Preliminary Report
of
The Hakuho Maru Cruise
KH-11-4,6

KH-11-4: May 20, 2011 - June 5, 2011
KH-11-6: June 24, 2011 - July 10, 2011
(Eel Cruise XVII)

Atmosphere and Ocean Research Institute
The University of Tokyo
2012

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Atmosphere and Ocean Research Institute
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2012

By
The Scientific Members of the Expeditions

Edited by
Shun Watanabe, Kazuki Yokouchi
and Katsumi Tsukamoto

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Preface

Progress of science is often not linear, but exponential. This is the case for research on the spawning area of the Japanese eel. The Ocean Research Institute of The University of Tokyo started conducting Eel Cruises using the R/V *Hakuho Maru* in 1973 to find where the Japanese eel spawns in the Pacific by organizing all eel biologists and some oceanographers in Japan, and then succeeded in outlining its spawning area in the North Equatorial Current by collecting almost a thousand tiny leptocephali in 1991, which was 18 years after we started the research.

After collecting more leptocephali in 1994 and 1995, attention shifted to the seamount chain of the West Mariana Ridge starting in 1998, when small leptocephali were collected near the northern seamounts. To further narrow down the estimated spawning area and predict an exact place and time where actual spawning behavior occurs, we proposed the Seamount Hypothesis and New Moon Hypothesis by analyzing all the historical collection data and hatching dates of leptocephali using otolith daily rings. Based on these two hypotheses, we successfully found preleptocephali a few days after hatching just west of the Suruga Seamount on the day of new moon in June 2005. Then 14 years after the discovery of the general spawning area in 1991, the first preleptocephali of the Japanese eel were collected. And, only three years later, the R/V *Kaiyo Maru* of the Fisheries Agency, Japan, also successfully caught adult eels just after their spawning near the Suruga Seamount and the southern tip of the seamount chain at new moon in 2008.

In the next year, 2009, after preleptocephalus collections in 2007 and 2008, the *Hakuho Maru* collected 31 eggs, and many preleptocephali just south of the salinity front near the southern end of the seamount chain two days before new moon, on 22 May. The eggs were confirmed genetically onboard to be the eggs that we had long been seeking for. Thus, we finally found a spawning event of the Japanese eel along the West Mariana Ridge during new moon, as we had estimated by the hypotheses.

Now, on 29 June 2011, two days before new moon, we successfully collected 147 eggs of the Japanese eel during this cruise at a crossing point of the seamount chain running north and south and a salinity front extending east and west at 13°N, which is the front that was hypothesized to affect the latitude of adult eel spawning and also crossed the seamount chain at around 13°N in May 2009. These timings and locations were predicted based on the above hypotheses and data from the previous cruises of the *Hakuho Maru*, and as a result, we could collect eggs on the fourth net tow at an early time during this cruise and validated the prediction scientifically. The depth distribution of eggs was then studied, which indicated that spawning occurs in shallower layers of about 150-200 m in waters of thousands of meters deep and not far below the surface as previously speculated. If considering such rapid progress in research on the spawning ecology of eels, we may be able to see the actual spawning behavior of eels during the darkness of new moon along the seamount chain of the West Mariana Ridge in the near future.

On behalf of all scientists on board, I sincerely thank the captain and his crew of the *Hakuho Maru* for their heartful cooperation. I also thank Ms M. Oya, Drs. T. Otake, T. Inagaki, T.-W. Lee, A. Fukui and other old eel cruise members who have been working with us, supporting and encouraging us continuously during this cruise and previous ones, and to all AORI and JAMSTEC staff who support the operations of the *Hakuho Maru*. Without their help, we could not come so far and so rapidly.

8 July 2011
In the cabin of *Hakuho Maru*

K. Tsukamoto

Katsumi Tsukamoto
Chief Scientist of KH-11-4,6

Station and Working log.: KH-11-4

2011	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
20-May (Fri.)										10:00 Leave Port Tokyo (Oi-huto) GMT+9					XCTD1			Passed Line 5a						
21-May (Sat.)	XCTD2 Passed Line 5b							Passed Line 5c												XCTD2				
22-May (Sun.)								Passed Line 5d																
23-May (Mon.)												Entered in 143-30 Line									St.1			
24-May (Tue.)			St.2										Cleared out 143-30 Line	8 shape rotation		St.3								
25-May (Wed.)					St.4					St.5				St.6				St.7				St.8		
26-May (Thu.)			St.9				St.10				St.11													
27-May (Fri.)	St.12				St.13			St.14				St.15				St.16				St.17			St.18	
28-May (Sat.)		St.19		St.20		St.21			St.22					St.23				St.24			St.25			St.26
29-May (Sun.)			St.27		St.28			St.29		St.30			St.31			St.32		St.33		St.34				St.33
30-May (Mon.)			St.35			St.36		St.37			St.38		St.39		St.40			St.41		St.42			St.43	
31-May (Tue.)		St.44		St.45			St.46		St.47			St.48			St.49			St.50		St.51		St.52		
1-Jun (Wed.)		St.53			St.54		St.55			St.56			St.57			St.58								
2-Jun (Thu.)																								
3-Jun (Fri.)														Passed Line 2d										
4-Jun (Sat.)															Passed Line 2c									
5-Jun (Sun.)				Passed Line 2b	Passed Line 2a							11:40 Arrivaled Port Tokyo (Harumi-huto)												

Station and Working log.: KH-11-6

2011	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
24-Jun (Fri.)										10:00 Leave Port Tokyo (Harumi-huto) GMT+9									Passed Line 5a					
25-Jun (Sat.)		Passed Line 5b							Passed Line 5c															
26-Jun (Sun.)										Passed Line 5d														
27-Jun (Mon.)													X1		X2		X3		X4		X5		X6	
28-Jun (Tue.)	X7		X8		X9		X10		X11				X12		X13		X14		St.1		St.2			St.3
29-Jun (Wed.)		St.4			St.4-2		C1	St.4-3	St.4-4		St.4-5	St.4-6	St.4-7		St.4-8	St.4-9		St.5		C2		St.6		C3
30-Jun (Thu.)		WC-1			C4	St.7		C5	St.8			St.9		C6	St.10			C7	St.11		C8			St.12
1-Jul (Fri.)		St.13			St.14		St.15			WC-2			St.16		St.17			St.18			St.19		St.20	
2-Jul (Sat.)	St.21		St.22		St.23		St.24		St.25			St.26		St.27		St.28		St.29				St.30		
3-Jul (Sun.)	St.31		St.32				St.33			St.34		St.35			St.36			St.37		Proton survey started	Passed P1	Passed P2	Passed P3	Passed P4
4-Jul (Mon.)	Passed P5	Passed P6		Passed P7	Passed P8		Passed P9	Passed P10		Passed P11	Passed P12	Passed P13	Passed P14	Passed P15	Passed P16	Passed P17	Passed P18	Passed P19	Passed P20	Passed P21	Passed P22	Passed P23	Passed P24	8 shape rotation Proton
5-Jul (Tue.)	survey finished St.38		St.39			St.40		St.41		St.42		St.43		St.44		C9	St.45		St.46		St.47		St.48	
6-Jul (Wed.)	St.49		St.50		St.51		St.52			St.53	St.54		St.55		St.56		St.57			C10	St.58			
7-Jul (Thu.)																								
8-Jul (Fri.)						Passed Line 6d																		
9-Jul (Sat.)																								
10-Jul (Sun.)																								

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Name	Affiliation
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KIMURA Shingo	Atmosphere and Ocean Research Institute, The University of Tokyo
AOYAMA Jun*	Atmosphere and Ocean Research Institute, The University of Tokyo
ZENIMOTO Kei	Atmosphere and Ocean Research Institute, The University of Tokyo
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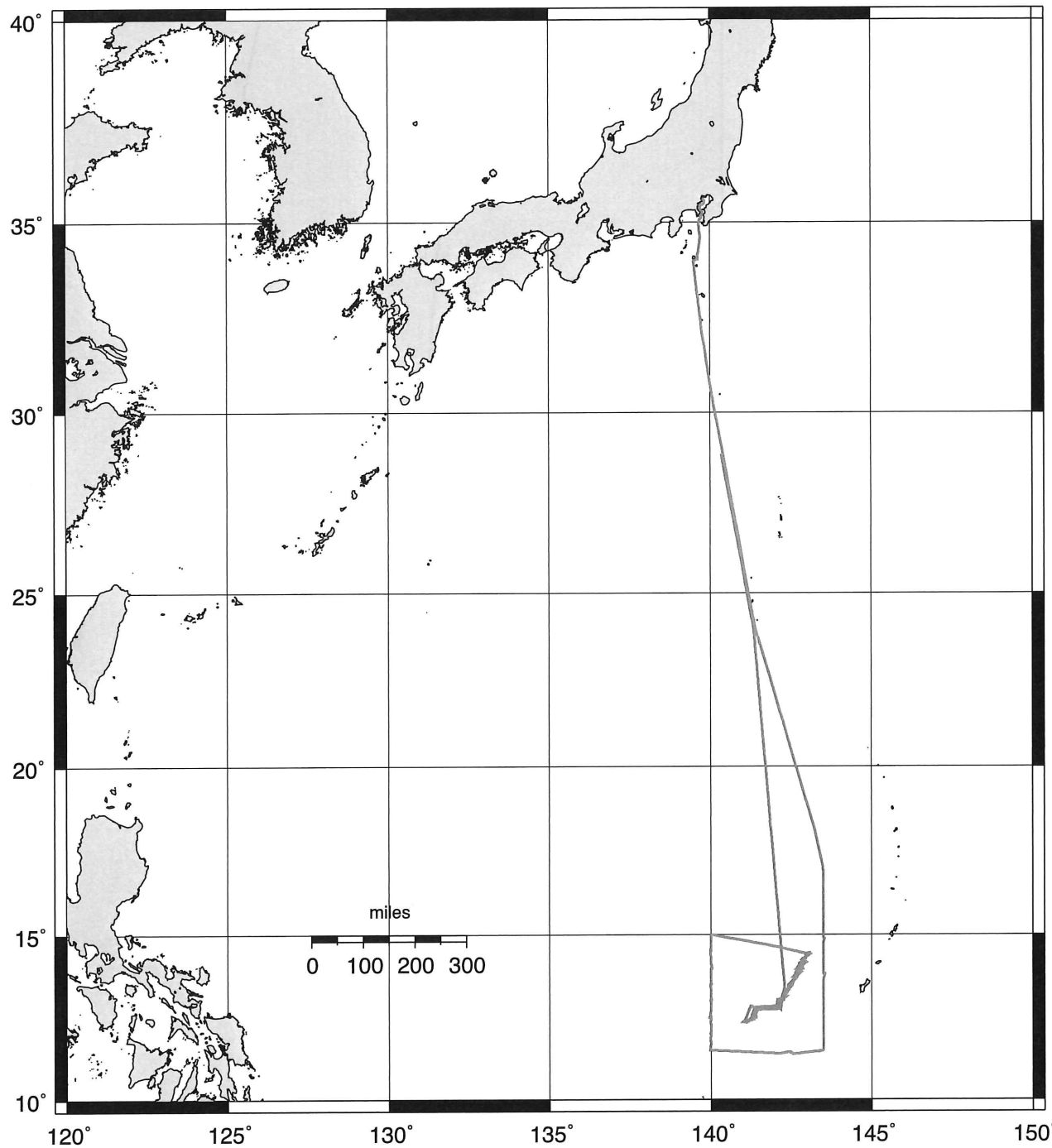
YOSHIJIMA Shigekane**	Japan Eel Society
HASHIDUME Tomoyuki**	Media Generalization Research Institute

* Onboard both KH-11-4 and 6; ** Onboard only KH-11-6.

KH-11-4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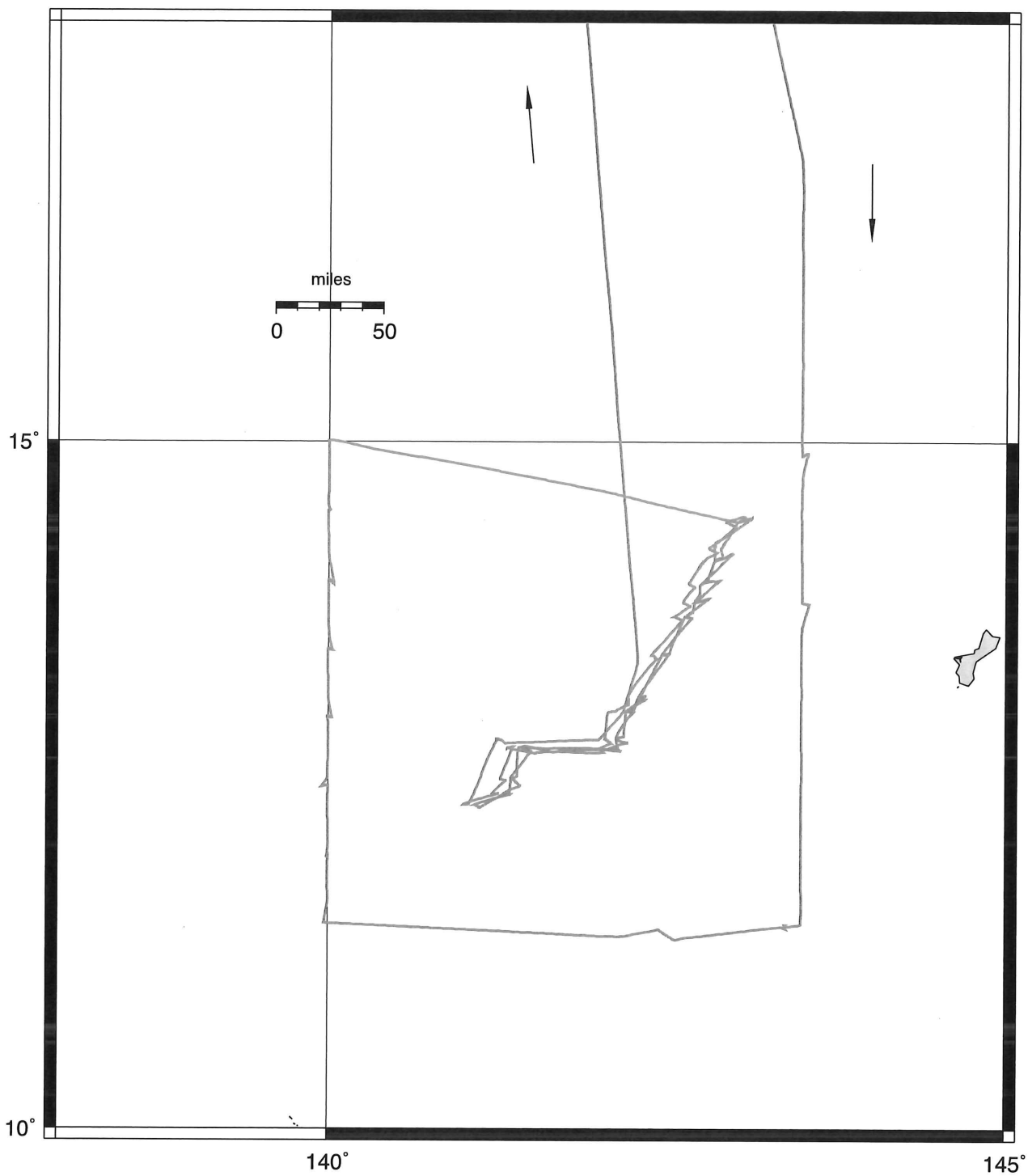
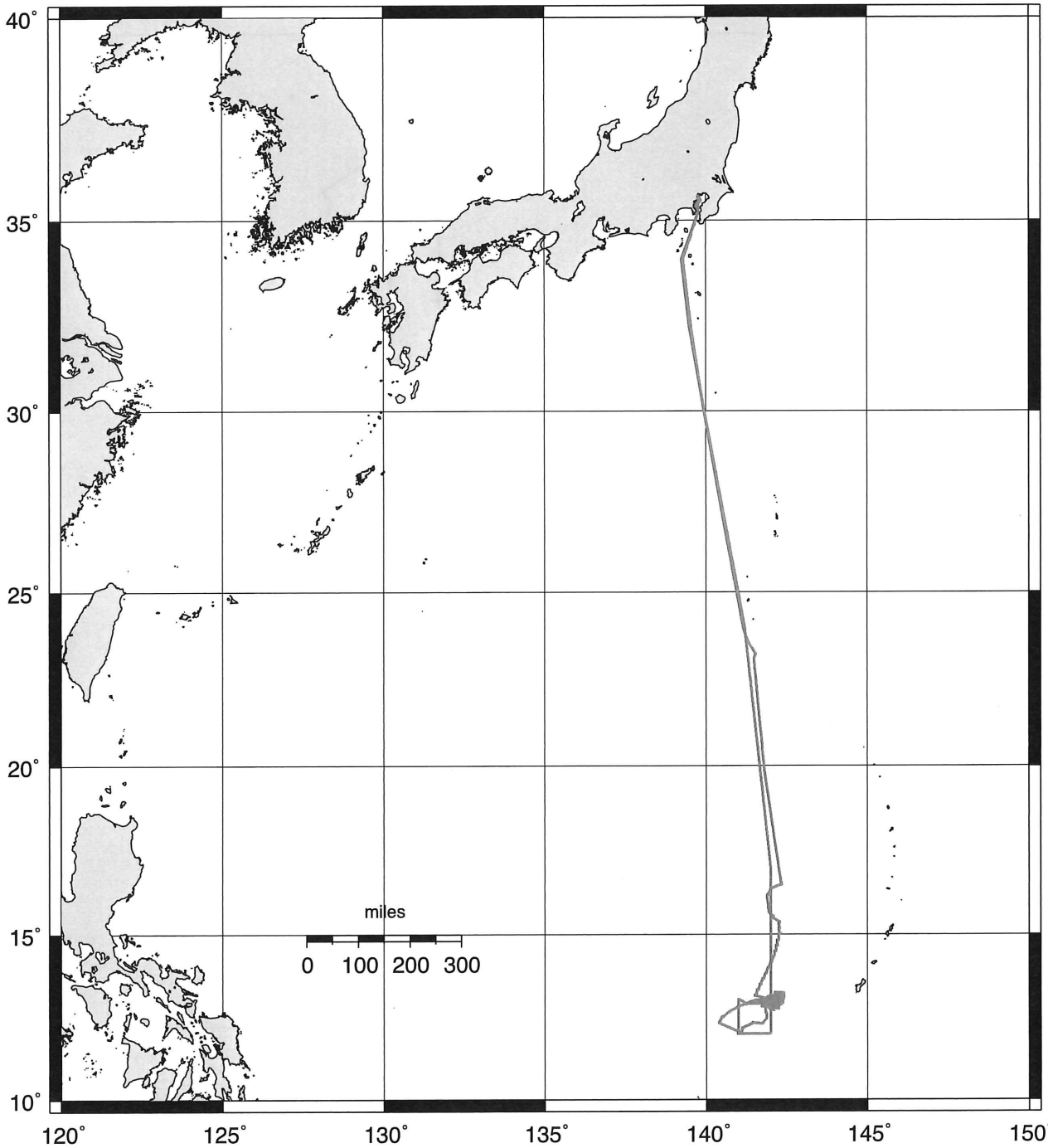


KH-11-6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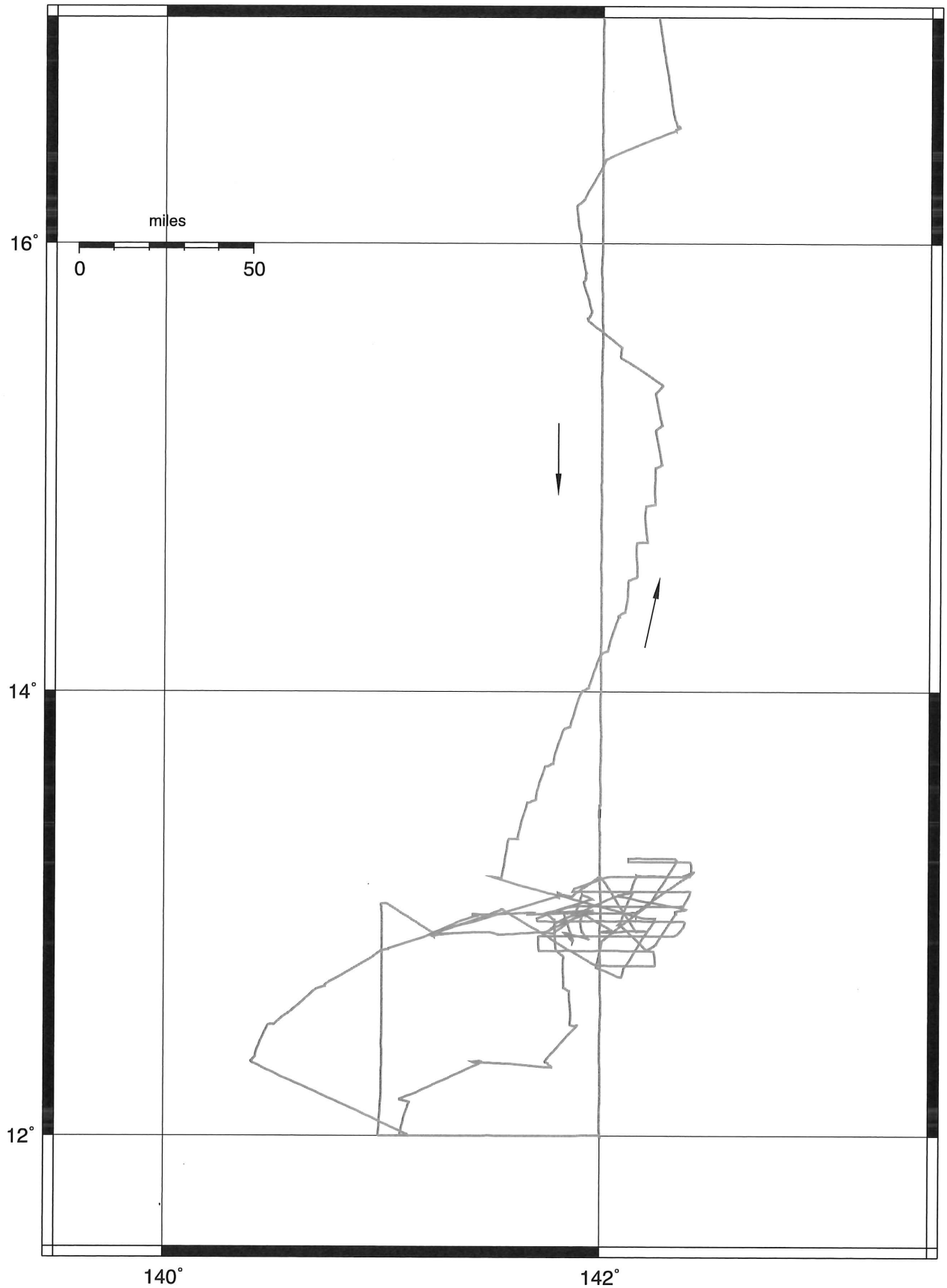


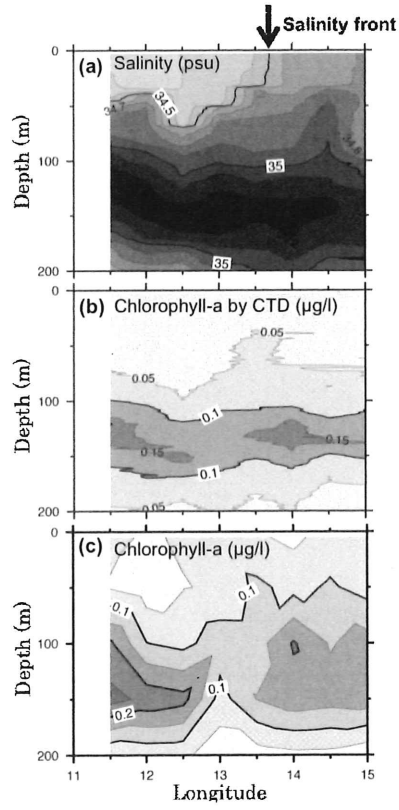
Physical and Biological Observations in the North Equatorial Current

Shingo Kimura, Kei Zenimoto, Hikaru Itakura, Aigo Takeshige,
Asagi Yagura, Nagato Iritani

Atmosphere and Ocean Research Institute
Graduate School of Frontier Sciences, The University of Tokyo

A salinity front considered as a landmark for termination of spawning migration of the Japanese eel adults (*Anguilla japonica*) is located around 15°N in the North Equatorial Current (NEC). After the spawning west of the Mariana Islands, this species starts 3000 km travel to their growth habitats in East Asia. Since southern region of the salinity front is suitable for appropriate larval transport into the Kuroshio region, this front functions as a physical survival strategy (Tsukamoto, 1992; Kimura et al., 1994). Therefore, larval transport rate from the spawning ground to growth habitats around the Japanese coasts is determined by location of the salinity front which fluctuates depending on appearance of El Niño (Kimura et al., 2001). During El Niño, the salinity front moves considerably farther southward in this region. In this case, since the southward movement causes southward movement of the spawning ground, rate of larval migration into the Japanese coasts becomes worse. In addition to the salinity front, bifurcation of the NEC plays an important role for larval transport into the Kuroshio regions. During El Niño, latitude of the bifurcation moves northward meaning large larval transport into the Mindanao Current regions and low recruitment of the Japanese eel in the nursery area (Kim et al., 2007; Zenimoto et al., 2009).

In this 2011 cruise (KH-11-4), physical and biological observations were conducted in the NEC region to survey distribution of leptocephali and the environment of their transport. Last year, La Niña occurred and the effect still remained in this year. Therefore, the salinity front was obscure and the latitudinal location was different on difference of observational lines along longitude. According to the 140°E observational line, the salinity front on the sea surface was located on 13°45'N which was slightly south of ordinal location (Fig (a)). Chlorophyll-a concentration was higher at the salinity front on the sea surface. Figures (b) and (c) are the chlorophyll-a distribution observed by CTD and measured by florescence analyses of sampled water, respectively. Chlorophyll-a observed by CTD was lower by approximately 0.05 μg/l than that



measured by florescence analyses of seawater which can be treated as the absolute values. High concentration regions in chlorophyll maximum layer at a 140 depth were divided into two parts north and south at 13°N just south of the surface salinity front.

Since some species of leptocephali have been found to ingest POM in the seawater as diet (Otake et al., 1993; Pfeiler, 1999), the latitude difference of POM would influence differences of larval body composition in the carbon and nitrogen stable isotope ratios through ingestion (Kimura and Tsukamoto, 2006). In present study, we aim to confirm this matter on the basis of hydrographic observation with net sampling around the spawning ground of the Japanese eel in the NEC. In addition to this purpose, since pre-leptocephali which are the larvae before starting ingestion seem to have large differences from the larvae which have already started ingestion, we also will try to conduct stable isotope analyses on the pre-leptocephali to compare them with those of adult and glass eel inhabiting around nursery grounds in the East Asia. This probably contributes to physiological and ecological understandings on adult spawning migration from nursery ground to spawning ground. For these analyses, water sampling at depths of 0m, 50m 100m, 150m and 200m were conducted along the 140°E CTD transect line and all water samples were filtered by GF/F filter during the cruise.

XCTD and CTD Observations During the KH-11-6 Cruise

Seishi Hagihara

Atmosphere and Ocean Research Institute, The University of Tokyo

In the western North Pacific, the spawning area of the Japanese eel *Anguilla japonica* was found to be located in the westward flowing North Equatorial Current to the west of Guam, by catching their small larvae and eggs (Tsukamoto, 2006; Tsukamoto et al. 2011). A steep north to south gradient of salinity (salinity front, 34.5 psu) is formed at the surface as a result of high precipitation in the southern area and high evaporation in the northern area. It has been inferred from larval distributions that Japanese eels spawn to the south of salinity front (Kimura et al., 2001; Kimura and Tsukamoto, 2006).

During the KH-11-6 cruise, XCTD observations were carried out along 141 and 142°E, and an additional 10 CTD observations were carried out (Fig. 1). The vertical temperature and salinity structures along the 2 lines were investigated using the data obtained from XCTD observations (Fig. 2). In the KH-11-6 cruise, the salinity front initially seemed to be located at around 15.5°N along 142°E because there was a changing point of 34.5 psu (Fig. 2). However, another point where salinity changed more drastically was found at around 13°N in both lines (Fig. 2).

In this cruise, eggs and preleptocephali were collected at around 13°N-142°E just to the south of the salinity front.

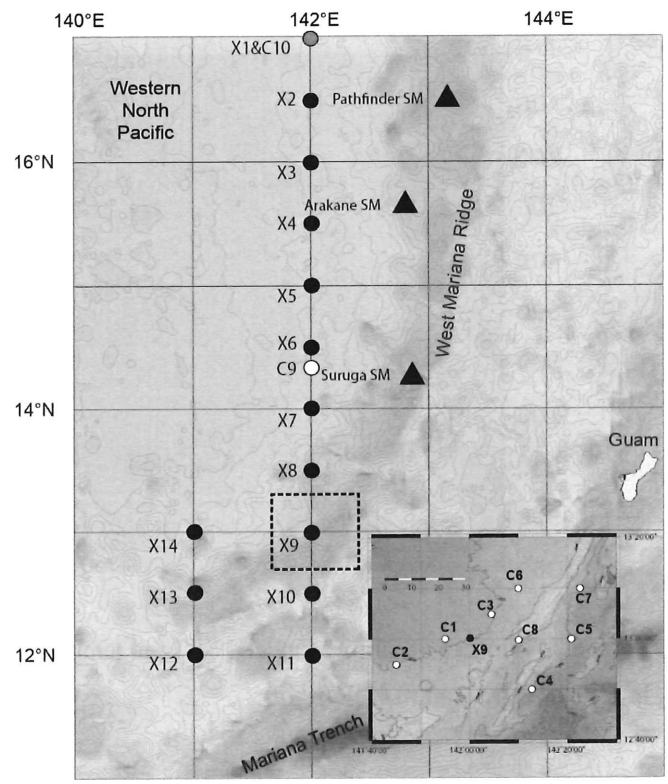


Figure 1 Location of XCTD (solid circle) and CTD (open circle) observation sites. Sites where both of XCTD and CTD observations were carried out are depicted by red circle.

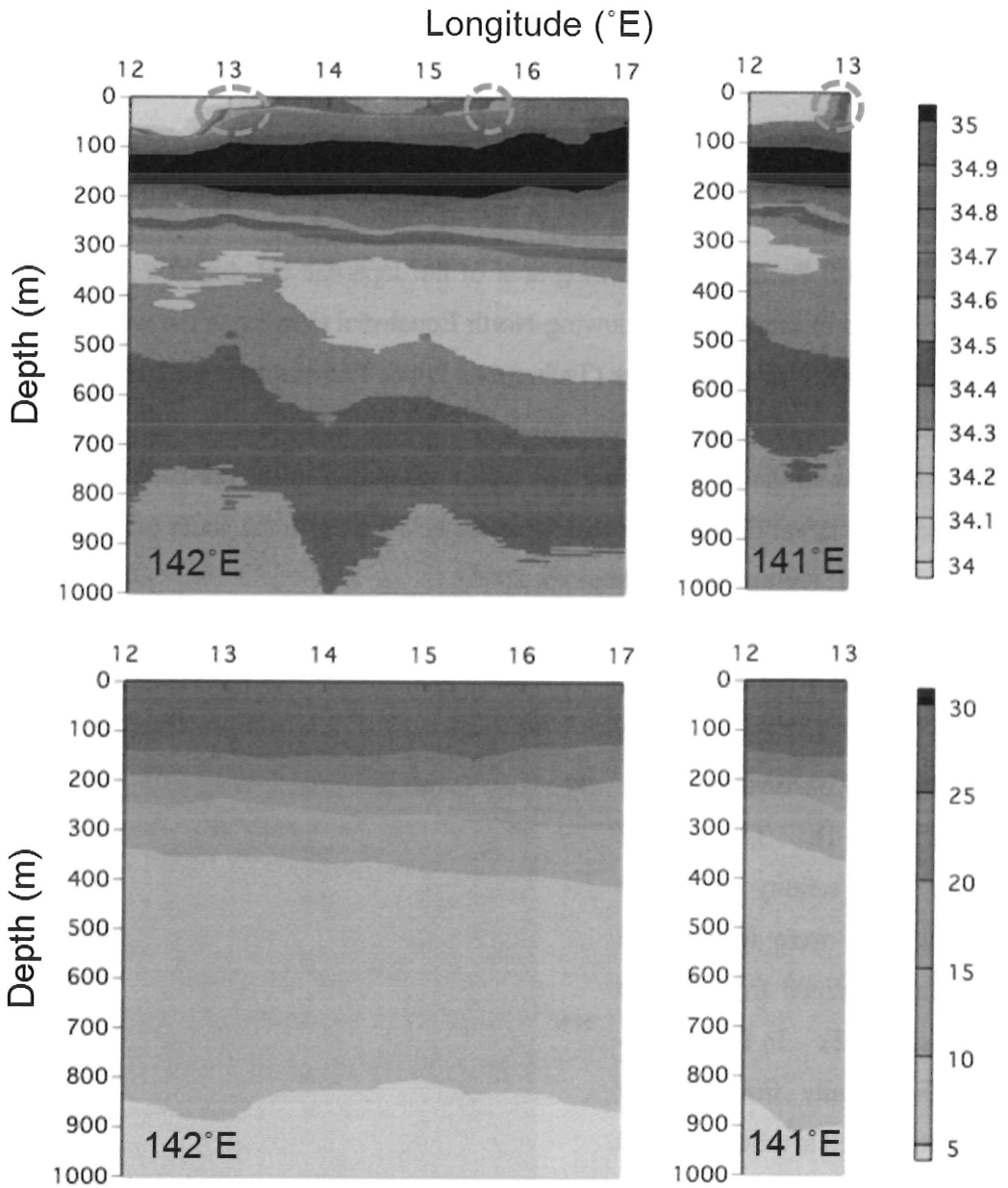


Figure 2 Profiles of salinity (upper, PSU) and temperature (lower, °C) along the XCTD observation lines, 142°E (left) and 141°E (right).

Distribution of the Surface Salinity in KH 11-4 and KH 11-6 Cruises

Takuma Terada[1] and Jun G. Inoue[2]

[1]*Agricultural Department, The University of Tokyo,*
[2]*Atmosphere and Ocean Research Institute, The University of Tokyo*

1. Introduction

The objective of this study is to detect the salinity front at the sea surface. The salinity front was used as a reference to determine the likely area for intensive sampling for larvae and eggs of Japanese eel, *Anguilla japonica*, just before the new moon when the salinity front crossed the seamount chain (Tsukamoto et al. 2010).

2. Method

Conductivity and temperature profiles were obtained using a Surface S-T system during each cruise. The position of the salinity front was distinguished by the location of 34.5 p.s.u. salinity at the sea surface.

3. Results

The profilesof conductivity and temperature were started on May 23 at 17°00'N, 134°30' E in KH11-4 and June 27 at 17°2'N, 141°60' E in KH11-6.The time series of conductivity and temperature of the surface are shown in Figs. 1 and 2. The data obtained will be carefully analyzed with the collection record of leptocephali and eggs.

Reference

Tsukamoto K. et al. 2010. Oceanic spawning ecology of freshwater eels in the western North Pacific. Nature Communications, 2: 179.

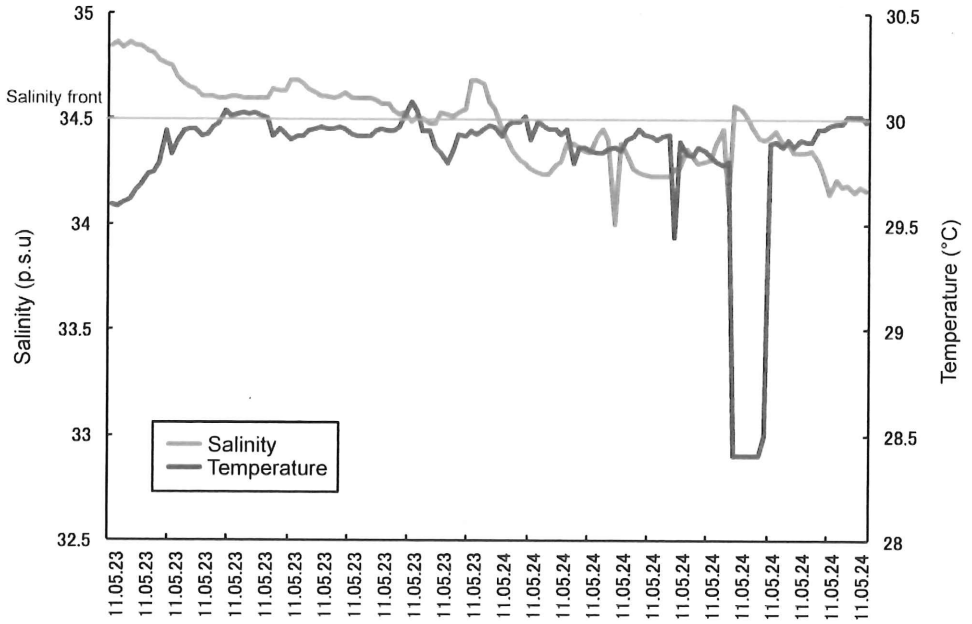


Figure 1 Distribution of the surface salinity and temperature in KH 11-4.

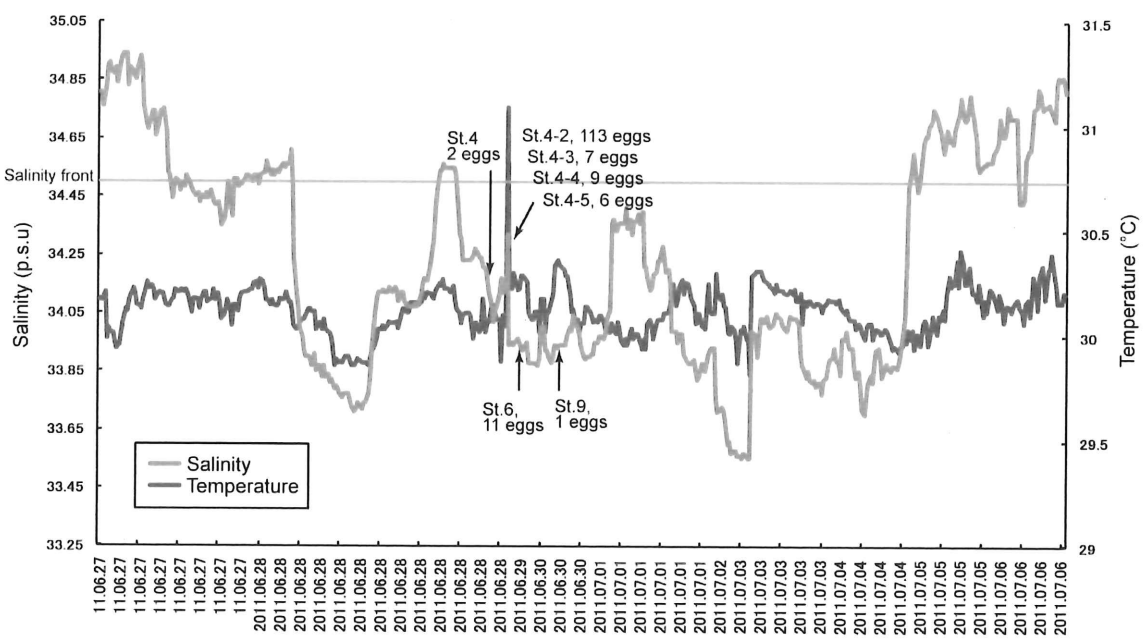


Figure 2 Distribution of the surface salinity and temperature in KH 11-6. The collections of Japanese eel eggs are shown with the station numbers. St. 4(34.12–34.17 p.s.u): June 29th, 1.34–2.47; St.4-2 (>34.32p.s.u): 4.29–5.45; St.4-3:7.38–8.22; St.4-4:8.55–9.39; St. 4–5:10.10–10.58. St. 6: 21.32–22.50; St. 9: June 30th, 11.42–12.57.

Distribution of *Anguilla japonica* Eggs and Evidence of Spawning Sites During the KH-11-6 Cruise

All Scientists Onboard

Eggs of the Japanese eel, *Anguilla japonica*, were collected in June 2011 during the KH-11-6 sampling survey along the West Mariana Ridge using the ORI-BF net. A total of 147 *A. japonica* eggs were collected at two stations (St. 4 and 6, Fig. 1), but St. 4 was sampled multiple times during a study of the depth distribution of eggs (see Yokouchi et al. report). Both stations where eggs were collected were over water greater than 3000 m deep to the west of the southern part of the seamount chain (Fig. 1,2), within an area just south of the salinity front at about 13°N (see Hagihara report).

The number of eggs, which were at various developmental stages (Fig. 3, 4), that were collected at the two stations were as follows: St. 4 (2 eggs), St. 4-2 (113), St. 4-3 (6), St. 4-4 (9), St. 4-5 (6), St. 6 (11). Geographically, St. 4 (13°00'N, 141°55'E) and St.6 (13°05'N, 142°05'E) were separated by about 15 km, so the eggs at those locations likely resulted from different spawning aggregations. A similar pattern of distribution of eggs and preleptocephali was observed in May 2009, when *A. japonica* eggs were also captured just south of the salinity front in this same general region of the southern part of the ridge (Tsukamoto et al. 2011).

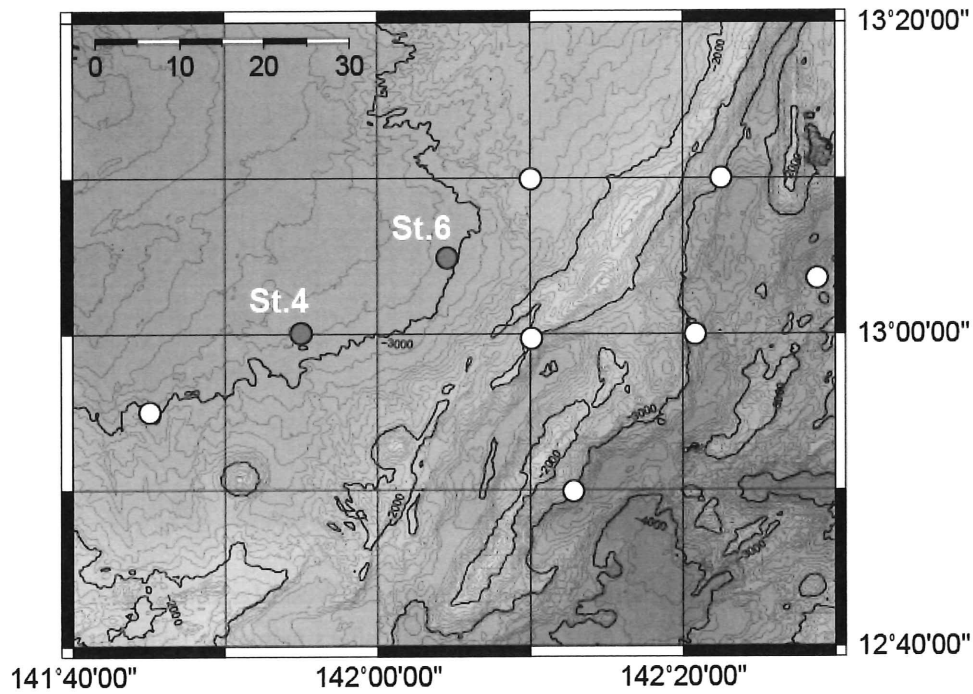


Figure 1. Map showing the locations where Japanese eel, *Anguilla japonica*, eggs were collected over a 20 hour time period on 29 June during the KH-11-6 cruise (red circles). Locations where CTD casts were made are shown with white circles, and CTD casts were also made at St. 4 and 6 where eggs were collected.

No *A. japonica* eggs or preleptocephali were collected anywhere else during the cruise, including further north along the west side of the seamount chain after the new moon period (Fig. 2). The distribution of preleptocephali was also consistent with spawning occurring south of the salinity front during the new moon period (Fig. 2).

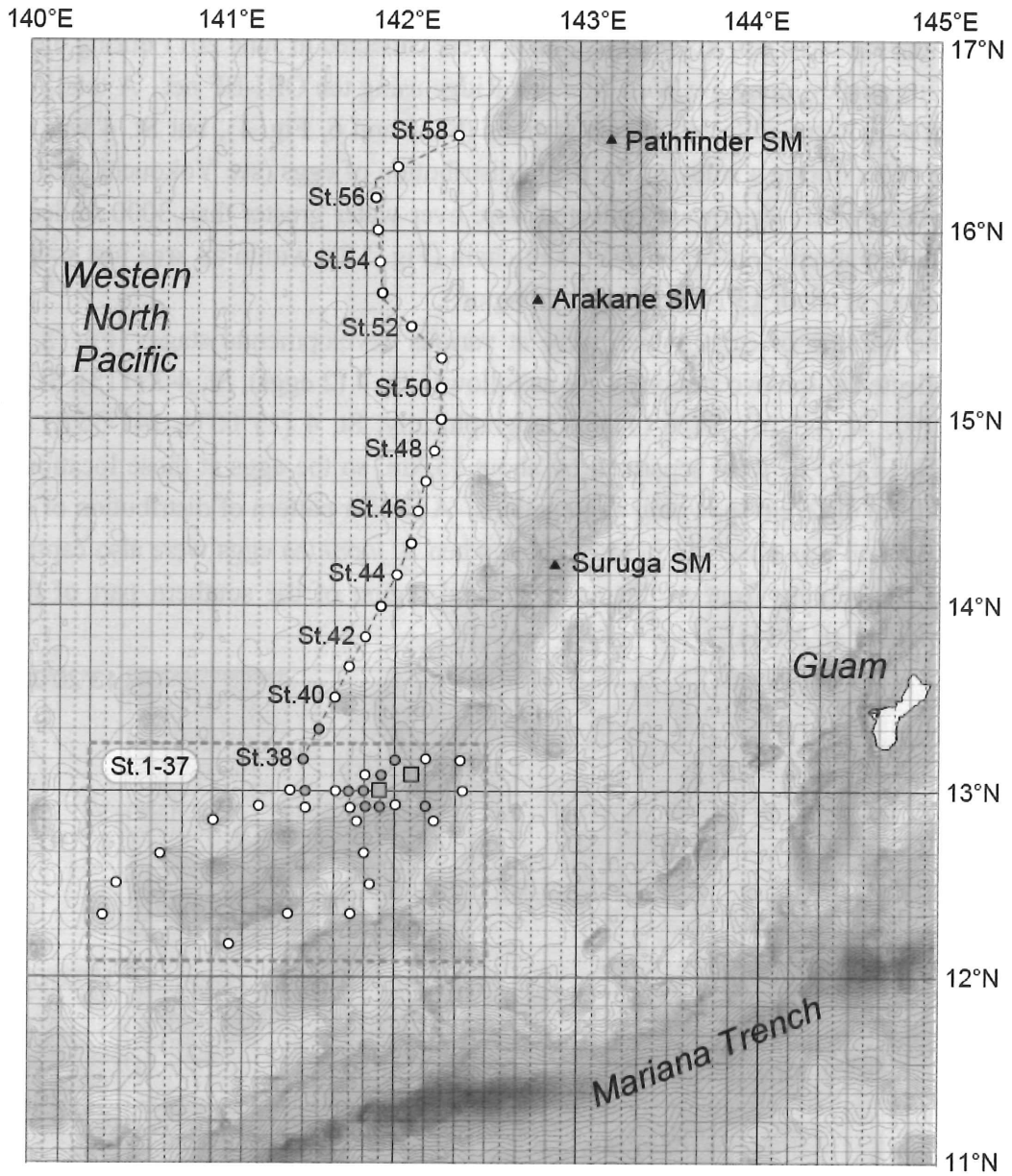


Figure 2. Map of the sampling stations of KH-11-6 along the West Mariana Ridge in June and July of 2011, showing the locations where eggs were collected (blue squares) and where preleptocephali were collected (green circles). Stations 1-37 were located within the rectangle (dotted red lines), and stations where no *Anguilla japonica* eggs or larvae were collected are shown with white circles. The salinity front was located at a latitude of 13°N just before the egg collections.

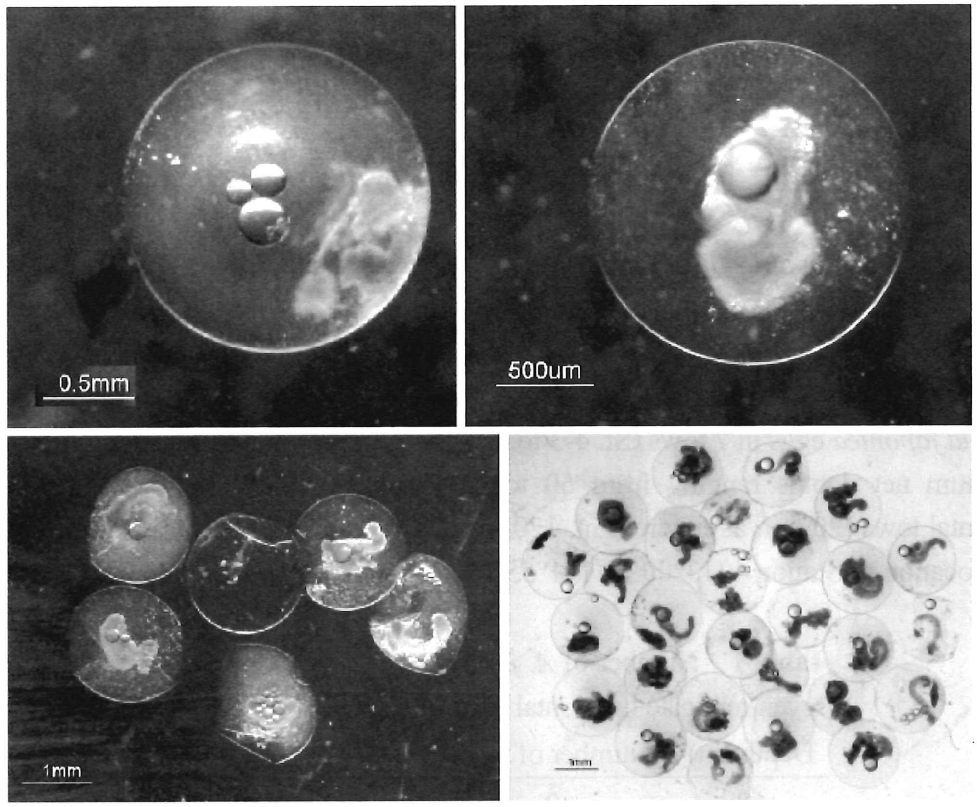


Figure 3. Photographs of early stage *Anguilla japonica* eggs collected during the KH-11-6 cruise of the R/V *Hakuho Maru*. These eggs may have stopped natural development due to the temperature shock of being transported into the warmer water near the surface.

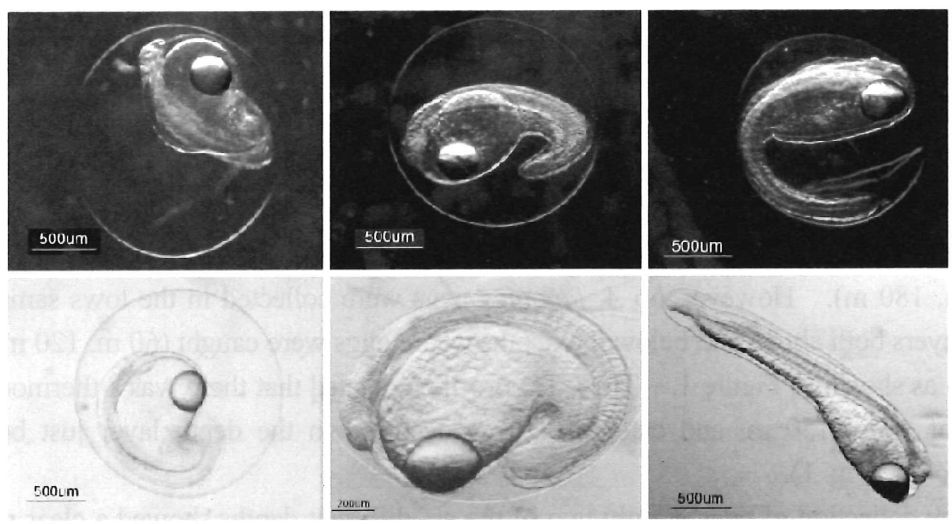


Figure 4. Photographs of late stage eggs *Anguilla japonica* eggs collected during KH-11-6 and an embryo that hatched out during observations onboard. These eggs may be more resistant to temperature shock and were still alive at the time of observations, showing clear structures of the embryo with yolk deposits and oil globules.

Vertical Distribution of *Anguilla japonica* Eggs Near the West Mariana Ridge

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During the KH-11-6 cruise the ORI-BF net was used to study the depth distribution of *Anguilla japonica* eggs in 7 tows (St. 4-3 to 4-9) that sampled at 6 different depth layers with maximum net depths ranging from 60 to 420 m. These stations were sampled with horizontal tows (20 min at either 60 m, 120 m, 150 m (n = 2), 180 m, 250 m, 420 m) at the same location of Station 4 (13°00'N, 141°55'E) during the day on 29 June 2011 (Table 1).

Table 1 Number of *A. japonica* eggs collected by the seven replicate horizontal tows of the ORI-BF net.

Depth (m)	Number of eggs	St. No.
60	0	4-8
120	0	4-7
150	6	4-3
150	9	4-4
180	6	4-5
250	0	4-6
420	0	4-9
Total	21	

A total of 21 *A. japonica* eggs were collected in 3 tows (St. 4-3, 4-4, 4-5) at two layers (150 m, 180 m). However, no *A. japonica* eggs were collected in the tows sampling the other layers both above and below the depths where eggs were caught (60 m, 120 m, 250 m, 420 m) as shown in Figure 1. The CTD profile indicated that there was a thermocline at a depth of about 150 m, and catches of eggs occurred in the depth layer just below the thermocline (Fig. 1).

The collection of eggs at only two of the six different depths showed a clear pattern in the catches, with eggs distributed in a vertically narrow depth stratum at 150 ~ 180 m (Fig. 1), whereas no eggs were collected at depths below 120 m or above 250 m. The depth distribution of eggs was quite similar to that of preleptocephali, which were collected during the KH-09-02 cruise (Fig. 1) and also to the depth of capture of adult eels that ranged from about 160 m to 300 m (Tsukamoto et al. 2011).

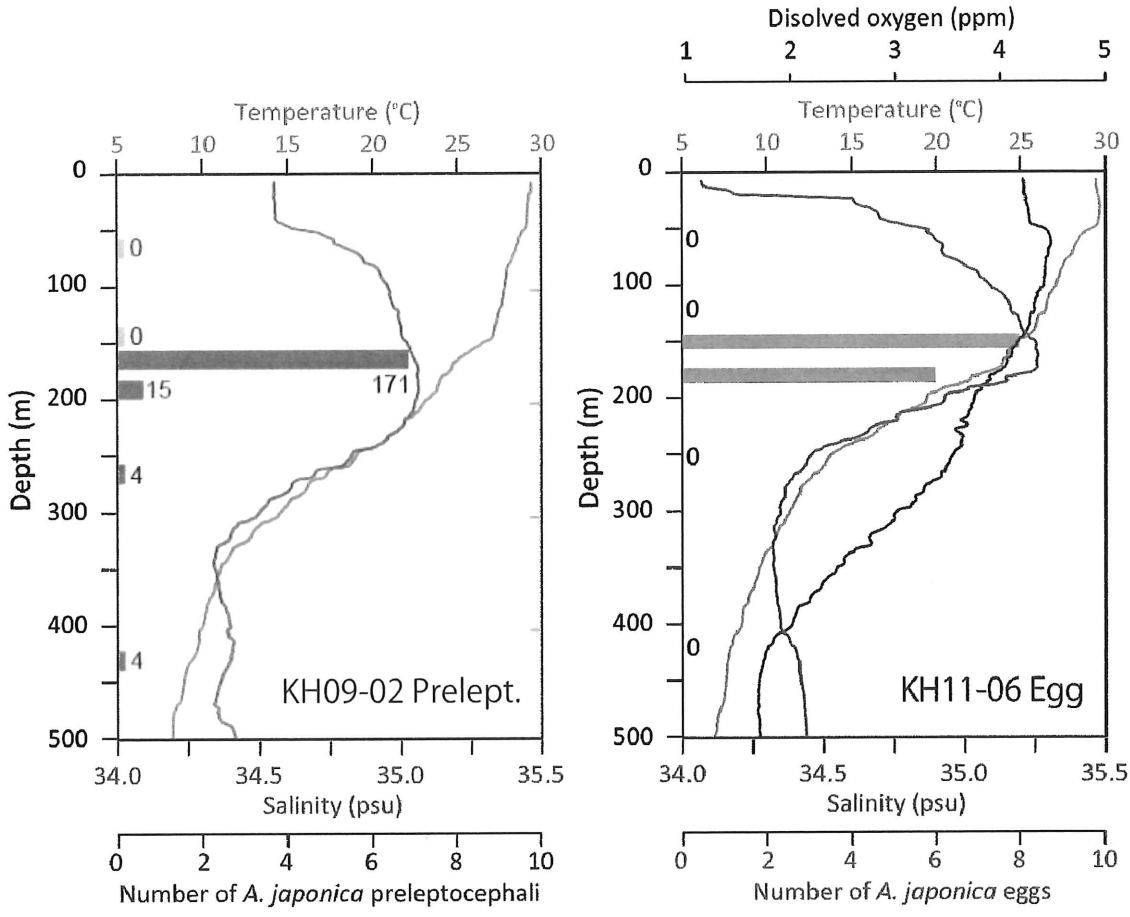

Figure 1. Plots of the catches of eggs at Station 4 of KH-11-6 in relation to the profile of hydrographic data at that location from the CTD cast (right), and of the preleptocephali catches (left) during the depth distribution study of KH-09-2 (Tsukamoto *et al.* 2011), showing the correspondence of the depth distributions of the eggs and recently hatched preleptocephali.

The 150 m depth layer was at the upper limit of the thermocline and because of the buoyancy of the eggs (Tsukamoto et al. 2009), it is likely that the eggs would have floated up to the top of the thermocline where they hatch and then they could stay at this layer until the end of the preleptocephalus stage. The concentrated vertical distribution of eggs and preleptocephali within a limited depth stratum shows the conditions of early development of this species and the possible speed of upward movement of eggs after spawning can be estimated based on the spawning depth of the adult eels.

References

Tsukamoto, K. et al. 2009. Positive buoyancy in eel leptocephali: an adaptation for life in the ocean surface layer. Marine Biology, 156:835–846.

Tsukamoto, K. et al. 2011. Oceanic spawning ecology of freshwater eels in the western North Pacific. Nature Communications, 2:179. DOI: 10.1038/ncomms1174.

Preleptocephali of *Anguilla japonica* Collected During the KH-11-06

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[2]*Institute of Fisheries Science, National Taiwan University*
[3] *Atmosphere and Ocean Research Institute, The University of Tokyo*

During the KH-11-06, ORI-BF net was used in 66 tows at 58 stations, where sampling was conducted mainly with step tows (10 min at 180 m, 150 m, 120 m and 90 m net depth or 8 min at 170 m, 145 m, 120 m and 95 m net depth). Among the preleptocephali collected during this cruise, 83 were either morphologically or genetically identified as *Anguilla japonica* (Sudo and Minegishi, report).

The morphological characters of the specimens were observed with a microscope and various stages of development were seen ranging from newly hatched larvae with no eye pigmentation nor teeth to fully developed preleptocephali (Fig. 1). This may indicate that there are some variations in individual development and growth, and that different spawning cohorts were mixed up in the specimens collected.

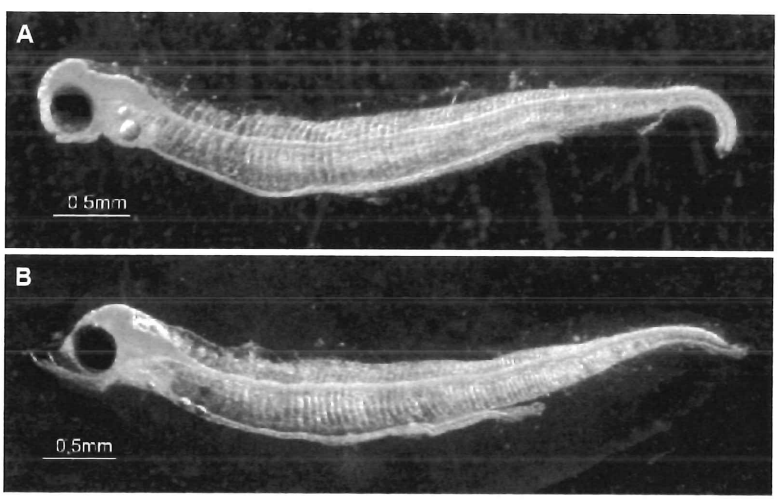

Figure 1. Photographs of an early stage 4.9 mm *Anguilla japonica* preleptocephalus (No. 119) with eye pigment but no teeth (A) and a later stage 5.0 mm preleptocephalus (No. 116) that had teeth and a smaller amount of oil globules (B) that were both collected at St. 37, where 33 preleptocephali were collected.

All preleptocephali were collected from the day of New Moon (July 1) to 5 days after New Moon (Table 1). This timing was longer in comparison with the collection data of in May 2009, in which preleptocephali were collected on one day before New Moon to two days after New Moon. Moreover, the catch of preleptocephali was most abundant on three days after New Moon in July 2011, which was later than the collection of preleptocephali in previous years.

Table 1 Catch data of preleptocephali

St.	Date	No. of specimens	TL (mm)
17	July 1	1	4.6
18	July 1	1	5.2
21	July 2	1	5.8
22	July 2	1	5.0
23	July 2	5	5.2 - 6.0
24	July 2	1	5.5
25	July 2	13	5.0 - 5.9
37	July 3	33	4.4 - 5.7
38	July 3	13	3.8 - 5.1
39	July 5	14	4.0 - 6.0

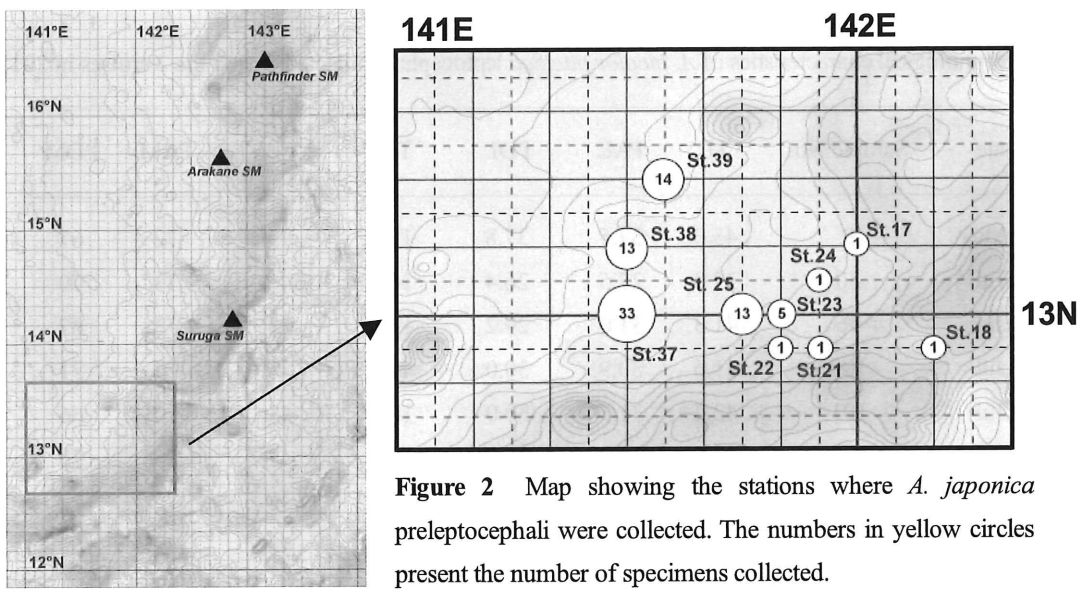

Figure 2 Map showing the stations where *A. japonica* preleptocephali were collected. The numbers in yellow circles present the number of specimens collected.

All preleptocephali were collected in the southern region of the western Mariana Ridge (Fig. 2), where the strong salinity front was observed (see Hagihara, report). The stations where preleptocaphli were collected during this cruise were located slightly north than the ones in 2009. This could be linked to the inter-annual changes of the oceanographic conditions around the spawning area of the Japanese eel.

Species identification by DNA sequencing will be done later in the laboratory for further detailed analysis.

Leptocephali of Anguillid Species Collected During KH11-04 and KH11-06

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There were a total of 8 leptocephali of *Anguilla bicolor pacifica* collected during the two cruises as shown in Table 1. All were collected from July 1 to July 3, except one individual that was collected in May, and their total length ranged from 37.0 to 45.5 mm. The body size range was quite uniform and consistent with the previous report (Kuroki et al., 2006). According to the growth rate of this species (0.48 mm/day, Kuroki et al. 2006), the specimens collected during the cruise were estimated to be born in March and April. Together with other specimens, these larvae will be used for further research such as population genetic and ecological studies.

Table 1 Morphological characteristics of *A. bicolor pacifica* leptocephali collected during the 2 cruises in 2011.

St.	Date	Sample No.	TL	PAL	PDL	TM	LVBV	PAM	PDM
5	25.May	9	43.0	37.5	37.8	109	43	72	67
19	1.Jul	50	37.0	27.5	26.4	109	46	73	70
29	2.Jul	96	41.3	31.0	29.2	107	44	73	67
30	2.Jul	100	41.6	30.9	30.0	111	47	74	71
30	2.Jul	101	42.4	31.7	30.0	105	43	70	66
31	3.Jul	103	40.5	30.3	28.7	104	47	75	70
31	3.Jul	104	45.5	34.2	33.0	105	46	75	72
32	3.Jul	111	41.2	30.8	28.8	111	48	75	69

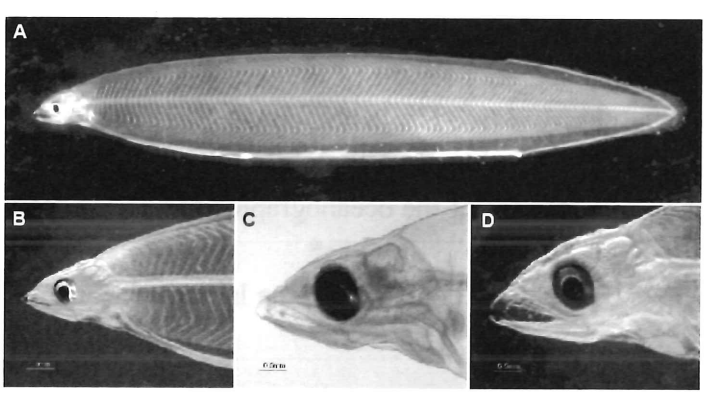

Figure 1 Photographs of *Anguilla bicolor pacifica* leptocephali collected during the KH-11-4 and KH-11-6 cruises showing a 41.6 mm specimen from St. 30 of KH-11-6 (A, D), 43.0 mm from St. 5 of KH-11-4 (B), and 37.0 mm St. 19 of KH-11-6 (C).

Onboard Genetic Species Identification of the Japanese Eel Eggs and Larvae Using Real Time PCR: KH11-6

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[2]*Institute Biology Leiden, Leiden University*

1. Introduction

Large well developed leptocephali of the Japanese eel (*Anguilla japonica*) can be identified by only morphological characteristics such as total number of myomeres. However, during *A. japonica* at early developmental stages such as the egg and preleptocephalus, it is difficult to identify *A. japonica* because of their undeveloped morphological characteristics. Indeed, some species of *serrivomer* have morphological features that are closely resembled those of *A. japonica* (Miller and Tsukamoto 2004). Accordingly, onboard genetic species identification of eggs and larvae (preleptocephali) using real time PCR *A. japonica* sequence detection system (Aj-SDS) based on real –time PCR has been employed since 2004, and contributed to the discovery of eggs and preleptocephali of this species in 2005 (Tsukamoto et al. 2011; Tsukamoto, 2006). Therefore, combined use of PCR with morphological examination is currently the most reliable strategy to identify *A. japonica* specimens onboard.

2. Materials and Methods

Larvae and eggs were collected by the ORI BigFish Trawl with 0.5mm mesh nets. After each tow, specimens were sorted fresh out of plankton samples, and morphological characters were measured and photographed according to Miller and Tsukamoto (2004). During the procedure, specimens were preserved in ice water bath to minimize degradation of nucleic acid.

Eggs and larvae, which were thought to be potentially *A. japonica* from morphological observation, have been analyzed. Whole eggs and posterior body of larva tissue was excised, and homogenized in 50 μL of 5% (w/v) Chelex-resin solution (Bio-Rad) with a disporsable plastic pestle. The homogenate was boiled at 98°C for 15 minutes, followed by spindown at around 2,000g and the diluted supernatant (1:10 or 1:50) was used as template DNA of PCR. 20 μl of PCR mixture consisted of 10μl of 2x TaqMan Fast Advanced Master mix (Applied Biosystems), 900nm each of Aja16S-L3 (F3) and Aja16S-H3 (R3) PCR primers, 200nM A. japonica-specific TaqMan probe, and 2μl of template DNA solution.

The reactions were carried out with an ABI PRISM 7300 Sequence Detection System (Applied Biosystems). Results were obtained as a Ct value that is a number of PCR cycles at the threshold, and based on the Ct value of a positive control sample. The samples with Ct < 24 were identified as *A. japonica*.

3. Results and discussion

During the cruise, total 5 assays have been carried out for 20 eggs and 8 preleptocephali. Specimens with *A. japonica* -positive were found at the stations 4, 4-2, 6, 17, 18, 22 and 23 (Table1). Thus these specimens were strongly suggested to be *A. Japonica*. Some of those were eggs at early developmental stages. This indicates that this system can identify A. Japonica, even if the sample has very few amount of DNA. The result will be further confirmed by determining the DNA nucleotide sequence of each specimen after the cruise.

4. Reference

Miller M.J. and Tsukamoto K. 2004. An introduction to leptocephali: Biology and identification. Ocean Research Institute, University of Tokyo, Tokyo.

Tsukamoto K. et al. 2011. Oceanic spawning ecology of freshwater eels in the western North Pacific. Nature communications, DOI: 10.1038/ncomms1174

Tsukamoto K. 2006. Spawning of eels near a seamount. Nature, 439: 929

Table 1 List of the result of all samples analyzed during KH-11-06

St	Specimen	stage	Result	St	Specimen	stage	Result
4	04001	Egg	+	6	06008	Egg	+
4-2	04-2001	Egg	+	6	06009	Egg	+
4-2	04-2002	Egg	+	6	06010	Egg	+
4-2	04-2003	Egg	+	6	06011	Egg	+
4-2	04-2004	Egg	+	9	09001	Egg	-
4-2	04-2005	Egg	+	17	46	Prelepotcephalus	+
4-2	04-2006	Egg	+	18	48	Prelepotcephalus	+
6	06001	Egg	+	22	62	Prelepotcephalus	+
6	06002	Egg	+	23	64	Prelepotcephalus	+
6	06003	Egg	+	23	65	Prelepotcephalus	+
6	06004	Egg	+	23	66	Prelepotcephalus	+
6	06005	Egg	+	23	67	Prelepotcephalus	+
6	06006	Egg	+	23	68	Prelepotcephalus	+
6	06007	Egg	+				

Ontogeny of Digestive Tract at Early Stage of Japanese Eel, *Anguilla japonica*

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1. Background

The digestive tract is closely related to the ontogeny in almost every living thing in term of their main function on nutrient absorption. In fish, digestive tract also plays a vital role on osmoregulation. Therefore research on digestive tract is highly important to understand the ontogeny of larvae. By histological experiment with artificial larvae, it is revealed that the differentiation of digestive tract as esophagus, intestine and rectum is completed within one week after hatching. In this period, larvae still have oil drop and assumed not start feeding, so called preleptocephalus. Since the development speed of natural larvae is about twice as fast as artificial ones, it is meaningful to compare the digestive tract between natural and artificial larvae. Therefore, it is planned to execute the histological observation on newly caught preleptocephalus at KH-11-6 cruise.

2. Material and methods

A total of 83 preleptocephali have been collected during the KH-11-6 cruise and considered as *A. japonica*. Among them 26 preleptocephali with least damage were sample for later experiment (Table. 1)

Preleptocephali were divided into head, body and tail (Fig.1). Heads were preserved in 70% ethanol for otolith analysis, so that the precise comparison on daily development would be carried out. Body parts were fixed by 10% PFA and moved to 70% ethanol for histological study. The microscopic observation will be performed with HE stained 4μm microsection. Tails were preserved in absolute ethanol for real-time PCR test to make sure that the species are *A. japonica*.

3. Possible results

Following the information that leptocephalus were usually caught in 100-200m layer (Miller and Tsukamoto 2004), water temperature varied about 18℃ to 27℃ and DPH (day post hatching) could be assumed as from day1 to day 8, under a postulation that all preleptocephli were *A. japonica*. The future otolith analysis will provide exact information. The development speed of digestive tract can be faster on natural preleptocephalus, because it only takes half a year before metamorphosis to glass eel in natural larvae, even though the artificial larvae need almost one year. However, it cannot be denied that the development

process of digestive tract is same on both natural and artificial ones, and another reasons affect the growth rate. Thus, study on digestive tract with natural preleptocephalus and compared to artificial samples would give a clue to find out the cause and effect of different growth rate.

Table 1 Information of sampled preleptocephalus during KH-11-6

Sample No	1	2	3	4	5	6	7	8	9	10	11	12	13
Species No.	75	81	83	85	116	118	119	120	122	123	124	126	127
Station No.	25	25	25	25	37	37	37	37	37	37	37	37	37
Sample No	14	15	16	17	18	19	20	21	22	23	24	25	26
Species No.	131	132	136	138	139	141	145	152	153	157	159	172	173
Station No.	37	37	37	37	37	37	37	38	38	38	38	39	39

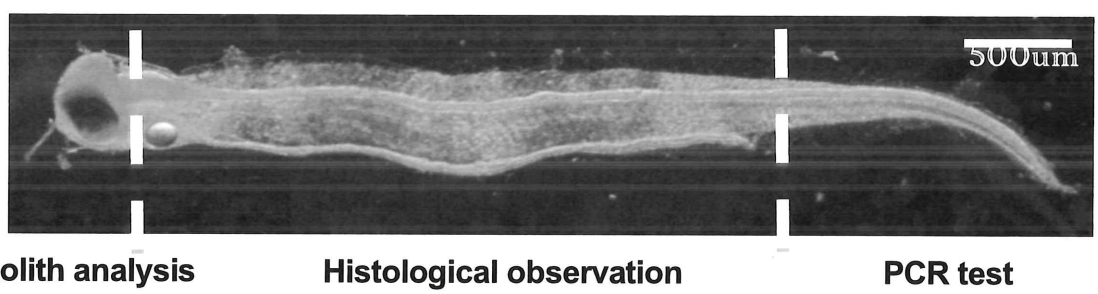

Figure 1 Schematic representation of sampling method according to purpose on collected preleptocephalus during KH-11-6.

Reference

Miller M.J. and Tsukamoto. K. 2004. An Introduction to Leptocephali Biology and Identification.

Transcriptional Differences of Japanese Eel Eggs and Preleptocephali Between Natural and Artificial Eels

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It is difficult to obtain high quality eggs from artificially matured Japanese eels *Anguilla japonica*, which causes the survival rate to be low. For producing glass eels with a high survival rate, improvement of egg quality is one of the most important aspects, because variability in egg quality is a limiting factor for the successful seed production of glass eels (Adachi et al. 2003). One of the possible causes of variability in egg quality is the use of hormonal treatments to obtain matured eggs and ovulated eggs for artificial propagation (Sato et al. 2003). Therefore, it is important to analyze the physiology of natural eels and clarify differences with comparison to artificial eels.

During the KH-11-6 cruise, over 147 Japanese eel eggs and 83 preleptocephali were collected (Fig.1). Among them, 8 eggs and 12 preleptocephali were preserved in RNAlater solution. These samples will make it possible to compare between natural and artificial eels using molecular biological analysis.

It is planned to carry out transcriptome analysis with newly caught eggs and preleptocephali of Japanese eel during KH-11-6, with the purpose of finding the specific gene of natural eels by comparing with artificial eels. The specific gene might have a high possibility to include the important gene that is involved with egg quality. Thus, this study is expected to contribute the improvement of artificial maturation of Japanese eels and help lead to successful aquaculture in the future.

References

Adachi et al. 2003. Oogenesis in the Japanese Eel, *Anguilla japonica.* "Eel Biology" pp. 301-317.

Sato et al. 2003. Induction of vitellogenesis. In "Eel Biology" pp. 387-399.

Figure 1. A Japanese eel egg (A) and a 4.9 mm preleptocephalus (No. 122) (B) from KH-11-6.

Daily Age Estimation of Japanese Eel Preleptocephali Collected During the KH-11-06 Cruise

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A total of 83 specimens of preleptocephali that were morphologically or genetically identified as being the Japanese eel were collected by the ORI-Big Fish plankton net with 0.5 mm mesh during the KH-11-06 cruise. Out of all the preleptocephali collected, 22 specimens were selected for studying their otoliths (Table 1). These leptocephali were collected from 2 sampling stations.

Thus, we will observe the microstructure and daily increments of the otoliths to determine their ages using both light microscope and scanning electron microscope photographs. These data will provide important information about their early life history and spawning ecology.

Table 1 Sizes, dates, and sampling stations of the KH-11-06 *Anguilla japonica* preleptocephali that will be used for otolith analysis.

St.	Latitude	Longitude	No.	Date	TL (mm)
37	13-00N	141-30E	117	3.Jul.11	5
37	13-00N	141-30E	121	3.Jul.11	5.5
37	13-00N	141-30E	125	3.Jul.11	5.4
37	13-00N	141-30E	128	3.Jul.11	5
37	13-00N	141-30E	129	3.Jul.11	5
37	13-00N	141-30E	130	3.Jul.11	5.3
37	13-00N	141-30E	133	3.Jul.11	5.1
37	13-00N	141-30E	134	3.Jul.11	5.1
37	13-00N	141-30E	135	3.Jul.11	5
37	13-00N	141-30E	137	3.Jul.11	4.9
37	13-00N	141-30E	140	3.Jul.11	4.8
37	13-00N	141-30E	142	3.Jul.11	5
39	13-20N	141-35E	170	5.Jul.11	5.6
39	13-20N	141-35E	171	5.Jul.11	5
39	13-20N	141-35E	174	5.Jul.11	4.8
39	13-20N	141-35E	175	5.Jul.11	4
39	13-20N	141-35E	176	5.Jul.11	4.3
39	13-20N	141-35E	177	5.Jul.11	4
39	13-20N	141-35E	178	5.Jul.11	4.2
39	13-20N	141-35E	179	5.Jul.11	5.5
39	13-20N	141-35E	180	5.Jul.11	6
39	13-20N	141-35E	181	5.Jul.11	4.8

Geophysical Environment of the Spawning Sites of the Japanese eel Around the West Mariana Ridge

Kazuki Yokouchi[1,2], Francoise Daverat[1], Shun Watanabe[2] and Katsumi Tsukamoto[2]

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[2]*Atmosphere and Ocean Research Institute, The University of Tokyo*

The geophysical features of the region along the West Mariana Ridge where spawning sites of the Japanese eel (*Anguilla japonica*) occurred during June 2011 were surveyed in a relatively fine spatial scale for the magnetic field, topography of the ocean bottom, current flow direction/velocity, and characteristics of the water column using the proton magnetometer, multi-beam bathymetric survey system (SeaBeam2012), ADCP, and CTD during the KH-11-6 cruise. This survey was undertaken in the area of 12°40'N – 13°20'N, 141°40'E – 142°30'E on 3 July 2011 – 4 July 2011. These measurements will contribute to clarifying the geographical and environmental conditions of the singular points corresponding to the spawning aggregations of eels. This survey will test the hypothesis of the existence of magnetic, or topographic clues allowing the eels to meet for spawning.

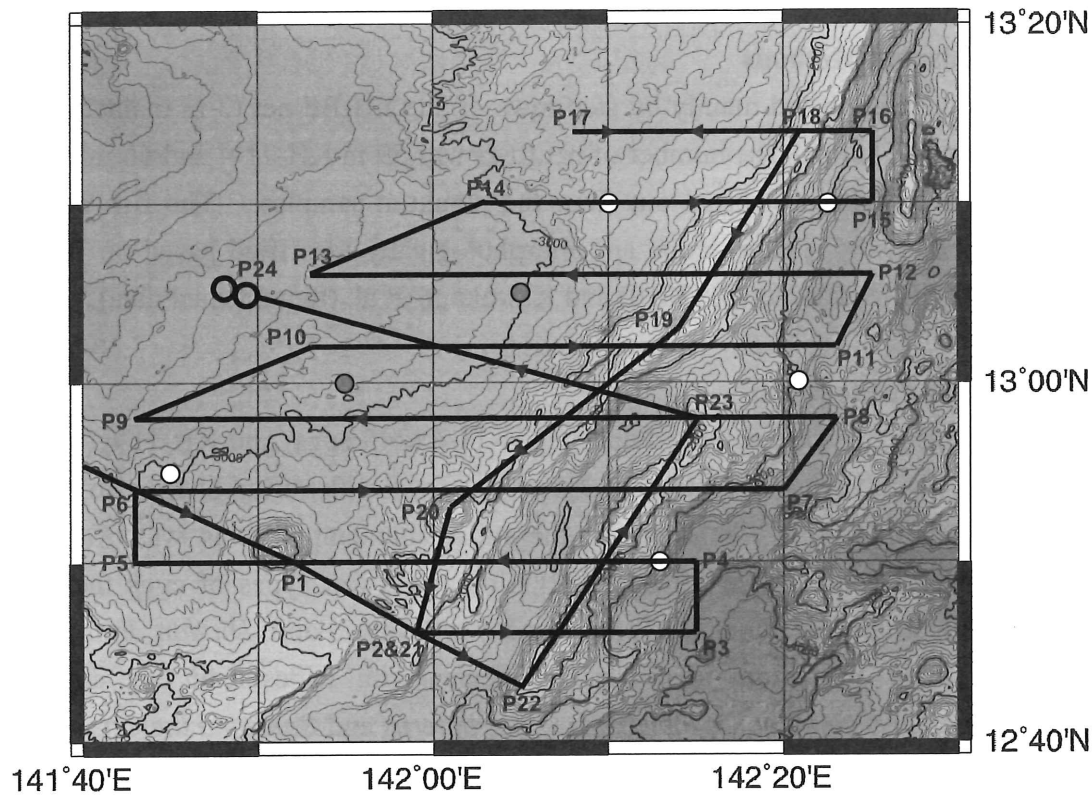

Figure 1 Map of the survey area along the southern part of the West Mariana Ridge during the KH-11-6 cruise. Red circles indicate stations where eel eggs were collected.

Species Composition of Pelagic Fish Eggs Collected in KH-11-4 and KH-11-6

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1. Introduction

The abundance and distribution of eggs is important information for determining the spawning areas and understanding the reproductive ecology of fishes. However, there is very little basic knowledge about the distribution, species composition and abundance of fish eggs in the open ocean. One of the main obstacles in research on fish eggs is difficulty in species identification based on morphological characters. However, recent progress in molecular genetics and bioinformatics, and increasing numbers of sequences in public DNA databases enables us to identify eggs that could not be discriminated by conventional morphological identification methods. Kawakami et al. (2010) used DNA sequences to identify fish eggs collected in the Mariana region, and described the egg morphology of 16 species and the distribution of 7 species. In this cruise, fish eggs were collected to reveal their species composition in the open ocean.

2. Materials and Methods

Fish eggs were collected mainly by step tows of an ORI-BF net (3 m diameter) with a mesh size of 0.5 mm. The number of net tows was 59 in KH-11-4 and 66 in KH-11-6. Fish eggs were visually sorted out from the plankton samples. The fish eggs were photographed using a microscope and morphologically identified based on Ikeda and Mito (1988), Kawakami et al. (2010) and Kawakami et al. (unpublished data).

3. Results and Discussion

A total of 805 eggs were collected during KH-11-4 (245 eggs) and KH-11-6 (560 eggs) (Table 1). Of all eggs collected, 71.9% of eggs were identified at least to the order level. Excluding *Anguilla japonica*, the most abundant species was *Katsuwonus pelamis* and they accounted for 35.9%. Although a few types of eggs that tend to concentrate around seamounts (*Scopelogadus mizolepis* and *Diodon hystrix*) were collected, most eggs were identified as species with widespread egg distribution patterns (*Lampris guttatus*, *Oxyporamphus micropterus* and *Ranzania laevis*).

There were differences in composition of eggs comparing KH-11-4, 6 and KH-02-2 (Table 1). Only a small number of anguilliform eggs were collected in KH-11-4 and 6, although they occupied 5.1% of eggs collected in KH-02-2. On the contrary, the

proportion of *L. guttatus* was considerably higher in KH-11-4 than in KH-02-2. These differences in occurrence of species could be due to differences in sampling areas among cruises, since the sampling area of KH-11-4 and 6 was located south of the shallower seamount area that was extensively researched in KH-02-2. Therefore, it is possible that *L. guttatus* spawns over wide areas of the open-ocean and many anguilliform fishes may spawn around shallow seamounts or more at higher latitudes. DNA species identification can provide a significant new tool for ecological studies on fish eggs. In the KH-11-4 and 6 cruises, 28.1% of the eggs could not even be assigned to any order. The number of DNA sequences accumulated in public DNA database is increasing rapidly, so it can be expected that further DNA analysis of the collected eggs would identify many of the unknown eggs. Furthermore, combining the results of DNA species identification with distribution patterns and the morphological information of eggs would reveal the spawning ecology and diversity of embryonic development of open-ocean fishes and provide new insights into the evolution of fishes.

Table 1. Species composition of fish eggs collected and morphologically identified in KH-11-4 and KH-11-6. Results of KH-02-2 was also shown for comparison.

	KH-11-4		KH-11-6		KH-02-2	
Taxon	No. of eggs	(%)	No. of eggs	(%)	No. of eggs	(%)
Anguilla japonica	0	0	147	26.3	0	0
Serrivomeridae	6	2.4	5	0.9	2	0
Other Anguilliformes	2	0.8	1	0.2	273	5.1
Lampris guttatus	51	20.8	20	3.6	81	1.5
Other Lampridiformes	9	3.7	2	0.4	14	0.3
Exocoetidae	6	2.4	2	0.4	130	2.4
Oxyporhamphus micropterus	0	0	11	2.0	70	1.3
Chauliodus sloani?	9	3.7	2	0.4	19	0.4
Scopelogadus mizolepis	1	0.4	0	0	46	0.9
Phtheirichthys lineatus	2	0.8	3	0.5	30	0.6
Katsuwonus pelamis	73	29.8	216	38.6	982	18.5
Diodon hystrix	9	3.7	1	0.2	50	0.9
Ranzania laevis	0	0	1	0.2	54	1.0
Others	77	31.4	149	26.6	3570	67.1
Total	245		560		5321	

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Kawakami, T., J. Aoyama and K. Tsukamoto. 2010. Morphology of pelagic fish eggs identified using mitochondrial DNA and their distribution in waters west of the Mariana Islands. Environ Biol Fish 87: 221–235

Anguilliform Preleptocephali Collected During the KH-11-4 and KH-11-6 Cruises

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There were various preleptocephali collected during the KH-11-4 and KH-11-6 sampling surveys in addition to the *Anguilla japonica* preleptocephali collected in the second cruise that are described in the Minegishi et al. report. The non-*Anguilla* preleptocephali were collected in the deployments of the ORI-BF net along the West Mariana Ridge from May to July 2011. In KH-11-4, there were 6 anguilliform preleptocephali collected including some that appeared to be those of the Serrivomeridae and Derichthyidae (Fig. 1). These ranged in size from 5.9 to 10.0 mm. In KH-11-6, there were 7 preleptocephali that were not classified as being likely to be *A. japonica*, which ranged in size from 4.5 to 13.5 mm.

Some of these preleptocephali can be estimated to belong to certain taxa if their last vertical blood vessels could be located, but the morphology of most marine eel preleptocephali is not yet know. The preleptocephali from these two cruises were all preserved in ethanol and will be genetically identified later in the laboratory using their DNA sequences. However, if the preleptocephalus shown in Figure 1a, is actually that of *Nessorhamphus* (Derichthyidae), it will be the first report of the early larva of this genus in this region.

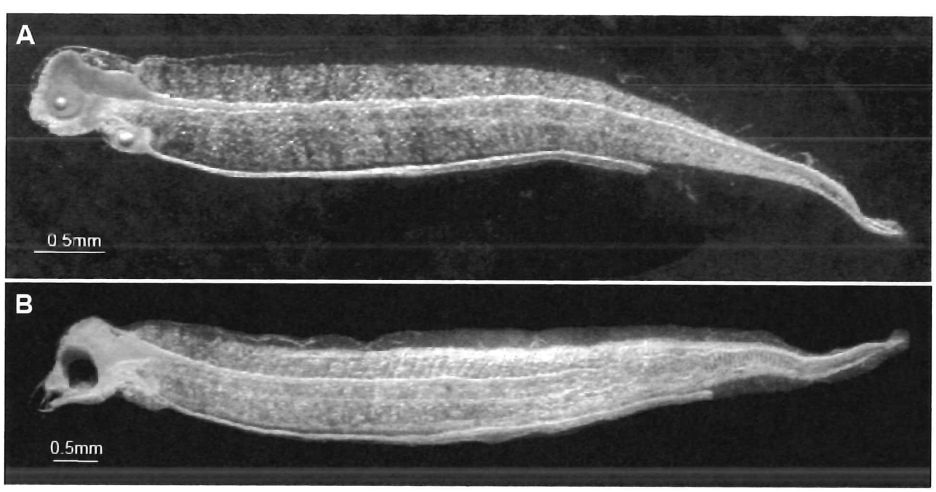

Figure 1. Photographs of preleptocephali of the Serrvomeridae (A, 6.4 mm, St. 58), and possibly of *Nessorhamphus* (B, 10 mm, St. 36) that were collected in KH-11-4.

Leptocephali Collected During the KH-11-4 and KH-11-6 Cruises

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A wide range of species of leptocephali were collected during the KH-11-4 and KH-11-6 cruises that sampled along the West Mariana Ridge from May to July 2011. Sampling for eggs and leptocephali was conducted using the large 3-m ORI-Big Fish (ORI-BF) net with 0.5 mm mesh and also with an IKMT with 5 mm mesh during the first cruise. The ORI-BF was mostly fished in horizontal step tows that sampled at different layers and collected most of the leptocephali obtained during the cruises (Table 1).

There were leptocephali of about 14 families of the Elopomorpha that were collected (Table 1), including 2 species of *Anguilla*, and at least 2 species of notacanthiform and 3 species of saccopharyngiform-type leptocephali (Cyematidae and Eurypharyingidae), with about 33 to 38 total species being collected in each of the two cruises. Leptocephali of *Ariosoma* sp. 7, Chlopsidae, and Muraenidae were the most abundant during the two cruises.

Various leptocephali were collected in good condition and were photographed using the dissecting microscope equipped with a digital imaging system. This enabled good images of the head or other body regions to be obtained (Fig. 1), or for multiple images to be combined to show the whole-body of smaller leptocephali such as of the Cyematidae (Fig. 2A).

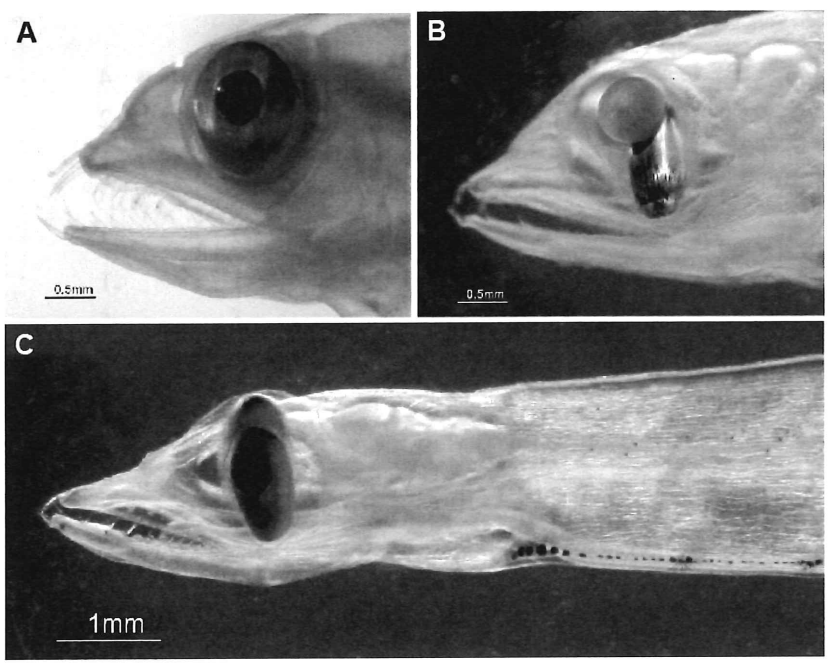

Figure 1. Photographs of *Derichthys* (A, 57.0 mm), Synaphobranchinae (B, 57.0 mm) and notacanthiform (C, 170.3 mm) leptocephali collected during the KH-11-4.

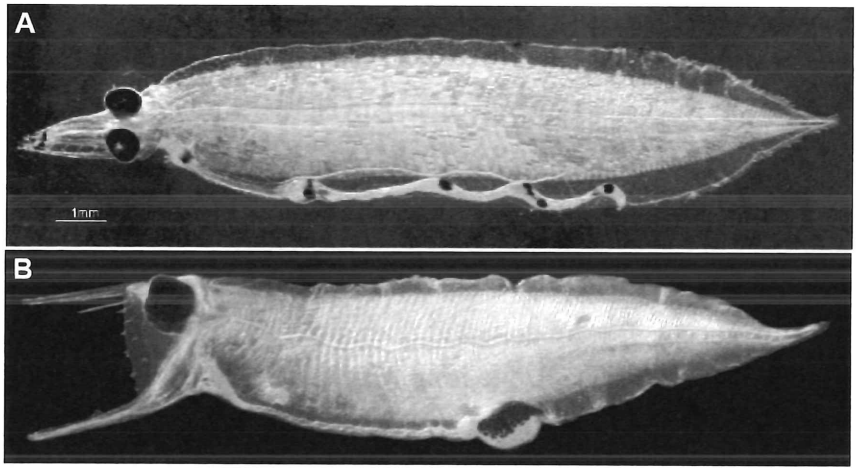

Figure 2. Photographs of a Cyematidae (A, 15.4 mm) and Eurypharyingidae (B, 8.7 mm) leptocephali collected during the KH-11-6 and KH-11-4 cruises, respectively.

Table 1. Catches of leptocephali and approximate number of species during the KH-11-4 and KH-11-6 cruises to the Japanese eel spawning area from May to July 2011, separated by type of net used. Only the ORI-BF net was used in the KH-11-6 cruise.

Taxa	KH-11-4 ORI-BF	KH-11-4 IK-5mm	Number of species	KH-11-6	Number of species
Anguilliformes					
Anguillidae					
Anguilla bicolor pacifica	1		1	7	1
Anguilla preleptocephali				76	1
Chlopsidae	10	1	5	23	5
Congridae					
Ariosoma sp. 7	5	3	1	24	1
Ariosoma sp. 4	4		1	5	1
Conger sp.	3		1		
Congridae spp.	2		2	4	3
Derichthyidae	5	1	2	2	1
Moringuidae	3		1	1	1
Muraenidae	11		~7	19	~11
Nemichthyidae	3		2	3	1
Nettastomatidae	1		1	1	1
Ophichthidae	1		1	6	6
Serrivomeridae	8		≥1	7	1
Serrivomerid juveniles/adults	7	12	≥1	5	≥1
Synaphobranchidae	4		2		
Saccopharyngiformes	8		3	3	3
Notacanthiformes	3		2	1	1
Unidentified				6	
			~33		~38

Occurrence of an Ophidiid Larva Collected During Leg 4 of the KH–11–04 Cruise

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Fishes of the family Ophidiidae, suborder Ophidioidei, are comprised of four subfamilies with 48 genera and about 222 species (Nelson 2006). Among them, larvae of 31 species have already been reported in the western central Atlantic by previous studies (Fahay and Hare 2006). Each subfamily is characterized as follows: elongate body, developed fan–shaped pectoral fins, and barbels on snout and tip of lower jaw in Brotulinae (Okiyama 1988); compressed body, moderately to extremely trailing gut, developed cartilaginous ventral process of the coracoid in Brotulotaeniinae (Fahay 2007); elongate body with relatively short preanus length, long dorsal and anal fins confluent with caudal fin in Ophidiinae (Fahay 2007); many variations in body shape and wide meristic characters in Neobythitinae.

In this cruise, an Ophidiid larva (44.9 mm in total length) was collected by the Isaacs-Kidd Midwater Trawl (wire out 710 m, mesh size 5.0 mm) at St.17 on 27 May 2011 (Fig. 1). The characters of the specimen are as follows: dorsal fin rays (D) 169+ (broken), anal fin rays (A)132+ (broken), pectoral fin rays (P_1) 20, pelvic fin rays (P_2) 3 [broken in caudal fin rays (C)]. Body (especially tail) extremely elongate. Head small. Melanophores absent on body. Although the combination of numbers of dorsal and anal fin rays of the specimen is similar only to the genus *Porogadus* (D 170-188, A 135-156) among the Ophidiidae, the

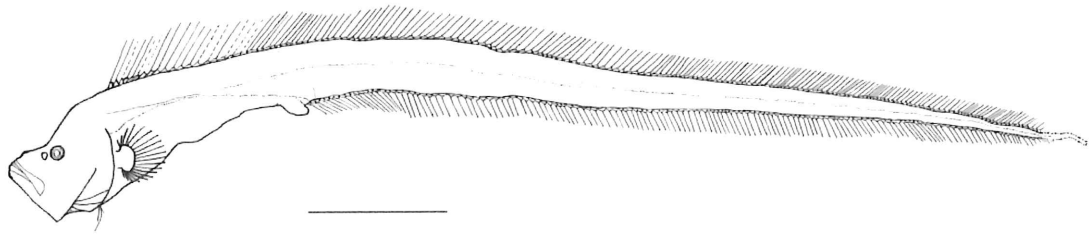

Figure 1 Ophidiidae sp., KH–11–04, St.17, 44.9 mm TL. *Scale bar* 5 mm.

pectoral and pelvic fin rays are different from this genus (P_1 16-19 and P_2 2). In addition, the melanophore pattern also is different from already known larvae of *Porogadus miles* (with 5 patches on the tail). Therefore, the taxon that the specimen belongs to is not clear at the present. I will study the morphological development of larvae belonging to family Ophidiidae, collected from the West Mariana Ridge region including specimens from past cruises.

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Studies on Feeding Ecology of Phyllosoma Larvae of Palinurid and Scyllarid lobsters

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1. Introduction

The larvae of palinurid and scyllarid lobsters are called "phyllosoma", having transparent and flattened body. Phyllosoma larvae are distributed in open ocean, have long planktonic period for over 6 months. The prey items of the phyllosoma larvae in the national environment have been unknown because their low natural density has also made wild specimens difficult to capture for experimental observations. The main purpose of the present cruise is to collect phyllosoma larvae for analyses of stable isotope ratios, fatty acid composition and gut contents, and for species identification using genetic markers.

2. Phyllosoma, plankton and POM sampling

A total of 39 phyllosoma and 1 puerulus larvae ware corrected using a ORI-Big Fish (7.1 m2 mouth opening and 0.5mm mesh aperture) towed step-wise during KH-11-4,6 (Table.1). Phyllosomas were sorted from the original zooplankton samples immediately after capture. These phyllosomas were fixed or frozen for the respective analyses as described below.

POM(particulate organic matter) were collected by filtered onto precombusted GF/F filters at depths 5m, 50m, Chl-a maximum , 100m, and 200m. After sampling, these filters were preserved at -80 $_{o}$C.

3. Food habitats of phyllosoma

Studies of mouthpart and gut morphology of phyllosoma larvae have suggested that they are suited for feeding on soft foods such as gelatinous zooplankton (Nishida et al., 1990; Johnston and Ritar, 2001). DNA analyses of gut contents have identified Cnidaria and Urochordata (Suzuki et al., 2006) as potential food sources. On the basis of these bodies of information, we collected gelatinous zooplankton samples (Cnidaria, Ctenophore, Chaetognatha, Heteropoda, Thecosomata, Gymnosomata, Thaliacea, Salpida), net zooplankton mixture (random sampling) and POM for stable isotope analysis to estimate trophic levels of phyllosoma. These zooplankton samples and sorted phyllosomas were identified to genera, families, or higher taxonomic groups and frozen at -80$_{o}$C

for the analyses of stable-isotope ratios and fatty acid composition on land. Some other phyllosomas were frozen or fixed in 100% alcohol for genetic identification of gut-contents, or fixed in 2% formaldehyde or in Karnovsky's fixative for light/electron microscopic observation on feeding structure and gut contents.

4. Species identification of phyllosoma with genetic markers

Species of scyllarid phyllosoma is difficult to identify, due to the limited morphological information, that are available only for the final stage larvae in most species. In addition, identification at the genus level is also difficult, particularly between *Parribacus* and *Scyllarides*, by morphological characteristics. Genetic species identification of invertebrate is often possible on the basis of mitochondrial cytochrome oxidase subunit I (COI) and/or 16S rDNA genes and coupled with a GenBank homology search. However, COI and 16S rDNA references of scyllarid lobsters are still very incomplete. With this circumstance, we also aimed at obtaining sequence references and morphological characteristics of the scyllarid lobsters. Some of the sorted scyllarid phyllosomas were identified to possible lowest levels and preserved in 100% alcohol for genetic analysis, while some other specimens were fixed and preserved formaldehyde as references for morphological analysis

Table 1 Number of phyllosoma larvae collected during KH-11-4,6 cruises.

	KH-11-4	KH-11-6
Panulirus spp.	_	17
Scyllarinae spp.	_	8
others	_	2
total	12	27

Evaluation of Whole-Body Photographic Techniques for Leptocephali During KH-11-4, 6

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Because leptocephalus larvae grow much larger than normal fish larvae, it is challenging to obtain publishable photographs of leptocephali onboard research vessels that are constantly moving. Photographing the head or other regions of leptocephali through a microscope is effective, but whole body images can not be obtained without combing multiple images manually using computer software.

It is especially difficult to obtain quality whole-body photographs of the larger sized larvae because if they are put inside water, they move side-to-side as the ship is moving with the ocean. If they are not in water, many reflections can form on the body surface and reduce the image quality.

The Miller and Tsukamoto (2004) book published many of the first whole-body photographs of leptocephali that are now available to the world, but these photographs were difficult to obtain using hand-held digital cameras, or through combining 3-6 digital images taken through a dissecting microscope equipped with a digital imaging system. This report briefly evaluates the use of new more advanced equipment for photographing leptocephali onboard the R/V *Hakuho Maru* during the KH-11-4 and KH-11-6 cruises.

The new method of obtaining whole-body photographs consisted of using a Nikon D-7000 digital SLR camera with a Nikon 60 mm macro-type lens for medium size leptocephali and a normal 35 mm lens for larger sizes. The camera was attached to a small-sized copy stand, and a 2-arm fiber optic light provided illumination of the leptocephali.

During the first cruise 12 specimens were photographed and 14 were photographed in the second cruise. Specimens of the Chlopsidae, Moringuidae, Muraenidae, Derichthyidae were successfully photographed, and some high quality photographs of freshly caught leptocephali were obtained (Fig. 1, 2).

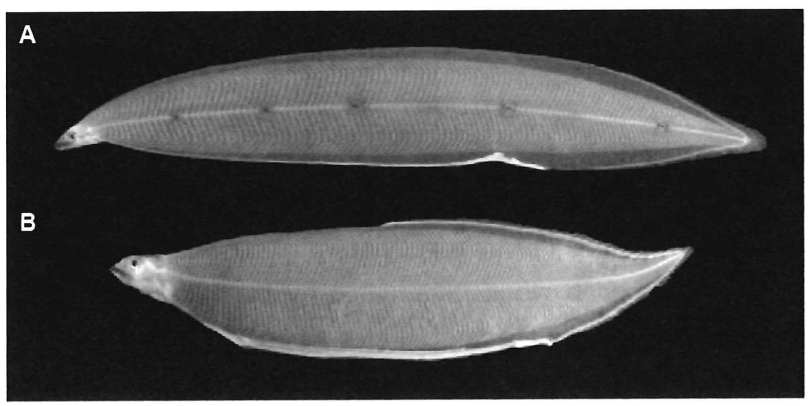

Figure 1. Whole body photographs of leptocephali of *Moringua* sp. (A, 58.3 mm) and *Derichthys serpentinus* (B, 57.1 mm) taken during KH-11-4 using the new camera and copy stand.

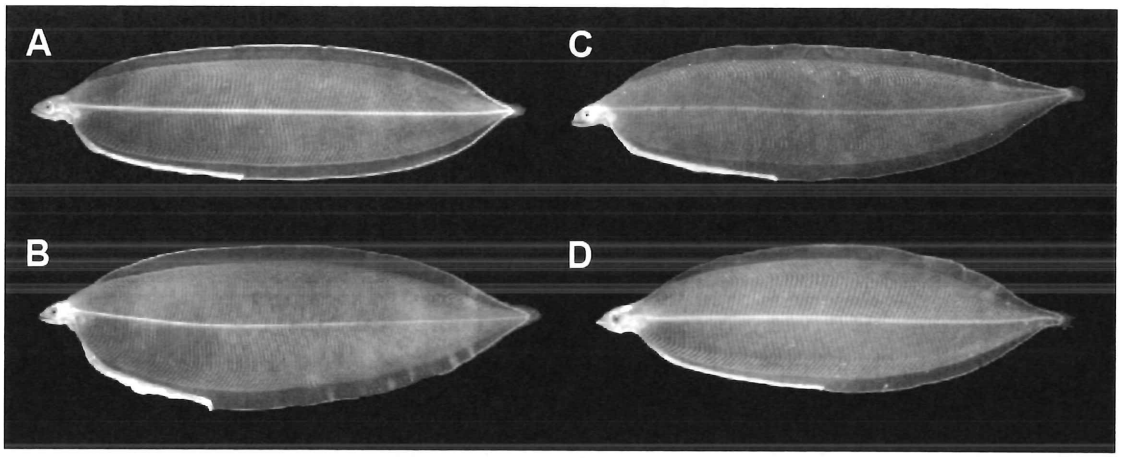

Figure 2. Whole body photographs of Chlopsidae leptocephali including *Kaupichthys* sp. (A, 51.5 mm), Chlopsidae sp. 4 (B, 66.0 mm), *Robinsia caterinae* (C, 68.0 mm), and a *Chlopsis* type (D, 45.5 mm) collected during KH-11-4. (Note: image resolution greatly reduced to minimize file size)

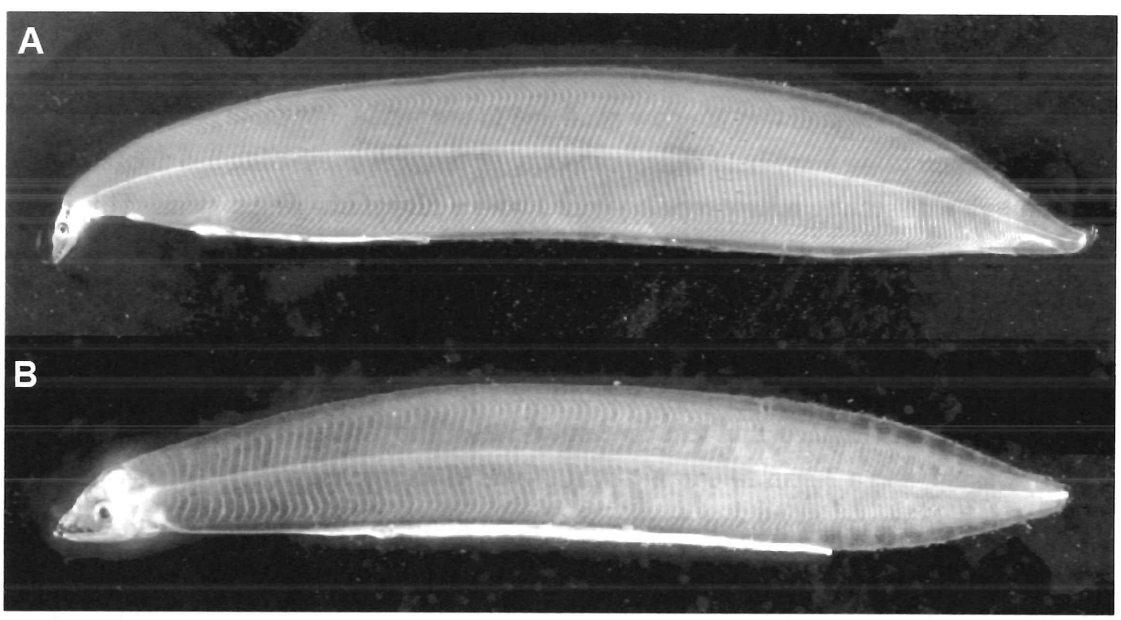

Figure 3. Whole body photographs of *Neenchelys* (Ophichthidae) (A, 68.6 mm) and Muraenidae (B, 25.3 mm) leptocephali that show examples of poor focus or reflections on the head or tail regions that were photographed during KH-11-6.

Several problems remain to be solved however, in order to be able to easily obtain quality images of large leptocephali onboard research cruises. During KH-11-6 seawater from the ship was filtered to reduce the amount of particulate material that shows up as bright spots on and around the leptocephali when illuminated by the fiber optic lights. Another persisting problem is the formation of small bubbles when the leptocephali are transferred to the dish for photographing. These image contamination issues increase the

time required to successfully photograph each specimen. Larger bodied species > 100 mm also continue to be challenging for obtaining quality photographs due to the lack of ability of the lens to focus adequately on the distant head and tail regions of the specimens (Fig. 3). Efforts to solve these problems will be made during future cruises to collect leptocephali.

Estimation of Spawning times of 3 Batches of Eggs Collected During KH-11-06 and Speculation about Northward Transport of Preleptocephali

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Batches of eggs were collected 2 times during the KH-11-6 cruise. 113 eggs were collected at the dawn of 29 June and 11 eggs were collected during the nighttime of the same day. The development stages of eggs collected at dawn were relatively synchronized, however the eggs collected at nighttime were not. The eggs collected at nighttime consisted of 2 groups that were one group of eggs just after fertilization and another group that already had the embryo formed. Eel eggs hatch 1~1.5 days after fertilizing, so based on that, it is likely that 3 batches of eggs were collected that included those spawned on 27 June, 28 June, and 29 June (Fig. 1). From the fact that the eggs just after fertilization were collected around 10 p.m., spawning might take place during the early part of the nighttime period.

In some previous years, spawning has appeared to take place further north than eggs were collected during this cruise and during 2009. In 1998, several small leptocephali were collected around 16°N, 143°E, which is farther north compared to the place eggs and preleptocepali were caught in recent years (Fig. 2). There are 2 possible explanations for the leptocephali around 16°N. The first reason could be that spawning took place along the northern part of Mariana ridge such as when the salinity front is at its northern limit of about 16°N. The salinity front hypothesis suggests that the latitude of spawning changes according to the latitude of the front. Also during this cruise, eggs were collected at different locations from the places where eggs were collected in 2009 (Fig. 2). In addition, from the distribution of preleptocephali, the spawning sites were considered to be located at different places in 2005, 2007 and 2008. This suggests that spawning sites of Japanese eel are not stable and the assumption that spawning occurred in the north in 1998 is possible.

The second reason could be that the spawning site is typically located in relatively southern areas in recent years and that the larvae become transported to the north. In 2008, the largest number of preleptocephali (113 individuals) was caught around 12.2°N, 141.8°E (Fig. 2) (A). The catch of preleptocephali decreased as the sampling location increased with distance from that location. The distribution of preleptocephali in 2007 shows the same trend too. Although the result of this cruise revealed that

spawning occurred several times at several places before the new moon, it is possible that the preleptocephali collected in 2007 and 2008 were spawned somewhere near where the largest number of individual were collected. When the period of the preleptocephalus stage is assumed to be 7 days, larvae would have to be transported by an average speed of 13.3 n-mile/day (28.6cm/sec) to reach from (A) to the northern end of distribution in 2008 (B). Similarly, preleptocephali would have to be transported by an average speed of 11 n-mile/day (23.7 cm/sec) and 12 n-mile/day to reach from (C) to (D) and (C) to (E), respectively. From the total length, the age of leptocephali collected in 1998 was estimated to be about 20 days. If the spawning site of these individuals is presumed to be the same as the place the eggs were collected in this cruise (13°N, 142°E), they would have to be transported at a speed of 10.2 mile/day to reach (F). This speed is slower than the estimated speed of transportation of preleptocephali mentioned above, and could be a realistic speed. From these results, both spawning places and transport histories could be possible and it is difficult to judge which one is correct at the moment.

In 1991, a large number of leptocephali were collected at 16°N, 137E°(Fig. 3). Previously these larvae were thought to have drifted directly east, but they also could have been transported from the area where eggs have been collected in 2011. If the hatch date of the larvae was assumed to be 3 days before the new moon of June, the larvae would have needed to be transported at 15 mile/day. Considering the fact that the average velocity of the North Equatorial current is 9.3~15 mile/day, this type of transport could happen.

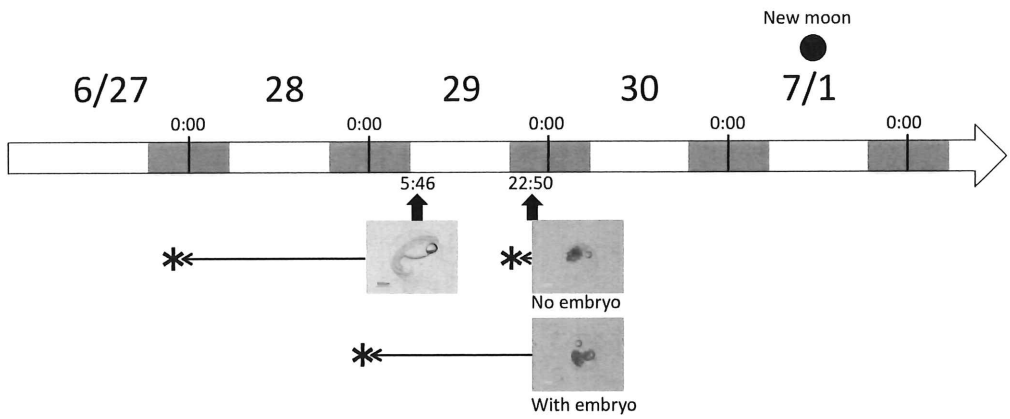

Figure 1 The timing of egg collection and the estimated spawning times of each batch in June 2011. * represents the estimated time of spawning. The gray part in the bar shows the nighttime.

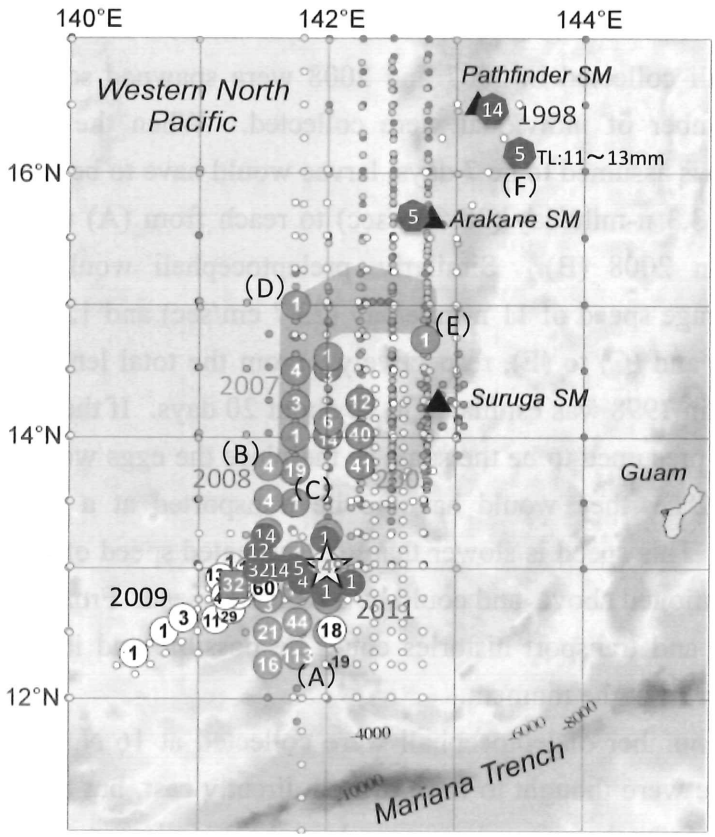

Figure 2. Historical catches of eggs, preleptocephali and leptocephali (Modified from Tsukamoto et al. 2011). Circles show the locations where preleptocephali were collected. Red square and star represent where eggs were collected in 2009 and 2011, respectively. Purple hexagons show where leptocephali were collected in 1998. Each number in the objects shows the number of individuals collected at each site.

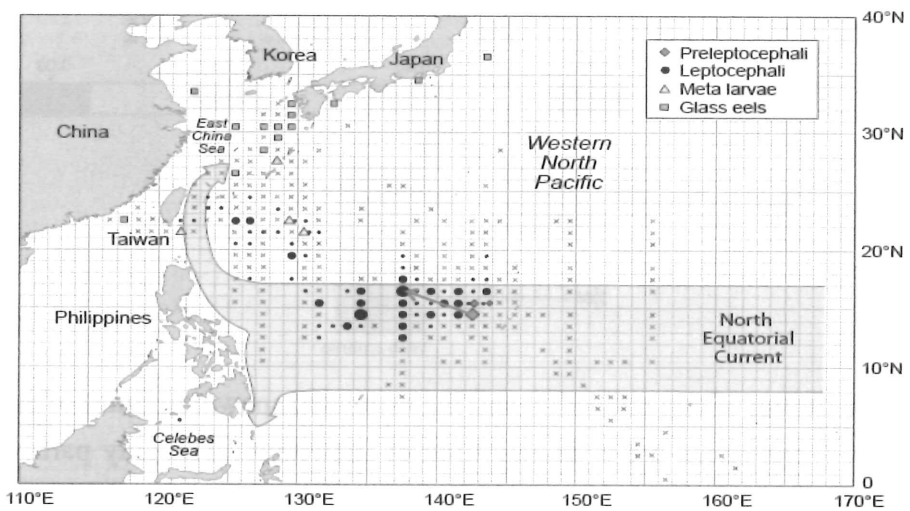

Figure 3. Historical catches of Japanese eel larvae and glass eels (from Shinoda et al. 2011). Red arrow indicates the transportation route discussed in this study.

Relationship Between Net-depth and Wire-out of the 3-m ORI-Big Fish Ring Net

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A 3-m ORI-Big Fish ring net (7.1 m^2 mouth opening and 0.5 mm mesh) and a 3 m Isaacs-Kidd midwater trawl (8.7 m^2 mouth opening, 0.5 or 1.0 mm mesh) have been used in spawning ground surveys for the Japanese eel *Anguilla japonica* Temminck & Schlegel. Though no relationships between net-depth and wire-out of these nets are known in detail. In this report, I described the relationship between net-depth and wire-out of the 3-m ORI-BF ring net for future reference.

The 3-m ORI-BF net was towed 110 times during the KH-11-4 and KH-11-6 cruises to collect eggs and larvae of Japanese eel and other samples. When the net was being deployed, wire out speed and ship speed were fixed at 1.0 m/sec and 2.5 nautical miles/hour, respectively. In 104 tows of the net, the maximum net depths and maximum wire lengths were recorded.

The relationship between net-depth (D) and wire-length (W) of the 3-m ORI-BF ring net showed a positive linear correlation (the intercept was fixed at 0) (Fig. 1) according to the relations:

$D = 0.4077\ W$

Mean ± SD (min - max) of the values of net-depth/100 m wire-out was 41.67 ± 6.23 m (29.06 – 61.15 m). Variation in the values may be caused by complex current directions, different current speeds and mesh clogging, but there is no data on that. In the future, it is important to evaluate possible causes of the instability of the net-depth/wire-out ratio.

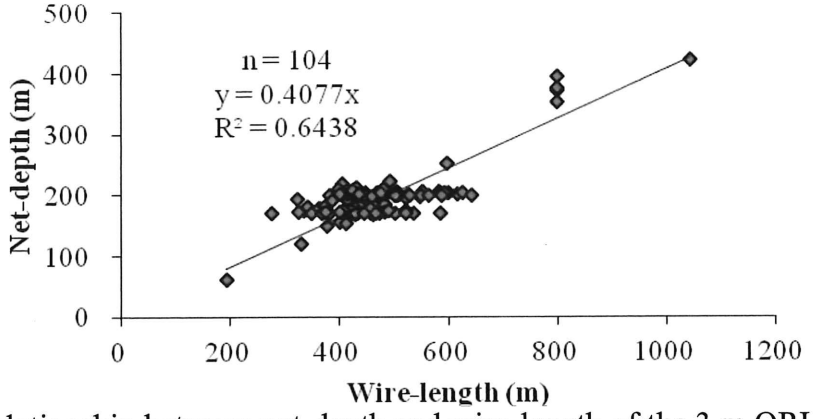

Figure 1. Relationship between net-depth and wire-length of the 3 m ORI-BF ring net towed during the KH-11-4 and KH-11-6 cruises.

Test of the Underwater Camera for Observing Spawning Behavior of the Japanese Eel

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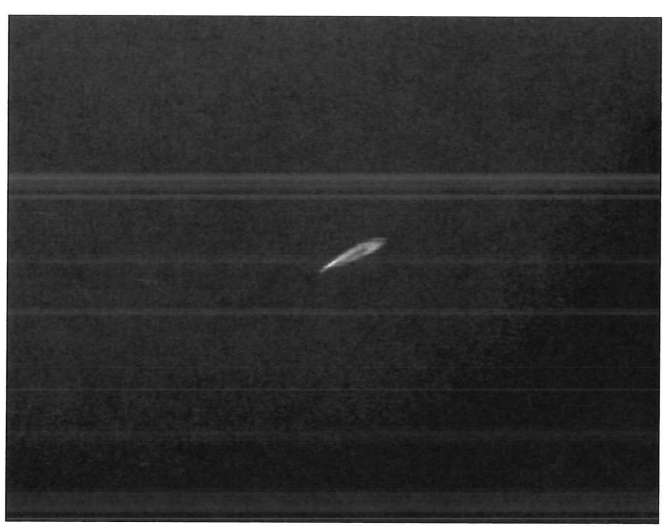
In order to observe the spawning behavior of the Japanese eel, *Anguilla japonica*, an underwater camera was tested. The camera system was 40 cm long, 40 cm wide, 78 cm in height, and 70 kg in weight (Fig. 1). The camera recorded video continuously during deployment and a light switched on for 1 minute at 4 minute intervals. The camera was operated on 27 May and 30 June 2011 during the KH-11-4 and KH-11-6 cruises (Table 1) in the spawning area. The maximum depth layer reached by the camera was 500 m and the camera operated well at all depths. The camera did not record any spawning behavior of the Japanese eel. However, many plankton were recorded, and on 30 June, a fish was seen in the 90 – 115 m layer (Fig. 1). The result suggests the camera is likely to be available to photograph eel spawning behavior in the open sea if a spawning aggregation can be located precisely.

Table 1. Locations, times, and depths of use of the underwater camera system.

Area	Latitude	Longitude	Date	In	Out	Wire out	Photographing layer
St. 13	14-14.7 N	142-51.5 E	5/27/2011	4:40	6:05	500 m	0-500 m
WC1	12-57.0 N	142-05.5 E	6/30/2011	1:09	3:12	300 m	0-300 m

Figure 1. The underwater camera (left) and a fish seen in the video recordings made by the camera that was at a depth of 90-115 m (right).



The Trial of Spawning Behaviors of the Japanese Eel *Anguilla japonica* using Dual-Frequency Identification Sonar (DIDSON)

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The resent study about the spawning ecology of the Japanese eel, *Anguilla japonica*, was remarkably progressed. For example, during new moon throughout the spawning season, Japanese eel eggs were collected near the West Mariana Ridge where adults and newly hatched larvae were also caught (Tsukamoto et al. 2011; Chow et al. 2009; Tsukamoto 2006). However, the spawning behavior of the Japanese eel has not been observed until now. To better understanding of eel ecology and improving their artificial reproduction, observations of spawning behavior of Japanese eel were important.

In general, eels exhibit strongly negative-phototaxis. Although the precise depth and time of spawning are still unknown, the spawning site is assumed to be unlighted condition. Therefore, it is difficult to observe using optical camera because these are needed very strong light to record images. In this situation, sonar camera which is no need to light is a strong tool for observation. In this study, we firstly try to observe the spawning behavior of the Japanese eel by using Dual-Frequency Identification Sonar (DIDSON) which has features written especially for fish behavior analysis and quantification.

The trial of observation had been done at July 1 on morning. Unfortunately, the trial was failed because the tow attachment has serious hydraulic problem. However, we succeeded recording the surface layer image using DIDSON (Fig. 1). In future, we have to improve the tow attachment.

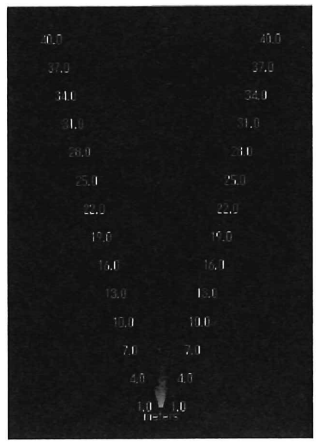

Figure 1 the surface layer image.

Reference

Tsukamoto K et al. 2011. Oceanic spawning ecology of freshwater eels in the western North Pacific. Nature communications, DOI: 10.1038/ncomms1174.

Chow et al. 2010 Discovery of mature freshwater eels in the open ocean. Fish. Sci., 75: 789-791.

Tsukamoto K. 2006. Spawning of eels near a seamount. Nature, 439: 929.