22	*Scorpaena onaria*	0	0	1	0
23	*Sebastes minor*	0	0	0	1
24	*Sebastes steindachneri*	0	0	4	0
25	*Sillago japonica*	0	0	2	0
26	*Stethojulis* sp.	0	0	3	0
27	*Thamnaconus modestus*	0	2	0	0
28	*Trachurus japonicus*	0	1	0	0
	Total count per month	**1**	**852**	**2034**	**50**
	Equivalent density per month (ind./1000 m^3)	**9.09**	**8322.89**	**19469.55**	**594.27**

Table 4. Molecular identification of fish larvae collected from Ulleung and Dokdo islands in 2016

Period	Scientific Name	Numbers	NCBI Accession No	Identity (%)	Photo
March	*Maurolicus* sp.	1	JX121820	90%	N/A
May	*Callanthias japonicus*	4	AP017451	99%	
May	*Callionymus enneactis*	2	AP012316	99%	
May	*Cleisthenes herzensteini*	2	KF386407	99%	
May	*Cololabis saira*	3	JQ354057	100%	
May	*Engraulis japonicus*	713	KY223630	99%	
May	*Girella punctata*	1	AP011060	99%	
May	*Glossanodon semifasciatus*	2	AP004105	99%	
May	*Maurolicus* sp.	105	KU199195	99%	

May	*Parapercis descemfaciata*	3	KU944764	100%	
May	*Scomber japonicus*	14	AB102724	99%	
May	*Thamnaconus modestus*	2	HM180917	100%	
May	*Trachurus japonicus*	1	JQ681512	100%	
August	*Auxis rochei*	10	KM651784	100%	
August	*Bothus* sp.	1	KJ433567	84%	
August	*Bregmaceros* sp.	3	AP004411	84%	
August	*Decapterus* sp.	1	KJ004518	84%	
August	*Engraulis japonicus*	1656	KF765500	100%	
August	*Gerres oyena*	1	AB355913	100%	
August	*Halichoeres tenuispinis*	3	EU082205	99%	

August	*Lutjanus* sp.	1	EF514208	95%	
August	*Maurolicus* sp.	340	JX121820	90%	
August	*Neobythites sivicola*	1	AB373006	100%	
August	*Paraplagusia* sp.	1	KF765500	82%	
August	*Repomucenus* sp.	5	AP012307	91%	
August	*Scomber australasicus*	1	AB102725	99%	
August	*Scorpaena onaria*	1	KR701907	99%	
August	*Sebastes steindachneri*	4	KJ834060	99%	
August	*Sillago japonica*	2	AP017438	100%	
August	*Stethojulis* sp.	3	AY850806	90%	
November	*Maurolicus* sp.	44	JX121820	90%	
November	*Repomucenus* sp.	5	AP012307	91%	

November	*Sebastes minor*	1	KJ834060	99%	

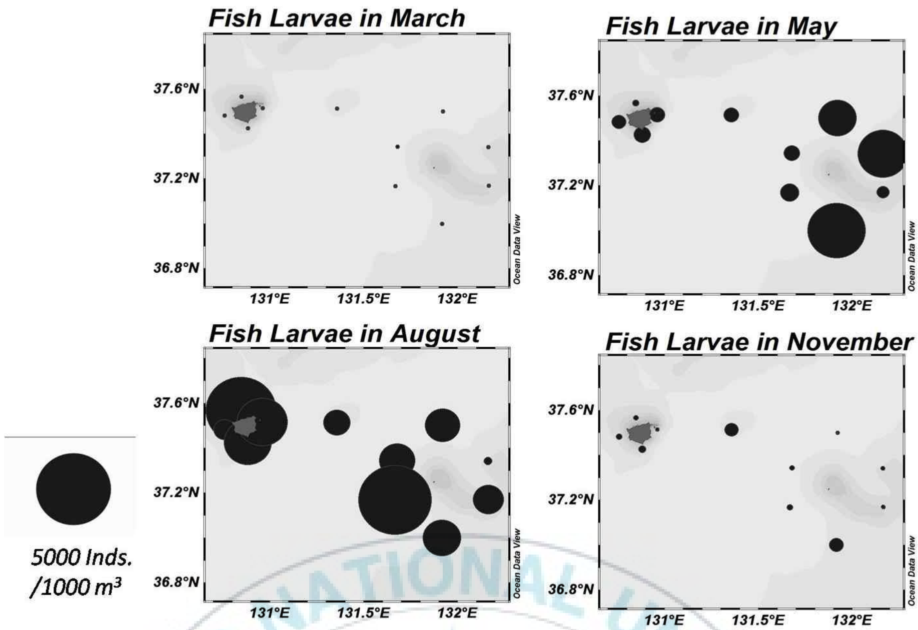


Fig. 4. Spatial and temporal distribution of fish larvae

Spatial and temporal distribution of fish larvae in March, May, August and November 2016.

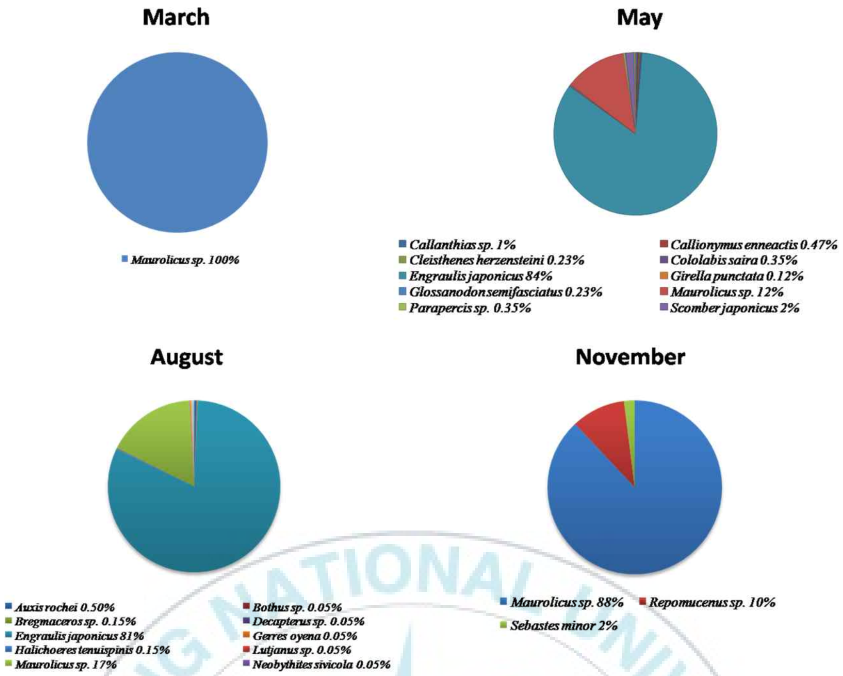


Fig. 5. Species proportions of fish larvae collected from Ulleung and Dokdo islands in 2016

Discussion

Fish larvae and eggs study is one of the methods in determining the fisheries productivity and fish species inventory of an area. Through this we can help establish management measures like implementation of closed season for fishing especially during spawning period to give chance for traditional fishing grounds to replenish its resources could be undergone(Casey et al., 2016). The islands of Ulleung and Dokdo has limited studies on fishes around its waters and additional studies regarding ichthyoplankton may better understand fisheries dynamics around the area (Kim and Choi, 2015).

Molecular approach to identification samples, which is an alternative method in identifying fish larvae and eggs was used in the study. Morphological sorting and counting per sampling sites of fish larvae and eggs greatly contributed to the quantitative analysis of samples in the study. The combination of morphological sorting and counting and following molecular identification can save the times and efforts for the ichthyoplankton survey, which enables researcher to perform a large scale survey. This method involves use of short, standard gene targets to create sequence profiles of known species against sequences unknowns that can be matched and identified (Zhang and Hanner, 2012). Universal primers for COI region were used in this study for molecular identification and the results yielded a significant species identification that may contribute to the spatial and temporal distribution of fish species inhabiting the waters around Ulleung and Dokdo Islands. However, we also identified many species collected in this study showed less than 98% sequence identity (Table 2 and 4). In present study, we were not able to identify 43 % of eggs and 29 % of larva to the species level.Previous morphological studies on ichthyoplankton particularly in Dokdo Island revealed the presence of unidentified species particularly for fish eggs (Kim et al., 2002; Lee et al., 2010b) and this is quite common for ichthyoplankton studies particularly for hard to identify species(Ahlstrom and Moser, 1996; Chamchang and Chayakul, 2000). In order to obtain a better result, a database of barcode for fish inhabiting in the area should be supplemented. Recently developed NGS technique can produce a high amount of data with a relatively low cost (Eiler et al., 2013; Lindeque et al., 2013), which enables large scale of genotype survey with a relatively low budget to supplement the barcode database.

1. Fish eggs occurrence in Ulleung and Dokdo Islands

Relatively high number of fish egg species were identified during the study compared to previous ichthyoplankton survey along the survey area (Kim et al., 2002; Lee et al., 2010b). Most of the species identified were coastal dwellers and subtropical species that are local inhabitants of the area and prefer to spawn where they usually live; in particular, the species M*aurolicus* sp. (dragon fish) that coincidentally found to be abundant in August and November, and may be indicating of long spawning period (Gjøsæter, 1981; Lee et al., 2010b). *Ditrema temminckii* (surf perch), *Gymnocanthus herzensteini* (sculpin) and *Sebastes* sp. (rockfish) are also some coastal dwellers that were present during March (Huh et al., 2013). Another inshore dwellers, the *Etruemeus teres* (round herring) is found to spawn all year round, except during summer, but was only seen in May during the study (Plaza, Sakaji, Honda, Hirota, & Nashida, 2007）. On the other hand, Japanese anchovy *Engraulis japonicus*, one of the most dominant species in Korean waters gave a significant number of eggs and was observed in the warm month of August (Lee et al., 2010a).

Fish egg results revealed highest variation of species during March and may indicate the preference of offshore and coastal fish with pelagic larvae to spawn during colder monthswhen surface wind drift is generally directed toward the coast (Boehlert and Mundy, 1988; Parrish et al., 1981) and prevailing winds or currents are at their weakest, thereby reducing long distance transport of larvae from where they originated(Johannes, 1978).

Warm water species *Cololabis saira* (Pacific saury) observed in May coincides with the previous studies which revealed that it was spawning all year round and heightened during March to June (Watanabe and Lo, 1989) and *Seriola quinqueradiata* (yellow tail) also observed in May are economically valuable species in the East Sea (Kim and Zhang, 2016).

Deep sea species breed seasonally but at different times of the year and spawning season cannot be related to spring plankton bloom (Merrett and Haedrich, 1997). Although eggs are spawned near the bottom, oil globules are present in the eggs to make it buoyant. During early life as pelagic larvae most deep sea species stay above the seasonal thermocline and descent in the benthic zone in later development (Haedrich and Merrett, 1988).

The presence of deep sea species *Coelorinchus* sp. (grenadiers or rattails) and *Trachipterus trachypterus* (ribbonfish) during the study maybe a new sighting in the waters near the islands that may need further investigation for future studies. Moreover, occurrence of *Trachipterus trachypterus* eggs, a typical Mediterranean and African species might be an indicator of increase sea surface temperature and local warming (Farias et al., 2010).

2. Fish larvae occurrence in Ulleung and Dokdo Islands

Several factors such as temperature, current and food source affect accumulation, growth and survival of fish larvae in a particular area and temperature plays a major role among these factors (Ahlstrom and Moser, 1996; Parrish et al., 1981). Fish tend to spawn during a period that ensures larvae rears at the right temperature usually during late spring to early autumn(Boehlert and Mundy, 1988). The sudden increase of temperature in East Sea influenced by global warming may cause shifting of native species in the area (Belkin, 2009) thus, monitoring of species inhabitant is essential to better understand the current status and to further know future management responses.

Ulleung Basin has been considered as a highly productive region compared to other areas in the East Sea and during summer where coincidentally highest abundance of fish larvae were observed in the study, eddy-induced upwelling of nutrients enhance high chlorophyll-a concentration (Kwak et al., 2013b; Yamada et al., 2004). This enhancement may in turn provide food source for fish larvae after yolk sac stage during that period. Moreover, primary production in Dokdo Island shows highest value in April and another small peak during November (Kwak et al., 2013a) hence may be a food source for fish larvae in the area.

Four fish species for both eggs and larvae were identified in the study including *Cololabissaira, Maurolicus* sp., *Engraulis japonicus*, and *Sebastes* sp. (Table 2 and 4). The study also revealed presence of *Maurolicus* sp. in all the months of the survey suggesting a long period of spawning for this species. The dominant species in this study, *Engraulis japonicus*, was found to be highest during the months of May and August and which the results coincides with previous fish studies in the area (Kim et al., 2002; Lee et al., 2010b). Waters around Ulleung and Dokdo Islands appears to be important species for spawning of two coastal fish species, *Sebastes* sp. *Cololabis saira* (saury), which are relatively abundant in East Sea (Kim and Zhang, 2016).

Many fish larva that were indentified in the survey area appeared to be transported from the southern china sea through the warm Tsushima Current originated from Kuroshio Current. A common silver-biddy (*Gerres oyena*) inhabits warm coastal waters of Jeju island and southern Kyushu and Okinawa, Japan and its spawning season is estimated between March and August (Kanak and Tachihara, 2008; Kim et al., 2014). Commercially important species, *Scomber japonicus* (chub mackerel), and *Scomber australasicus* (spotted mackerel) found during May and August in this study were said to be spawning around East China Sea and may be driven to the East Sea by the warm Tsushima Current(Yukami et al., 2009). *Auxis rochei* (bullet tuna) found during August and May respectively could confirm spawning periods for these species.

3. Significant findings

Morphological counting and sorting and molecular identification of fish larvae and eggs has the potential of producing a more accurate number of individuals or stocks and precise species identification that is a requirement for effective fisheries management. New species sighting of the deep sea species *Coelorinchus* sp. (grenadiers or rattails) and *Trachipterus trachypterus* (ribbonfish) during the survey may contribute to the East Sea fish species inventory, though further studies are still needed to verify their existence in the area. The occurrence of ichthyoplankton species during different months of survey suggests of a spawning period for these species. However further studies are still needed to verify and confirm this result.

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