

**Preliminary Report  
of  
The Hakuho Maru Cruise  
KH-06-2 Leg 2,3,5**

Leg 2: June 26, 2006 - July 12, 2006

Leg 3: July 15, 2006 - July 27, 2006

Leg 5: Aug. 15, 2006 - Sep. 05, 2006

(Eel Cruise XIII)

Atmosphere and Ocean Research Institute  
The University of Tokyo  
2012

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By  
The Scientific Members of the Expeditions

Edited by  
Shun Watanabe, Kazuki Yokouchi  
and Katsumi Tsukamoto

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## KH-06-2 Cruise Report

### Introduction

At the end of 20<sup>th</sup> century, the mystery of spawning of the Japanese eel was partly revealed by collecting their tiny transparent larvae called leptocephali in the Philippine Sea, which showed their remarkable migration of thousands of kilometers out into the ocean for spawning. In the latest research cruise of the R/V Hakuho-Maruk KH-05-1, we successfully collected many genetically identified newly-hatched preleptocephali of the Japanese eel near a seamount during the new moon of June 2005, and located the spawning area of the Japanese eel precisely. This was the first clear evidence of a pinpoint spawning location of an anguillid eel, which insures entrainment into a specific current that transports its larvae properly to their growth habitat in East Asia and avoids southward transport outside of its species range.

Based on this result, we planned this research cruise KH-06-2 to reveal the spawning and migration of the Japanese eel. The main objectives of Legs 2, 3, 5 were to examine the relationship between eel spawning and migration by (1) determining the precise spawning area of the Japanese eel by collecting preleptocephali and eggs in Leg 3 and 5 (July 15-27 and August 15-September 5, respectively) and (2) understanding the process and mechanism of larval migration in relation to oceanographic conditions in Leg 2 and 5 (June 26-July 12 and August 15-September 5, respectively).

We have eventually met with many Typhoons because the spawning area of the Japanese eel is mysteriously located in the birthplace of many typhoons, so observations of the oceanographic conditions in Leg 2 and 3 were difficult and the location of the salinity front may have been affected. In spite of this difficulty, however, we could make substantial observations and sampling during the cruise, for example we made 219 tows of ORI-Big Fish and IKMT Net by filtering as much as 5 million cubic meters of ocean water, and 761 leptocephali or juvenile eels of 16 families of eels and their relatives were collected during the three legs. Many specimens were collected for studies on leptocephali, and extensive water samples were taken for research on the biological characteristics of the spawning and larval development areas of the Japanese eel. All the data and samples are precious and important, and will be precisely analyzed in laboratory after cruise.

We acknowledge the captain and his crew of the R/V Hakuho Maru for their heartfelt cooperation.

September 5, 2006

In the cabin of Hakuho Maru

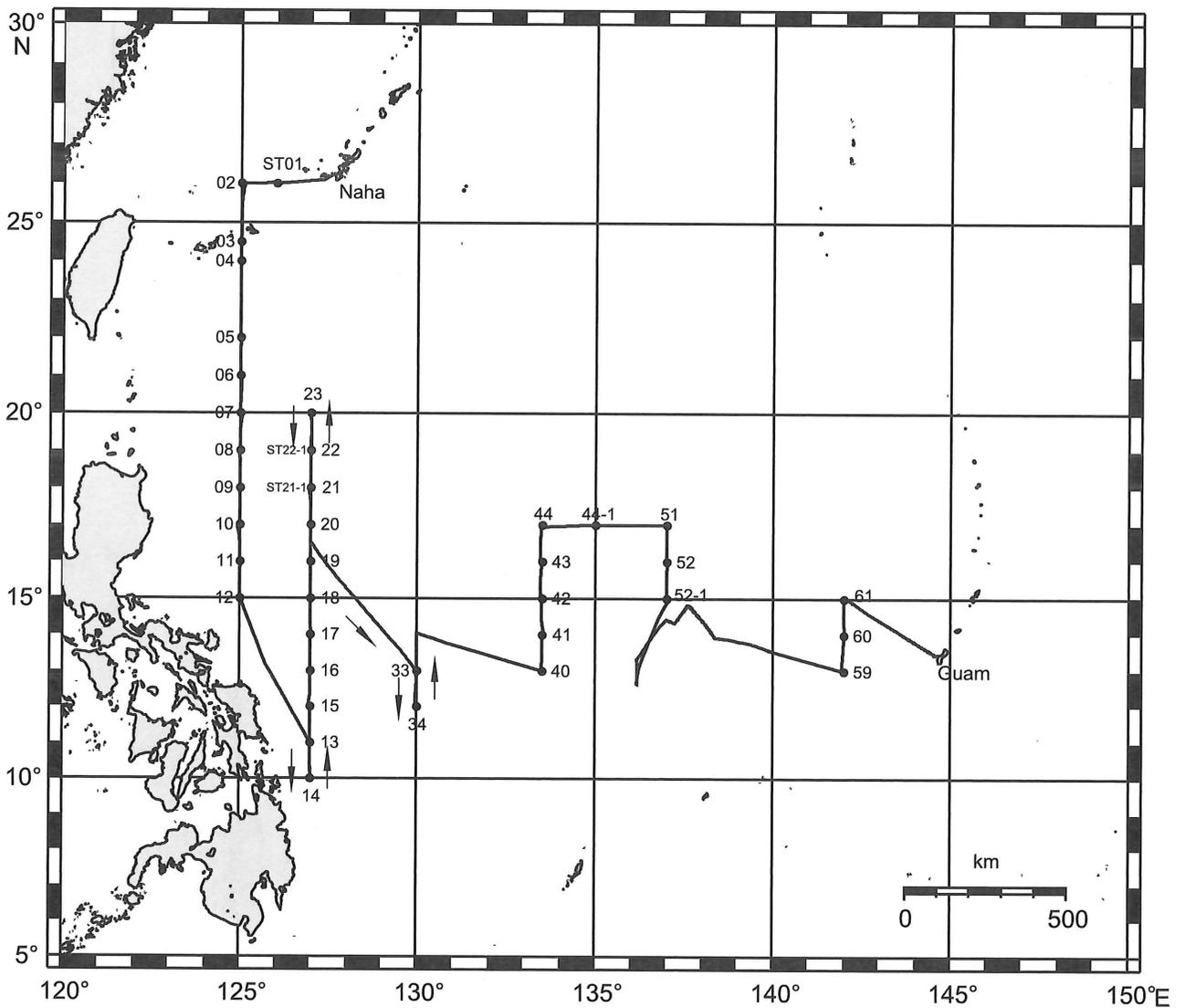
Katsumi Tsukamoto  
Tsuguo Otake  
Shingo Kimura

## List of Participants

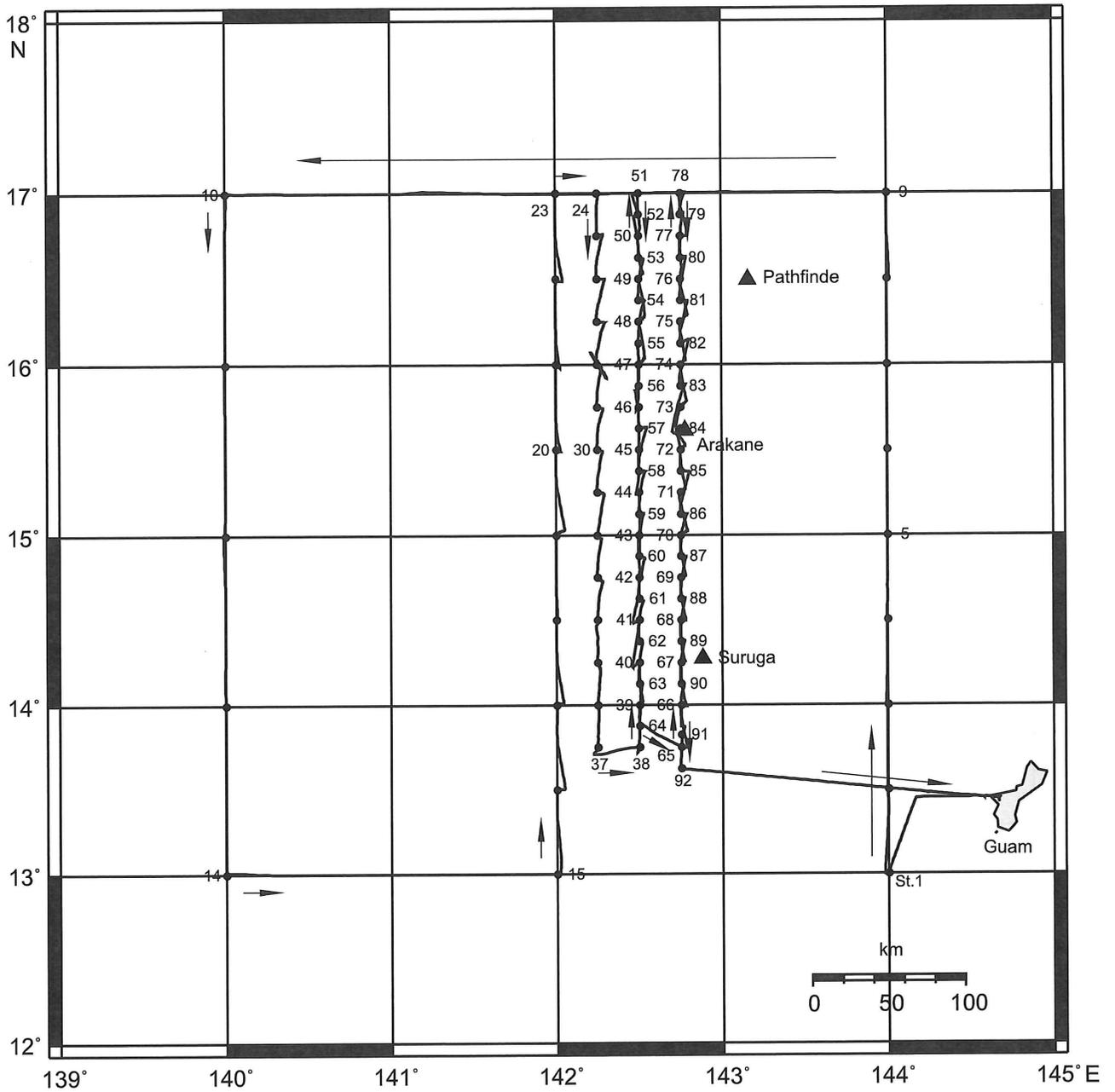
Affiliation	Position	Name	Leg		
			2	3	5
			6/26 -7/12	7/15 -7/27	8/15 -9/6
Ocean Research Institute, The University of Tokyo	Professor	Katsumi Tsukamoto		○	
	Professor	Tsuguo Otake			○
	Associate Professor	Shingo Kimura	○	○	
	Research Associate	Jun Aoyama	○	○	○
	Research Associate	Sachihiko Ito	○		
	Research Associate	Takashi Kitagawa	○		
	Technical Officer	Machiko Oya		○	○
	Technical Officer	Hideo Nagae	○		○
	Technical Officer	Kenji Oguma			○
	Technical Officer	Hideo Ishigaki	○	○	○
	Research Fellow	Shun Watanabe	○	○	○
	Research Fellow	Akira Shinoda	○	○	○
	Research Fellow	Michael J. Miller	○	○	○
	Research Manager	Asako Matsumoto		○	○
	JSPS Fellow	Hee Yong Kim	○		○
	Postgraduate Student	Yoshiki Kato	○	○	
	Postgraduate Student	Nobuto Fukuda	○	○	○
	Postgraduate Student	Youichi Miyake	○		○
	Postgraduate Student	Mari Kuroki	○	○	
	Postgraduate Student	Kei Zenimoto	○	○	○
	Postgraduate Student	Naoki Yamaoka	○	○	○
	Postgraduate Student	Sachie Miyazaki	○	○	○
Postgraduate Student	Etsuko Sawada	○	○	○	
Postgraduate Student	Seishi Hagihara	○	○	○	
Postgraduate Student	Tomita Yasuo			○	
Department of Agricultural and Life Sciences, The University of Tokyo	Professor	Yuzuru Suzuki			○
	Postgraduate Student	Motoko Kawabe		○	
Graduate School of Marine Science and Technology, Tokai University	Postgraduate Student	Nozomu Eto		○	
	Postgraduate Student	Munehiro Takami			○
Graduate School of Biosphere Science, Hiroshima University	Professor	Kazumasa Uematsu			○
	Associate Professor	Tetsuo Harada			○
	Postgraduate Student	Takao Inoue			○
Faculty of Education, Kochi University	Postgraduate Student	Takamasa Ishibashi			○
	Associate Professor	Noritaka Mochioka		○	
Faculty of Agriculture, Kyushu University	Associate Professor	Hisayoshi Yokose		○	
Faculty of Science, Kumamoto University	Associate Professor			○	

Affiliation	Position	Name	Leg		
			2	3	5
			6/26 -7/12	7/15 -7/27	8/15 -9/6
Department of Oceanography, Chungnam National University NFRDI, Korea Aberdeen University, Ocean Lab.  Irago Institute Journalist NHK  Setonaikai Broad Casting LTD Kaiyo Denshi LTD. MARINE WORKS JAPAN LTD.	Professor	Tae Won Lee		○	
	Researcher	Sun-Do Hwang	○		
	Post Doctral Fellow	Camila Henriques			○
	Post Doctral Fellow	David Bailey			○
	Vice Director	Yoshiaki Yamada		○	
		Bunpei Ai		○	○
	Director	Yasuhiro Koyama		○	
	Camera man	Hiroshi Ebisawa		○	
	Camera man	Toshinori Ariyoshi		○	
	Engineer	Taichi Kawakami		○	
Engineer	Shinichiro Yokokawa	○		○	
Engineer	Satoshi Ozawa		○		

# KH-06-2\_Leg2 Track Chart

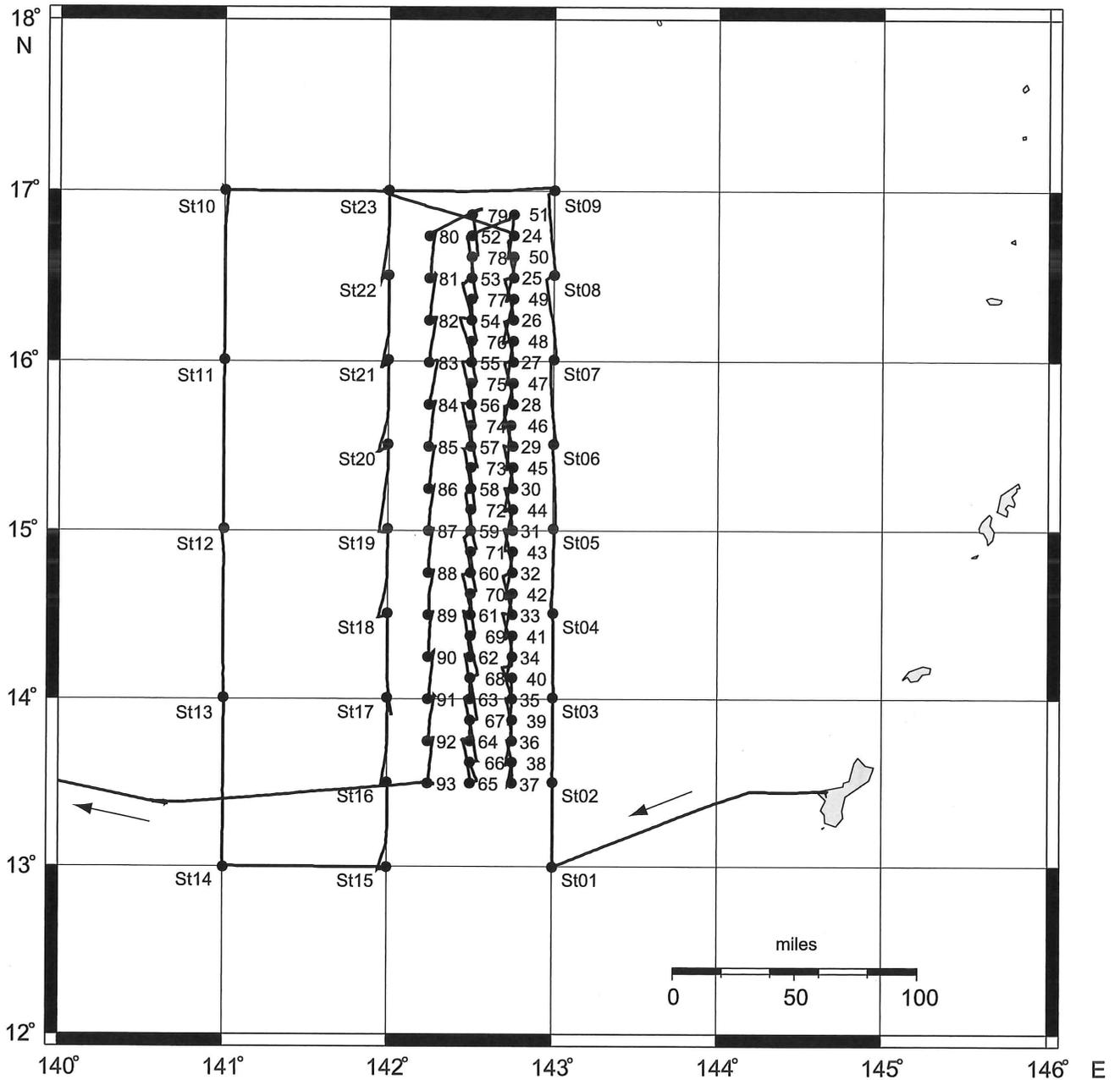


# KH-06-2\_Leg3 Track Chart

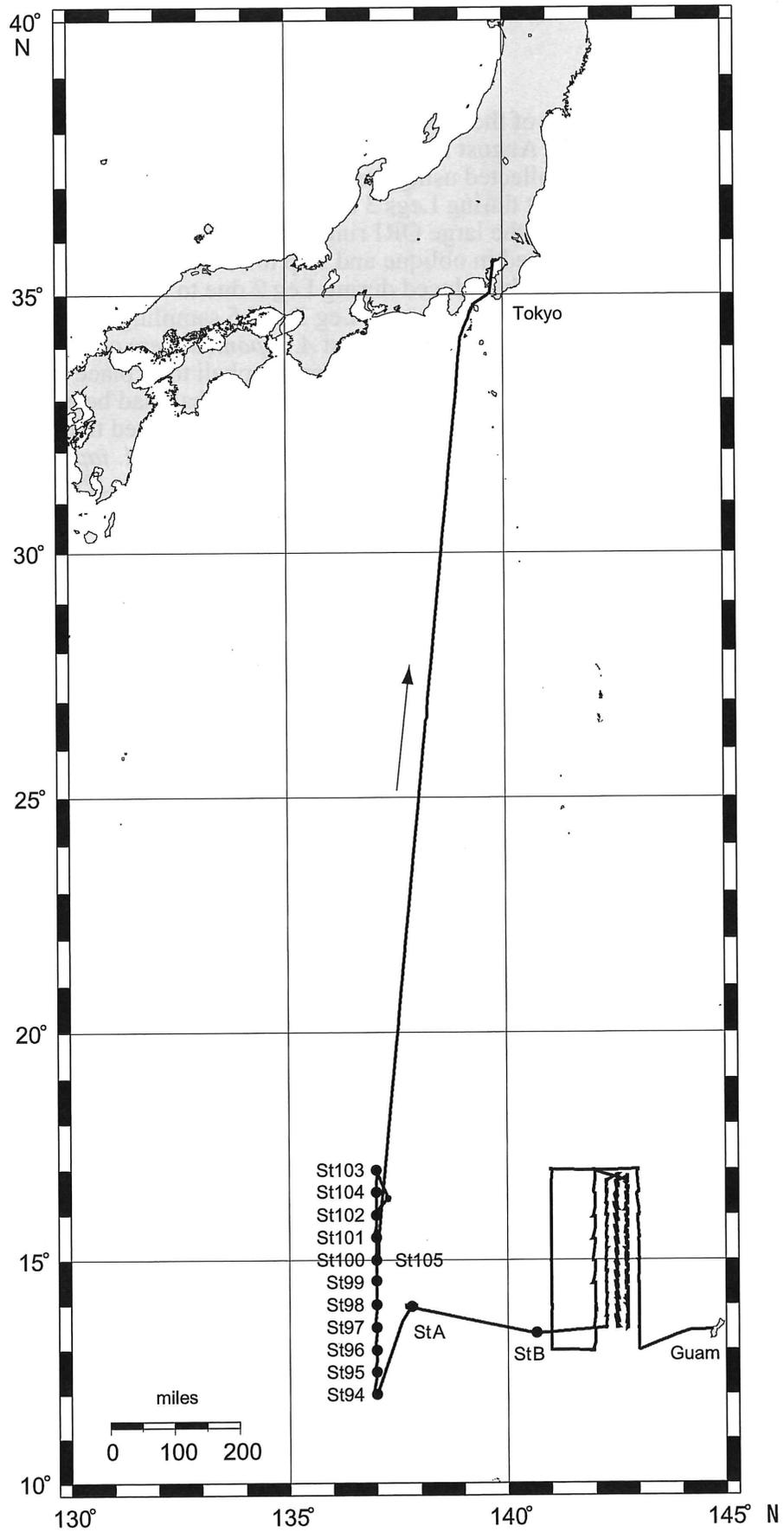


# KH-06-2\_Leg5 Track Chart

## - Grid Survey -



# KH-06-2\_Leg5 Track Chart

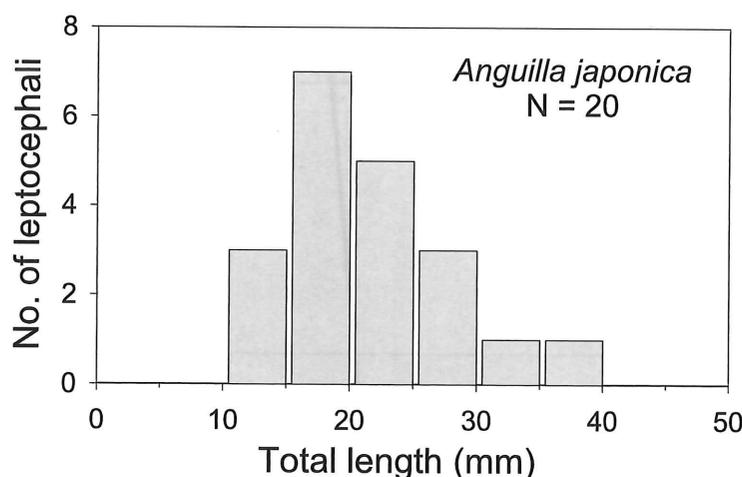


## *Anguilla japonica* Leptocephali Collected During the KH-06-2 Cruise

### All Scientists on Board

Sampling for the leptocephali of the Japanese eel, *A. japonica*, was carried out using 219 net tows between 26 June and 31 August 2006 during the KH-06-2 cruise. A total of 20 *A. japonica* leptocephali were collected using the Isaacs Kidd Midwater Trawl during Leg 2, and using the 3-m ORI ring net during Legs 3 and 5. The IKMT had an 8.7 m<sup>2</sup> mouth opening and 0.5 mm mesh and the large ORI ring net had a 7.1 m<sup>2</sup> mouth opening and 0.5 mm mesh. Both nets were fished in oblique and step tows during daytime and nighttime. The sampling grid was drastically reduced during Leg 2 due to 2 typhoons, but 14 *A. japonica* leptocephali were collected. During Leg 3 and 5 sampling was primarily targeting eggs and preleptocephali, but 2 and 4 larger sized *A. japonica* were collected during each leg, respectively. The sampling for eggs and preleptocephali took place to the west of the seamounts of the West Mariana Ridge where small leptocephali had been previously collected (Ishikawa et al., 2001), and where it has been hypothesized that the Japanese eel may spawn (Tsukamoto et al., 2003). In 2005, preleptocephali of *A. japonica* were collected in the area to the west of the Surgua Seamount (Tsukamoto, 2006), so the present cruise was designed to collect eggs and preleptocephali based on the 2005 finding.

The size range of these leptocephali collected during all three legs was 11.3 – 38.3 mm TL (Fig. 1). Sampling at the end of Leg 5 included tows targeting *A. japonica* leptocephali during both the day and night. The day tows were designed to collect leptocephali during the day when they are feeding. One *A. japonica* was collected during the day, and its gut contents will be genetically analyzed to determine what it had been feeding on.



**Figure 1.** Length frequency distribution of the *Anguilla japonica* leptocephali collected during the KH-06-2 cruise in the Philippine Sea region of the western North Pacific.

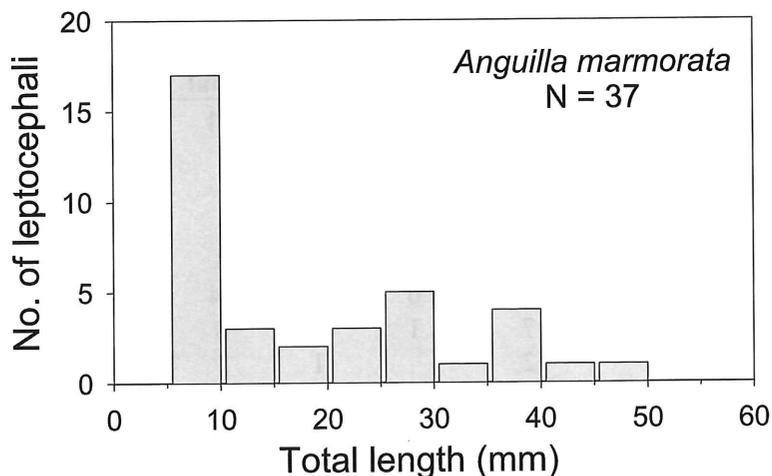
### References

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- Tsukamoto K., T. Otake, N. Mochioka, T. W. Lee, H. Fricke, T. Inagaki, J. Aoyama, S. Ishikawa, S. Kimura, M. J. Miller, H. Hasumoto, M. Oya, and Y. Suzuki. 2003. Seamounts, new moon and eel spawning: the search for the spawning site of the Japanese eel. *Environ. Biol. Fish.* 66: 221-229.
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## Tropical Anguillid Leptocephali Collected During the KH-06-2 Cruise

### All Scientists on Board

Two species of tropical anguillid eel leptocephali were collected during the KH-06-2 cruise between 26 June and 31 August 2006. These leptocephali were collected using the Isaacs Kidd Midwater Trawl during Leg 2, and using the 3-m ORI ring net during Legs 3 and 5. The IKMT had an 8.7 m<sup>2</sup> mouth opening and 0.5 mm mesh, and the large ORI ring net had a 7.1 m<sup>2</sup> mouth opening and 0.5 mm mesh. Both nets were fished in oblique and step tows during daytime and nighttime. A total of 37 leptocephali of *Anguilla marmorata* were collected during the three legs of the cruise at a size range of 6.2 – 50.9 mm TL (Fig. 1).



**Figure 1.** Length frequency distribution of the *Anguilla marmorata* leptocephali collected during the KH-06-2 cruise in the Philippine Sea region of the western North Pacific.

The large number of small *A. marmorata* leptocephali that were collected in the region to the west of the Mariana Islands confirms the presence of a spawning area of this species in this region as has been previously reported (Aoyama et al., 1999; Miller et al., 2002; Kuroki et al., 2006). The leptocephali spawned in this region would first be transported westward by the North Equatorial Current and then some would be transported northward into the Kuroshio and others transported southward by the Mindanao Current. In addition to the *A. marmorata* leptocephali, 2 large sized *Anguilla bicolor pacifica* leptocephali were collected (44.0, 47.8 mm TL). The spawning area of this species is not known, however. The otolith microstructure of both species will be analyzed as part of an ongoing larger study on the early life history of tropical anguillid leptocephali (Kuroki et al., 2006) and studies on the feeding ecology of *A. marmorata* will also be conducted using the leptocephali collected during the cruise.

### References

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## Leptocephali Collected During the KH-06-2 Cruise

Michael J. Miller, Noritaka Mochioka, Tsuguo Otake, Mari Kuroki,  
Seishi Hagihara, Etsuko Sawada, Jun Aoyama and Katsumi Tsukamoto

A total of 761 leptocephali of at least 65 species of 16 families eels and their close relatives were collected between 26 June and 31 August 2006 during the KH-06-2 cruise (Table 1). These leptocephali were collected using the Isaacs Kidd Midwater Trawl during Leg 2, and using the 3-m ORI ring net to collect eggs and preleptocephali during Legs 3 and 5. The IKMT had an 8.7 m<sup>2</sup> mouth opening and 0.5 mm mesh and was fished in oblique and step tows during both daytime and nighttime. The large ORI ring net had a 7.1 m<sup>2</sup> mouth opening and 0.5 mm mesh and was fished in oblique and step tows during both day and night.

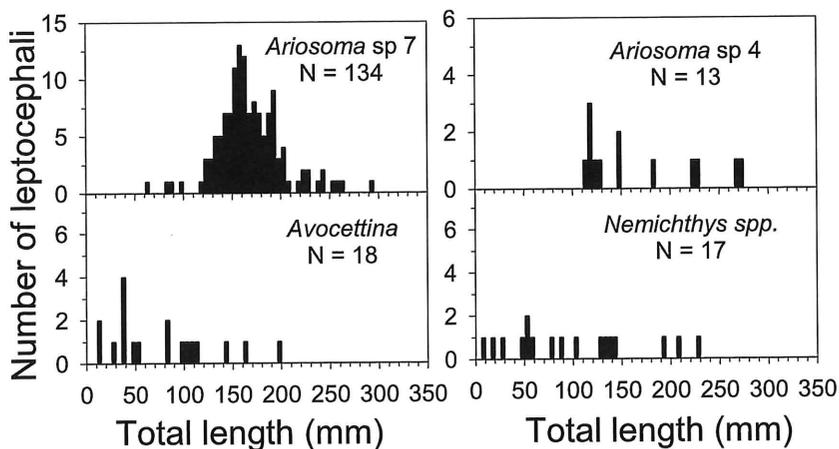
**Table 1.** List of the numbers and size ranges of the leptocephali collected during the 3 legs of the KH-06-2 cruise in the Philippine Sea region of the western North Pacific. The number and size ranges of the juveniles or glass eels of some taxa are shown in parentheses.

	Leg 2	Leg 3	Leg 5	Total	Total length range (mm)
ウナギ科 (Anguillidae)	21	24	16	61	6.2 - 50.9
アナゴ科 (Congridae)					
<i>Ariosoma</i> sp. 1	1			1	190.0
<i>Ariosoma</i> sp. 2	1			1	78.3
<i>Ariosoma</i> sp. 3	3	1		3	108.0 - 125.0
<i>Ariosoma</i> sp. 4	1	6	7	14	112.0 - 274.0
<i>Ariosoma</i> sp. 5	7	1		8	26.7 - 139.0
<i>Ariosoma</i> sp. 6	2		1	3	73.0 - 237.0
<i>Ariosoma</i> sp. 7	21	55	63	139	61.7 - 293.5
<i>Ariosoma</i> sp. 8		2	1	3	148.0 - 257.2
<i>Ariosoma</i> sp.	1	3		4	-
<i>Conger</i>	9		17	26	21.2 - 60.7
<i>Gnathophis</i>	1	3	2	6	29.8 - 48.4
<i>Gorgasia</i>	2	3	5	10	20.0 - 65.4
<i>Heteroconger</i>			1	1	109.3
Congrinae spp.	1	1	2	4	18.6 - 95.4
Congridae total	50	75	99	223	18.6 - 293.5
イワアナゴ科 (Chlopsidae)	2	6	11	19	22.8 - 82.0
ヘラアナゴ科 (Derichthyidae)	5	2	9	16	7.4 - 93.6
ハリガネウミヘビ科 (Moringuidae)		2	1	3	59.6 - 71.2
ウツボ科 (Muraenidae)	35	43	40	119	7.5 - 96.6
シギウナギ科 (Nemichthyidae)					
<i>Avocettina</i>	3	13	3	19	12.8 - 196.7
<i>Nemichthys</i>	9	4	5	18	7.0 - 192.0 (N=1: 126.0)
Nemichthyidae total	12	17	8	37	7.0 - 196.1
クズアナゴ科 (Nettastomatidae)	1	1		1	129.8
ウミヘビ科 (Ophichthidae)	41	4	4	49	6.0 - 116.0
ノコバウナギ科 (Serrivomeridae)	49	99	39	187	5.1 - 64.5 (N=20: 90.0-224.0)
ホラアナゴ科 (Synphobranchidae)	1	3	10	14	33.1 - 74.9
セムシウナギ科 (Cyematidae)		3	1	4	11.7 - 33.3
<i>Thalassenchelys</i>		1	1	2	34.0 - 251.0
Saccopharyngiformes		1	3	4	73. - 14.9
Elopiformes	1			1	21.0
Notacanthiformes			3	3	69.5 - 232.0
Unidentified		3	4	7	3.0 - 10.3 (N = 1: 71.4)
<b>Total</b>	<b>218</b>	<b>289</b>	<b>254</b>	<b>761</b>	

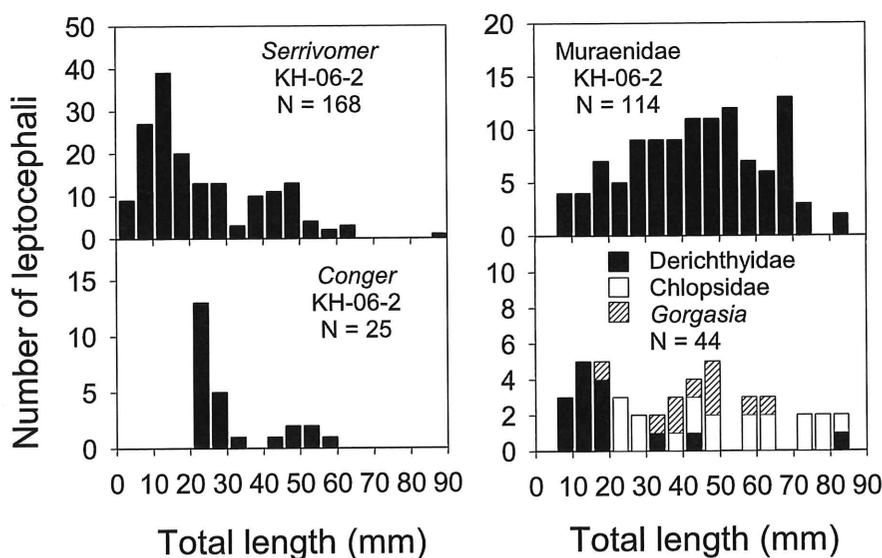
The leptocephali of the Congridae (N = 223) whose adults live in shallow water or on the upper continental shelf were the most abundant family, with those of the genus *Ariosoma* being collected in the greatest numbers (Table 1). Two species, *Ariosoma* sp. 7 and sp. 4, were collected mostly at very large sizes up to 274 and 293 mm TL, respectively (Figure 1), whereas other taxa such as *Gorgasia*, *Conger* and most other Congrinae, whose maximum sizes are shorter than *Ariosoma* species, were collected at various size ranges down to as small as 18.6 mm TL (Table 1, Figure 2). The leptocephali of the shallow water eels of the Muraenidae were the third most abundant family (N = 119) and were collected at a wide range of sizes. Various species of the other shelf and slope eel families were also collected (Chlopsidae, Moringuidae, Ophichthidae, Nettastomatidae, Synphobranchidae).

The leptocephali of the mesopelagic eels of the family Serrivomeridae (N = 187) were the second most abundant family, which also included 20 juvenile eels in the collections. Various other species of mesopelagic or bathypelagic eels or deep-sea fishes were collected, such as *Nemichthys* (N = 18), *Avocettina* (N = 19), the Derichthyidae (N = 16), the Cyematidae (N = 4), other Saccopharyngiformes (N = 4), and the Notacanthiformes (N = 3).

There were also 61 leptocephali of the freshwater eels of the genus *Anguilla* (family Anguillidae) that were collected during the cruise (see other reports in this volume).



**Figure 1.** Length frequency plots of species of leptocephali of the family Congridae and Nemichthyidae during KH-06-2, which all reach large sizes.



**Figure 2.** Length frequency plots of leptocephali of five families of eels collected during KH-06-2, showing the sizes of three taxa of the family Congridae (lower right).

## **Studies on the migration conditions of Japanese eel and Bluefin tuna**

**Shingo Kimura, Sachihiko Itoh, Takashi Kitagawa, Hideo Nagae, Heeyong Kim, Yoshik Kato, Youichi Miyake, Kei Zenimoto, Naoki Yamaoka, Sachie Miyazaki**

*Ocean Research Institute, the University of Tokyo*

Recent collection of 2 day-old pre-leptocephali of Japanese eel (*Anguilla japonica*) in the Pacific Ocean around the West Mariana Ridge gives us a clue for the further understanding of long-term and long-range migration of this species (Tsukamoto, 2006). For the migration of the Japanese eel, salinity front in the North Equatorial Current (NEC) has been considered as an important oceanic condition and its interannual variability associated with ENSO probably lead to a reduction in the transport of the larvae to the Kuroshio, causing poor recruitment in East Asia (Kimura et al., 2001). Although there is a hypothesis which the salinity front works as a factor for adult eel to detect a spawning site, just the north-south shift of it does not decide a success of the transport of the eel larvae to eastern Asian countries. In addition to the fluctuation of the salinity front, the NEC bifurcation into the northward flowing Kuroshio and the southward flowing Mindanao Current east of the Philippines should be considered as another important feature influencing the migration of *A. japonica* in the NEC.

We established two observation lines near the Philippine coast along which we conducted CTD hydrographic observations with water sampling and the sampling of *A. japonica* leptocephali, zoo plankton and the larvae of mackerels, to understand the biophysical system at the western boundary of the Pacific where the NEC bifurcation generates. The observation of this ocean area is the first time for us, which will give us much information for the transport of several organisms along the Kuroshio and the Mindanao Current. The low-salinity waters by high precipitation in the equatorial Pacific formed a distinct salinity front at 13.5 °N on the easternmost line (133.5 °E) but the front shifted to the more north as going to the more western observation line (Fig. 1-1). There were more complicated current structures at the western boundary region near

the Philippine coast than the eastern region, although a mean current observed by Acoustic Doppler Profiler indicated the westward NEC (Fig. 1-2).

CTD observation lines in Leg3 and Leg5 were established around the spawning site of Japanese eel. In Leg3, 34.5 psu, which is an index of the salinity front, is shown between 16.5 oN and 17 oN on 140 oE and 144 oE line but between 15 and 15.5 oN along 142 oE line (Fig. 2-1). Compared with the horizontal distribution of salinity at each depth (Fig. 2-2) and current vectors (Fig. 2-3), the meridional displacements of the salinity front were probably due to the formation of meso-scale eddy or a large meandering generated by the eddy. Such phenomena were detected in the hydrographic structure in Leg5 (Fig. 3-1, 3-2, and 3-3). The formation of meso-scale eddy makes the hydrographic structures be complicated around the spawning ground of the Japanese eel, which means that not only the salinity front but also the smaller scale structure need to be investigated for the ecology of the Japanese eel. For example, in the cruises of KH04-1 and KH05-1, the stations showing many collections of the pre-leptocephali and the leptocephali were located around the meso-scale eddy. Finally, we suggest that the salinity front and the NEC bifurcation decide the general migration route of *A. japonica* in the western North Pacific, and the formation of meso-scale eddy affects the dispersion of the eel larvae from the spawning ground.

In addition, to investigate the migration conditions of Bluefin tuna, *Thunnus orientalis*, we conducted the larval samplings by IKMT with CTD observation at each station around the Ryukyu Islands where the spawning ground is formed. The investigation in the spawning ground of this species with the samplings of mackerels in west North Pacific will provide us with much useful information for how the tunas and the mackerels inhabiting around Japan Islands migrate from Ryukyu Islands or the Pacific.

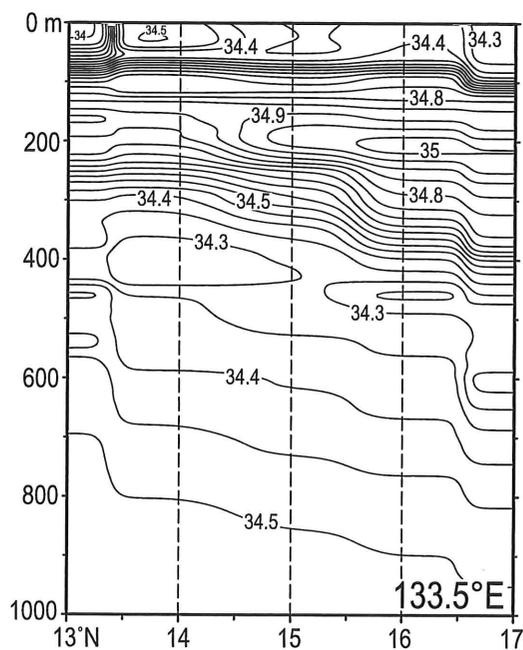
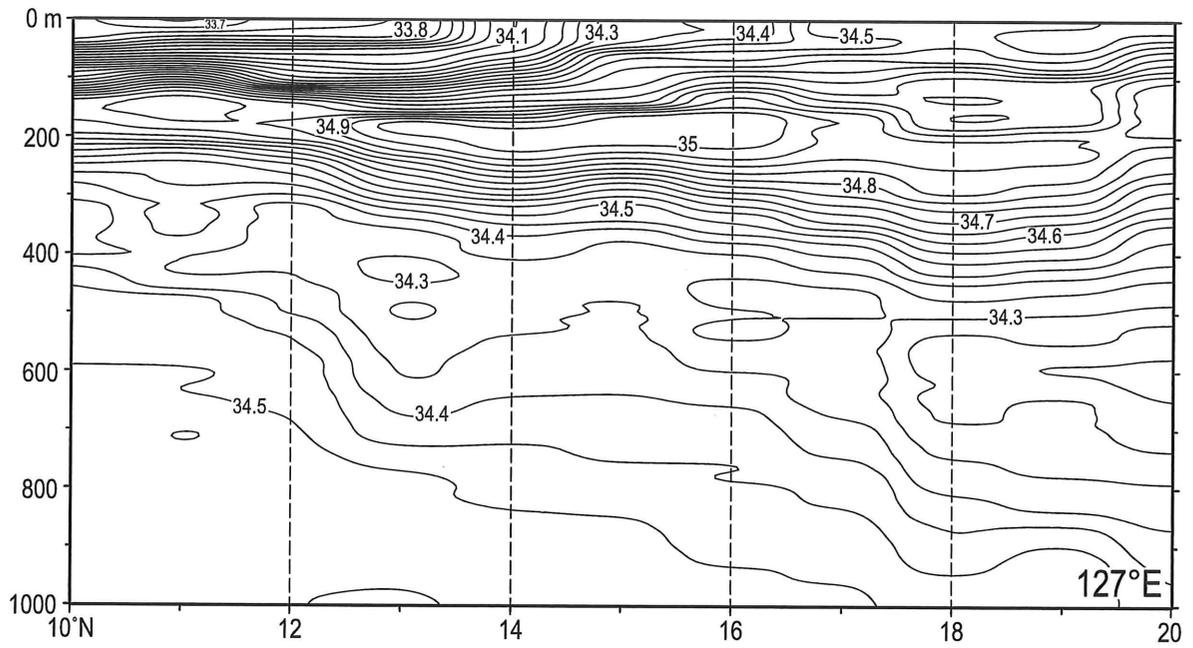
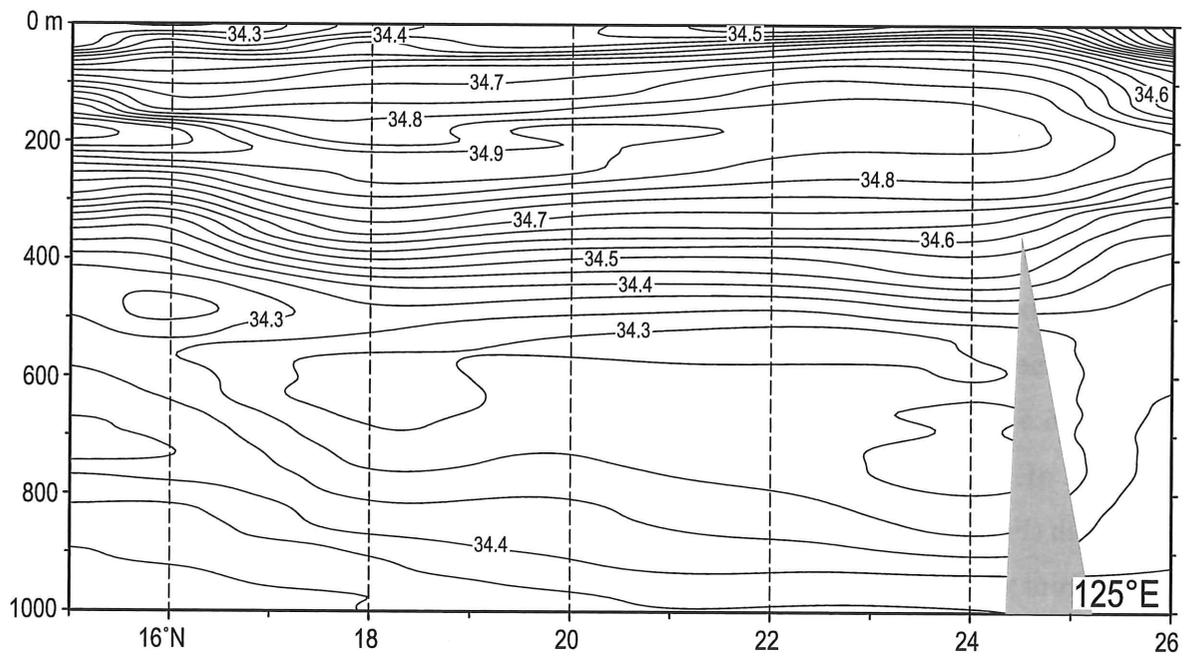


Fig. 1-1. Profiles of salinity (psu) during KH06-2-Leg2

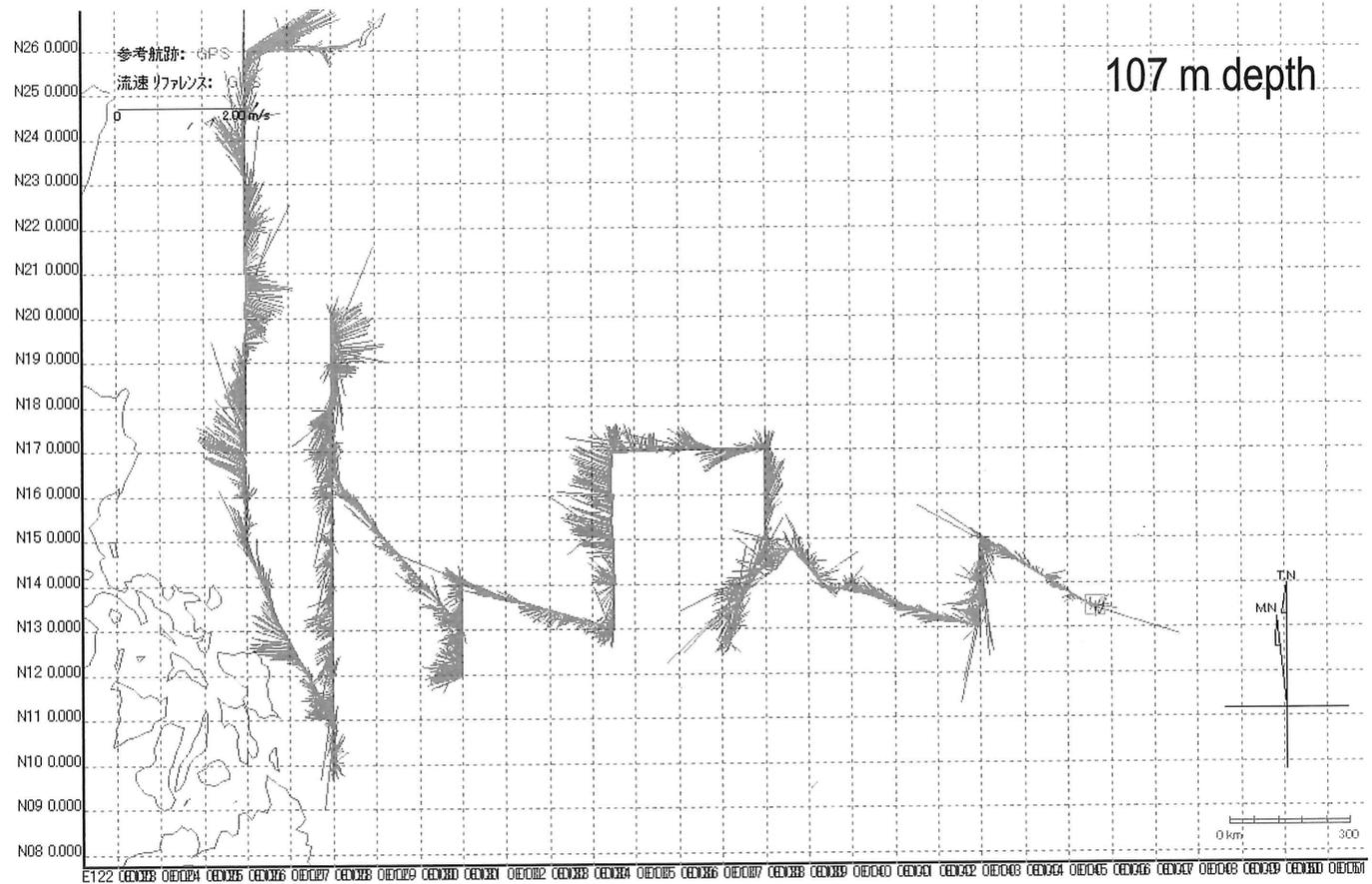
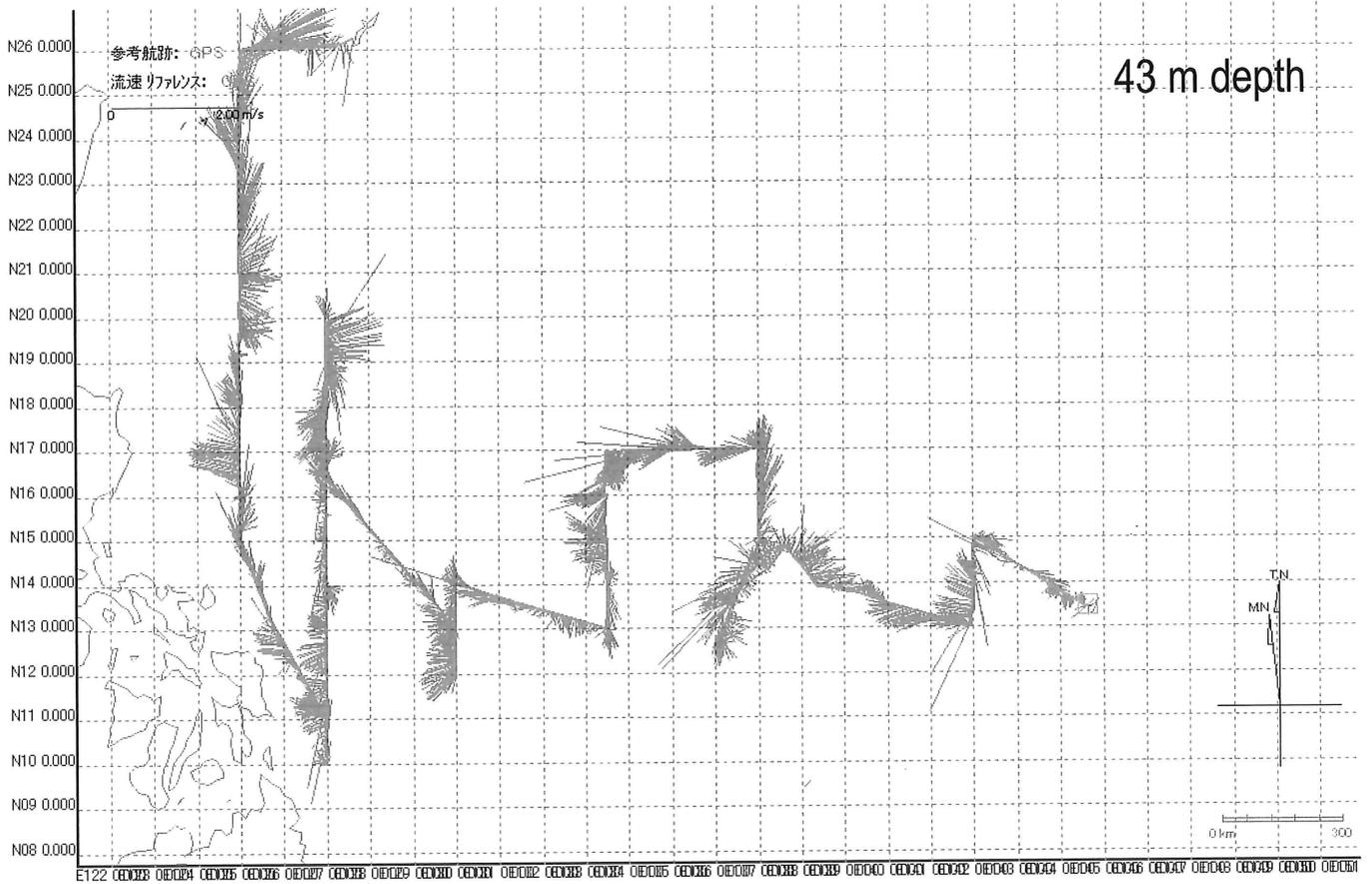


Fig. 1-2. Current vectors observed by ADCP during KH06-2-Leg2

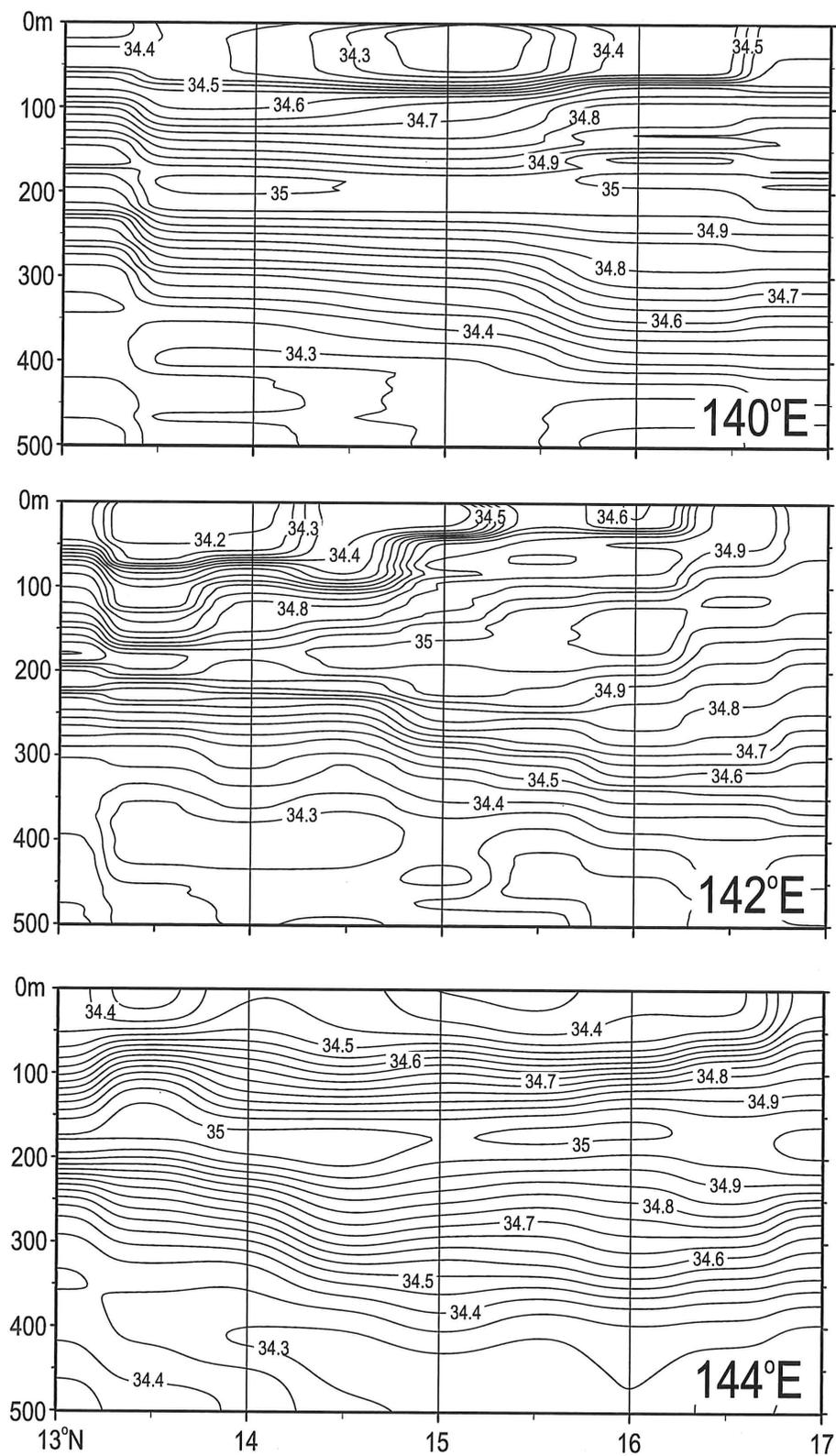


Fig. 2-1. Profiles of salinity (psu) during KH06-2-Leg3.

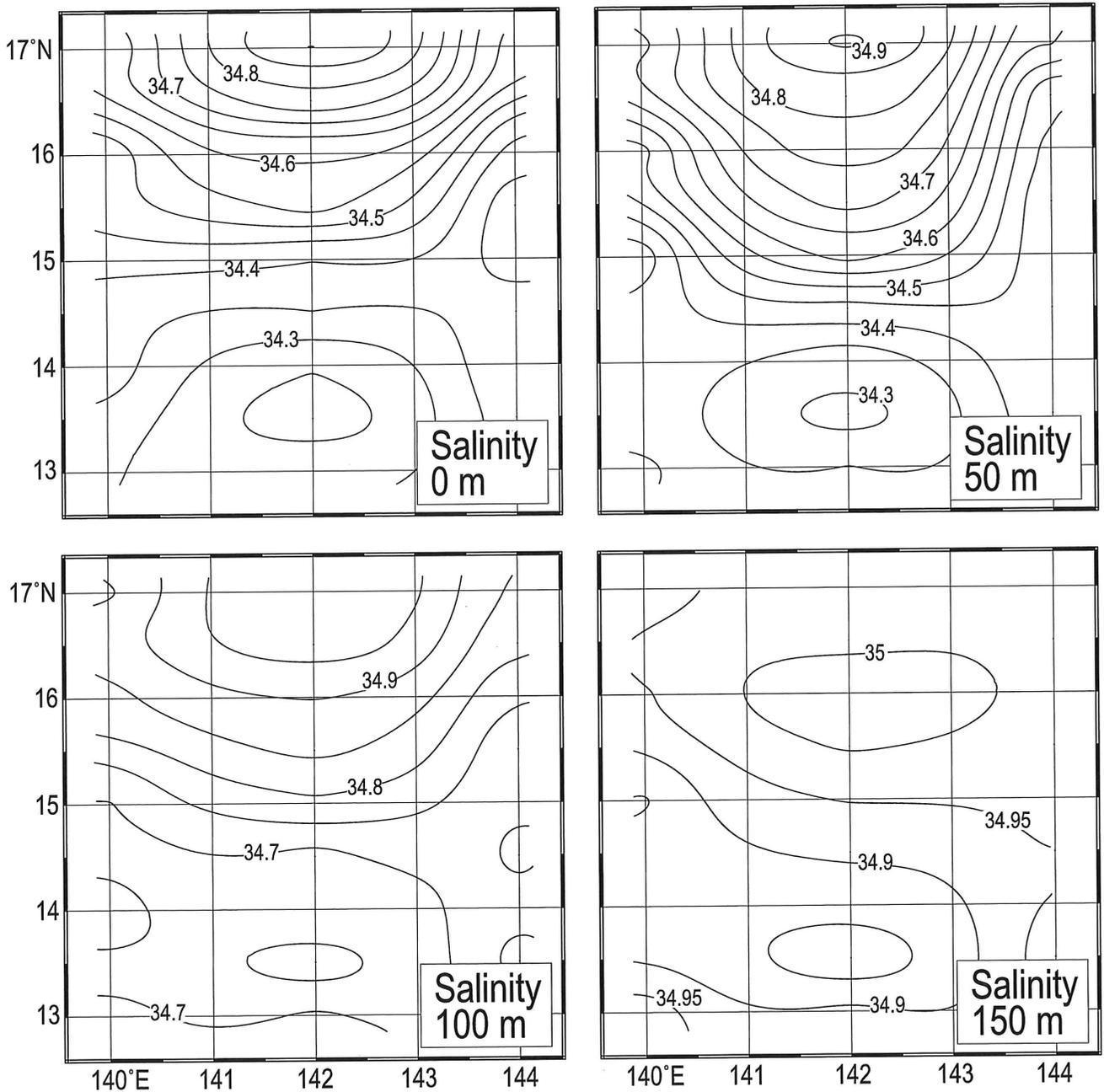


Fig. 2-2. Horizontal distributions of salinity (psu) during KH06-2-Leg3.

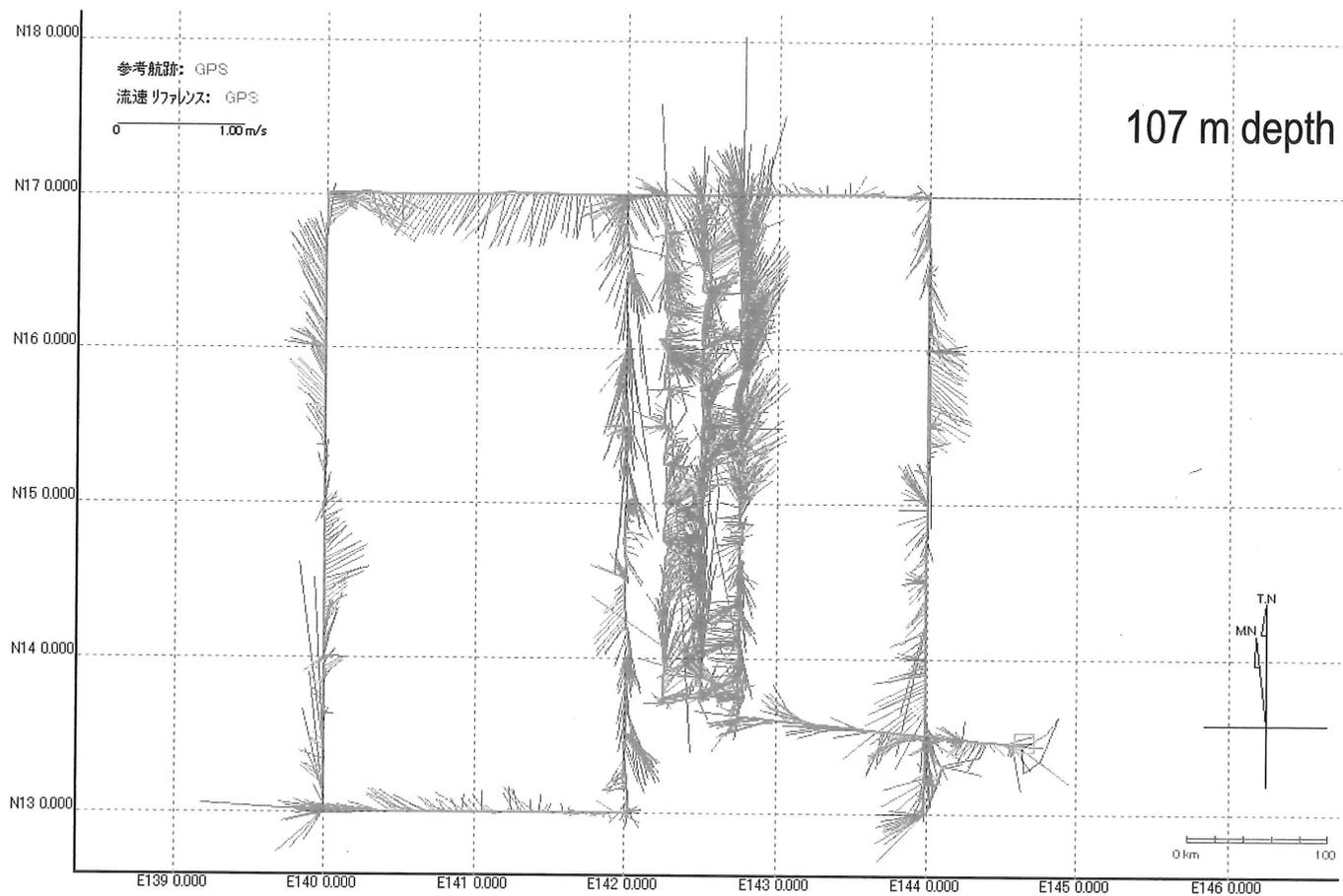
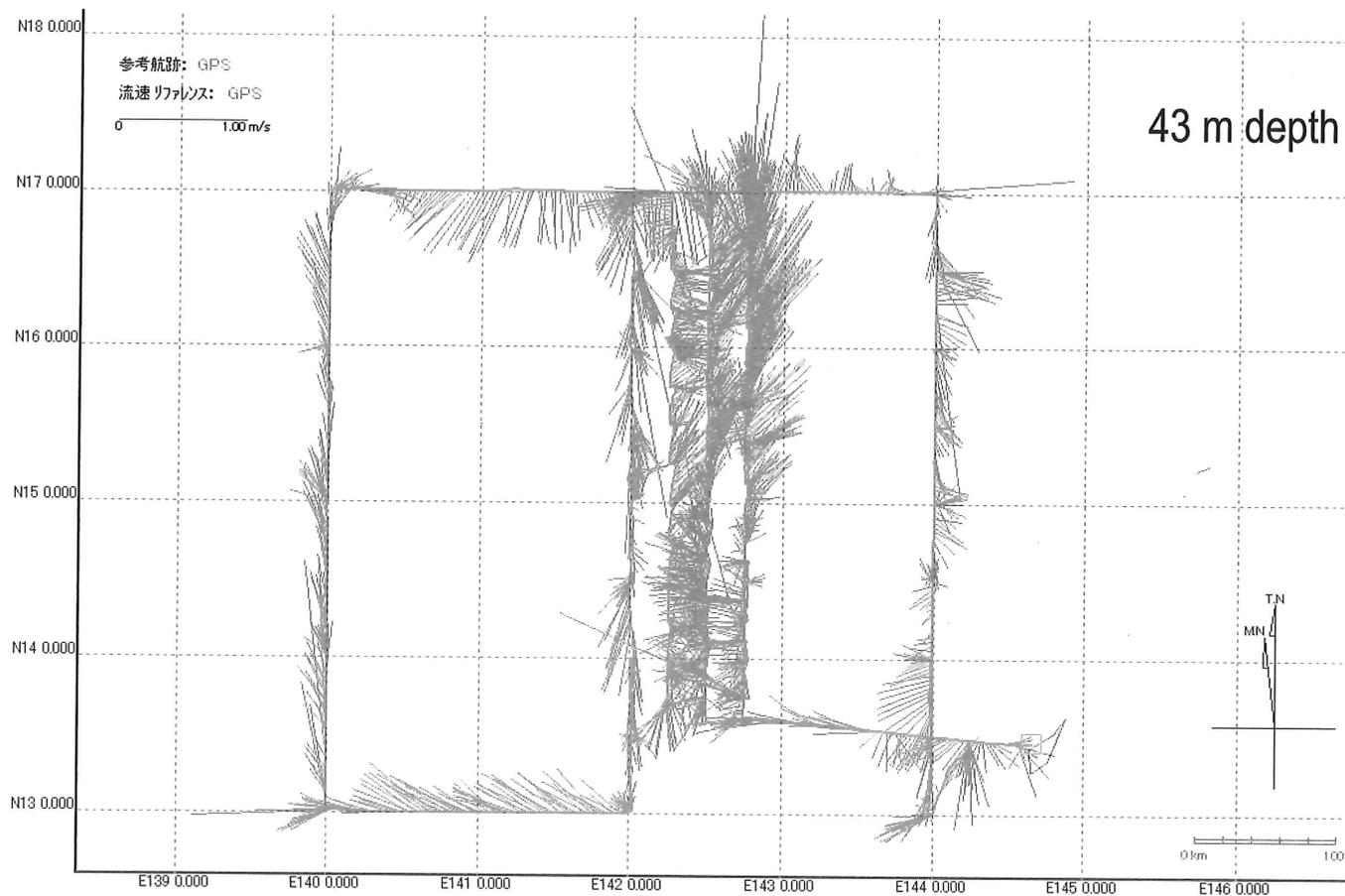


Fig. 2-3. Current vectors observed by ADCP during KH06-2-Leg3

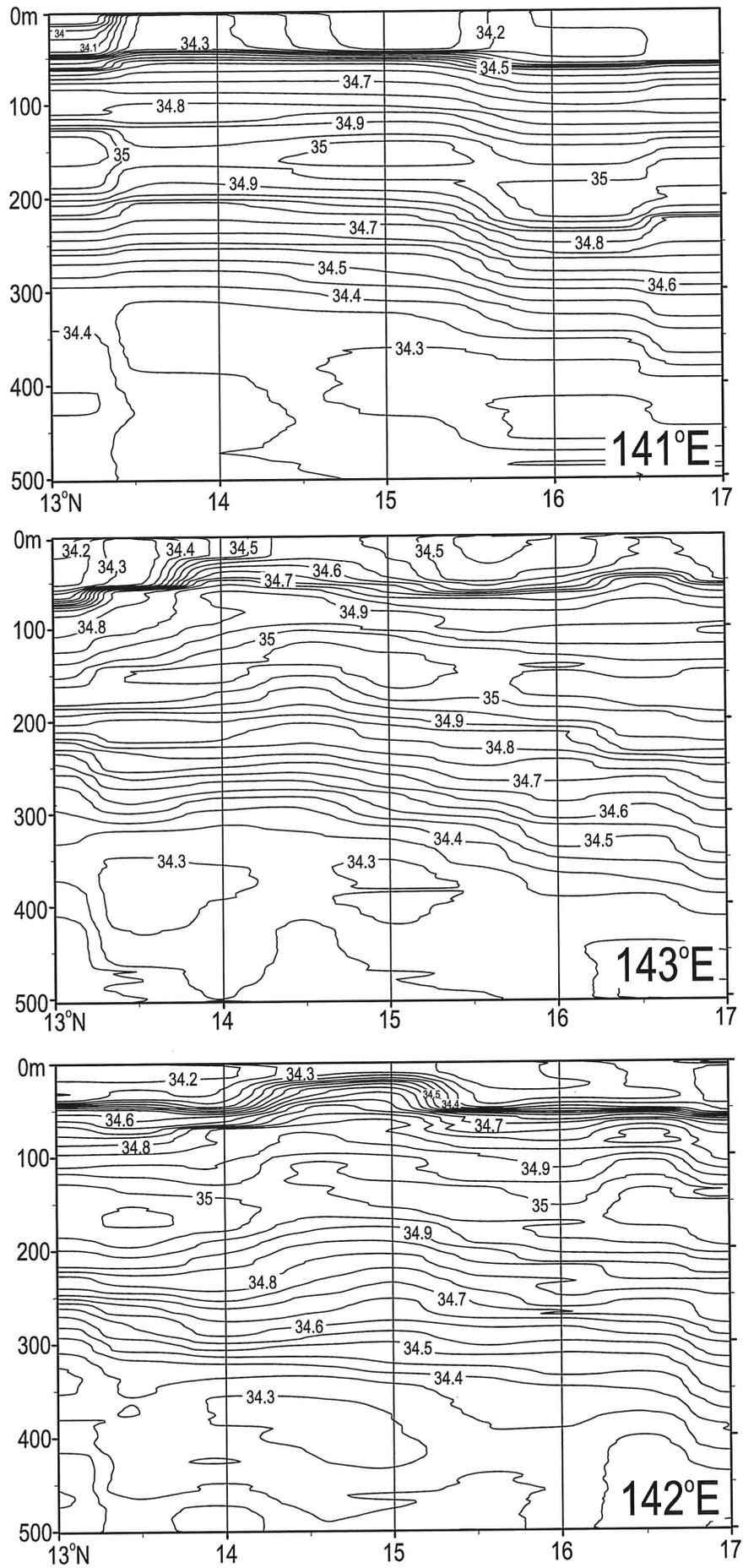


Fig. 3-1. Profiles of salinity (psu) during KH06-2-Leg5.

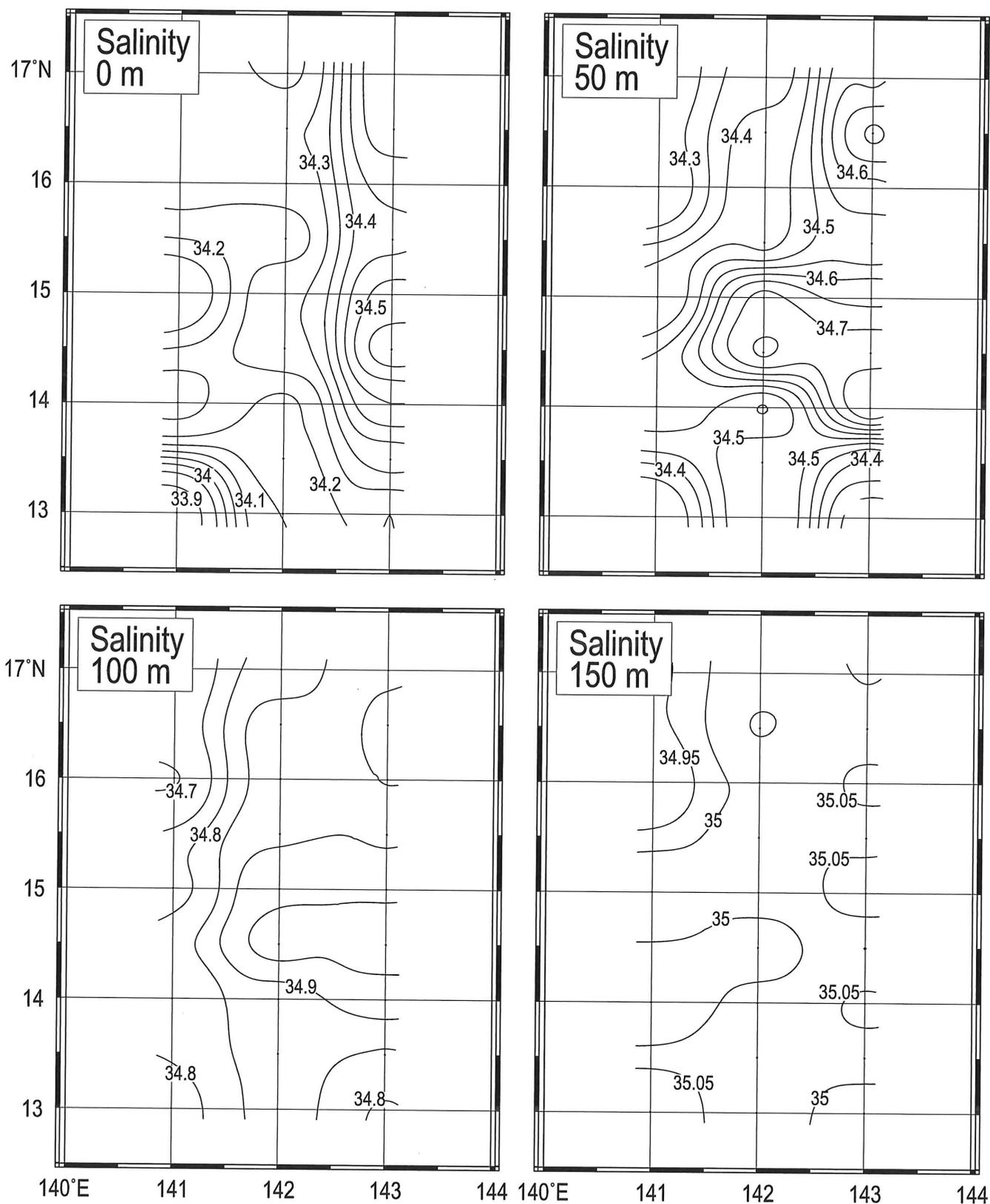


Fig. 3-2. Horizontal distributions of salinity (psu) during KH06-2-Leg5.

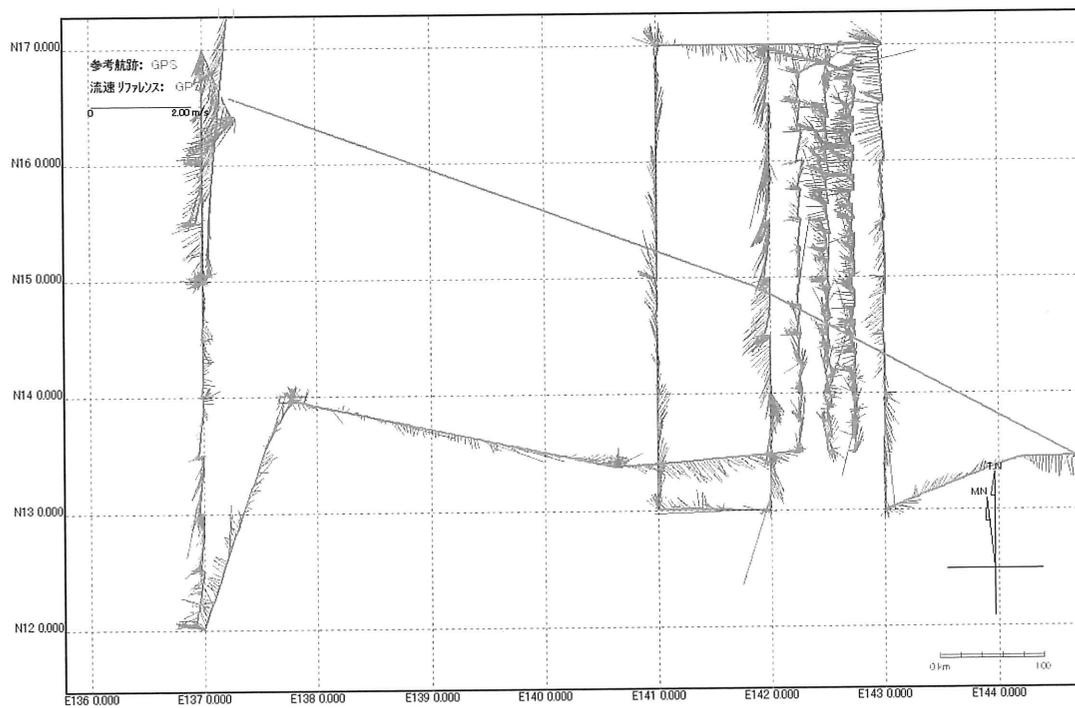


Fig. 3-3. Current vectors observed by ADCP at 107 m depth during KH06-2-Leg5.

# Geographical distribution and heat-tolerance in three oceanic *Halobates* species (*Heteroptera: Gerridae*)

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## INTRODUCTION

The only insects that inhabit the open sea are five species of sea skaters: *Halobates micans*, *H. sericeus*, *H. germanus*, *H. splendens*, and *H. sobrinus* (Cheng 1985). Three species, *Halobates sericeus*, *H. micans* and *H. germanus* inhabit tropical and temperate areas of the Pacific Ocean in the northern hemisphere, including The Kuroshio Current and the East China Sea (Andersen & Polhemus 1976, Cheng 1985). *Halobates sericeus*, *H. micans* and *H. germanus* are reported from a latitudes of 13°N-40°N, 0°N-35°N and 0°N-37°N, respectively, in the Pacific Ocean (Miyamoto & Senta, 1960, Andersen & Polhemus 1976, Ikawa et al., 2002). However, this information was collected on different cruises and different times of the years. There has been only one ecological study based on samples collected in a specific area in a particular season. One sea skater species, *Halobates sericeus*, was collected at 18 locations in the East China Sea area (27°10' N- 33°24' N, 124°57' E - 129°30' E) (Harada, 2005), and *H. micans* and/or *H. germanus* at only 8 locations in the area south of 29° 47'N, where water temperatures were more than 25°C. At three locations, where the water temperature was less than 23°C, neither *H. micans* nor *H. germanus* were caught. However, there have been no such ecological studies performed in the wide area ranged 0°N to 35°N in the Pacific Ocean. One purpose of this study is to make it clear that how the species components and life history dominance among the three oceanic sea skaters appear in such wide latitude area especially in tropical area. Fresh water species in Gerridae can be proposed to have temperature tolerance from -3°C to 42°C, because water temperature in fresh water in ponds and river highly changes daily and seasonally. However, water temperatures in the ocean are relatively stable and it only ranges from 24°C to 30 °C in the center of Kuroshio current in southern front of western Japan.

Adults of *Halobates germanus* showed heat-paralysis (static posture with no or low frequency to skate on water surface), when they were exposed to temp. higher than 32°C (Harada unpublished, data in the TANSEIMARU cruise: KT-05-27). In the tropical ocean area, water temperature is more stable around 30°C rather than that in temperate ocean. Therefore, the tropical species of *H. micans* are hypothesized to have lower tolerances to temperature changes than the temperate species, *H. sericeus*. This study tries to examine whether this hypothesis is true by laboratory experiment during the cruise of KH-06-02-Leg 5.

## **MATERIALS AND METHODS**

### *Samplings*

Samples (20 mins at 2.0-3.0 knots) were collected from 18-31 August, 2006 using a NEUSTON NET (rectangular parallel pipes, width of the opening: 57 cm) trailed along on sea water surface 10-15 m from the side of the Hakuohomaru (3991t), which is owned by JAMSTEC (Japan Agency for Marine-earth Science and TECHnology), and the cruise was organized by the Ocean Research Institute, University of Tokyo. Samples were taken at 26 locations in the western area of the pacific ocean ranged 12°00'N-17°00'N and 137°00'E - 142°00'E (Table 1). Surface which was swept by NEUSTON NET was expressed as value of flow-meter x width of NEUSTON NET x compensation value=0.71 which is based on the slanting angle of the body of NEUSTON NET from the shipping direction.

### *Laboratory experiment*

Adults and 5th instar larvae collected in the samplings were moved and reared under natural conditions on the 3rd deck. Within 48 hours from the collection, the laboratory experiments on the responses to temperature change (increasing) were performed in the room where air temp. was kept at 26°C. First, 2 or 4 adults or fifth instars of *Halobates micans*, *H. sericeus* or *H. germanus* in the aquarium (round and transparent type: 30cm diameter and 15cm height) were adapted for 1 hour to 28 or 29°C which was near to the average water temp. in shade on the 3rd deck. For the second half hour or the last 15min., behaviour of the specimens were recorded by handy-video-camera (Sony). Water temperature was regulated by heater linked to

digital-electric-thermo-regulator (Gex Co Ltd.). After the adaptation to 28°C or 29°C, water temp. was stepwise increased every 1 hour by 1 °C and kept 1 hour in which the last half or 15 min. their behavior was again recorded by the video camera. If the specimen was paralyzed by heat (static standing or captured by water surface: standing unable), temperature was stepwise decreased and the behaviour of the specimens were recorded as well to check whether they recover from the paralysis. If the specimen was not paralyzed even under 35°C, the temp. was decreased stepwise and the behaviour was recorded as well to check whether the skating activity (frequency and distance of skating) changes, because the thermo-regulator can control only up to 35°C. Through the laboratory experiments, the behaviour of the specimens were observed. Halobates collected were fed on shrimps collected with NEUSTON net and commercial foods as half-fresh mosquito larvae while kept in natural conditions before the experiments.

## RESULTS

### *Distribution*

14° 30' Halobates micans were caught at 6 of 7 locations, while H. germanus and H. sericeus were caught at only 3 and 1 location(s), respectively (Fisher's Exact Probability test:  $P=0.024$ )(Table 1). However, at 15 ° 00'N or northern area, H. germanus were caught at 14 of 19 locations, whereas H. micans and H. cericeus were caught at only 8 and 6 locations, respectively ( $\chi^2$ -test on species component:  $\chi^2$ -value=7.3,  $df=2$ ,  $P=0.026$ )(Table 1). In the area south of 15° 30'N, the number of specimen of Halobates spp. collected was very small and 3.10 on average (SD: 1.97), while in the area north of 16° 00'N, the number is relatively high and 10.20 (SD:±7.4)(Mann-Whitney U-test:  $z=-1.782$ ,  $P=0.075$ ).

### *Laboratory experiment*

When the water temperature increased stepwise 1°C every 1 hour, heat-paralysis occurred at 29 to >35°C (increase by 1 to >7°C). Three of four specimens in Halobates sericeus were not paralyzed even at 35 °C and resistant to temperature change, while one of nine in H. micans H. and four of twelve in H. germanus were not paralyzed at 35 °C. On average, H. sericeus, H germanus and H. micans were paralyzed at >35.6 °C (SD:

0.89), >32.9 °C (SD: 2.17) and >31.6 °C (SD: 2.60) on average, respectively (Kruskall-Wallis test:  $\chi^2=6.705$ ,  $df=2$ ,  $P=0.035$ ).

#### *Additional analysis*

The video data will be analyzed very soon after the cruise to examine the frequency and speed of skating and their responses to the temperature differences.

## **DISCUSSION**

Halobates micans seems to, predominantly, inhabit the area of 12-15° N. in the Pacific Ocean. With a critical line of 15° N, H. germanus may be predominant instead in the northern area. Higher amplitudes of seasonal fluctuations in temperature and photoperiod and biological conditions related to those physical conditions, eg. the components of zooplanktons as preys of sea skaters, were proposed to affect the changes in dominant species in Halobates. The key factor(s) to affect the change in the dominant species remain(s) to be examined.

The north current of equator goes on from East to West in 10-15° N in the Pacific Ocean. Sea water in the current is highly transparent and with low biological productivity with low level of nourishments. However, in the north area out of the current, the biological productivity seems to be higher than that inside the current. High number of Halobates species collected in 16-17 ° N seems to be related to the high biological productivity in this area, because Halobates were reported to feed on the zooplanktonic crustaceans and fish larvae trapped in the surface film (Andersen and Polhemus, 1976) .

Heat-paralysis can be used as the index to show a resistance to the temperature changes. Even in the specimens inhabiting this common tropical sampling area (12-17° N ) in the Pacific Ocean, Halobates sericeus were more dominant to the temperature changes than the other two species. The high resistance to temp. change may be related to the northern distribution of this species which are easily exposed to seasonal change in temperature. Genetic potential of physiological capability of the high resistance seems to be kept even in the tropical colonies of H. sericeus. Such high potential may be adaptive when such tropical colonies are transferred from tropical to temperate zones, riding on the north current of equator and the black current (The Kuroshio Current).

Adults of *Halobates germanus* collected at the station of 30° N showed heat-paralysis at 32°C (Harada unpublished). In the tropical ocean area, water temperature is more stable around 30°C rather than that in temperate ocean. Therefore, the tropical species of *H. micans* seems to have lower tolerances to temp.-change than the temperate species, *H. cericeus*.

#### ACKNOWLEDGEMENT

We would like to thank Prof. T. Ohtake (Head Scientist on Leg 5 of the cruise: KH-06-02) for the permission to do this study during the cruise on the *Hakuhomaru*, and his warm encouragement and help. The samplings and the experimental study were also possible due to supports of all of the crew (Captain: Mr. S. Suzuki) and scientists on the cruise.

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# ***Species identification of Japanese Eel (*Anguilla japonica*) eggs and preleptocephali using real-time PCR during the KH-06-2 cruise***

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## **INTRODUCTION**

Relatively large leptocephali of *A. japonica* (>30 mm TL) can be easily identified by morphological characteristics such as their body shape and total number of myomeres (Tabeta and Mochioka, 1998). But during the early developmental stages such as the egg or preleptocephalus, it is difficult to identify *A. japonica* because of their undeveloped morphological characteristics. Watanabe et al. (2004) established the method of species identification for eggs or preleptocephali of *A. japonica* using the molecular genetic technique of real-time PCR. This method is convenient and robust, and can be used on board the ship during research cruises. However, this method has in the past identified some specimens of the Serrivomeridae as *A. japonica* mistakenly. Therefore, we introduced a new method that can distinguish between *A. japonica* and the Serrivomeridae to establish a strict identification method of *A. japonica* eggs and small leptocephali. The new method is carried out only on specimens identified as *A. japonica* by the conventional method.

## **MATERIALS AND METHODS**

### *Materials*

8 specimens of preleptocephali collected during the KH-06-2 cruise (Table 1) were used for real-time PCR on board the ship.

### *Methods*

Total DNA was extracted by incubation at 98°C in 50µl of a 5% chelex solution. That concentration was measured with a spectrophotometer and diluted for optimum concentration (4~5 ng/µL). Then, real-time PCR using the new and conventional methods were carried out simultaneously by means of the ABI PRISM 7000 Sequence

Detection System (Applied Biosystems Japan Ltd.) in a 20 $\mu$ l reaction volume (Table 2). After activation at 50 °C for 2 minutes and 95°C for 10 minutes, 40 cycles of denaturation at 95°C for 15 seconds, and annealing and extension at 60°C for 1 minute were performed. For the new method, fluorescent intensity was measured before and after that reaction.

#### *Identification in the conventional method*

We decided that  $Ct \leq 35$  is *A. japonica* based on the conventional method. But specimens that showed a Ct at around 35 could not be exactly identified. So we will determine their mtDNA nucleotide sequence in the laboratory for species identification.

#### *Identification in the new method*

Only specimens that were positive in the conventional method will be analyzed using the new method. We identify specimens based on a specific amplification being indicated using *A. japonica* and Serrivomeridae.

## **RESULTS**

Unfortunately all 8 specimens examined here showed no amplification in the conventional method, indicating that they were not *A. japonica* or Serrivomeridae. Therefore, all 8 specimens could not be examined by the new method. Because of that, we could not validate the new method. Hereafter, we need to evaluate the new method using pseudopositive specimens.

**Table 1.** Number of *Halobates* collected at 26 locations in the western region of Pacific Ocean, August 18-31, 2006 (N: Total number of individuals collected, *H.m.*: *Halobates micans*; *H.g.*: *Halobates germanus*; *H.s.*: *Halobates sericeus*; Stat: Station number; WT: Water temperature; AT: Air temp.; L: N of larvae, A: N of adults, E: N of exuviae; Date: sampling date; SS: Area of water surface over which the NEUSTON were trailed by the ship; ○ caught; ×: not caught) (1): 1 exuviae, (○): exuviae of *H.m.*

<u>Latitude</u>	<u>N</u>	<u>L</u>	<u>A</u>	<u>H.m.</u>	<u>H.g.</u>	<u>H.s.</u>	<u>E</u>	<u>Stat</u>	<u>WT(°C)</u>	<u>AT(°C)</u>	<u>Time of day</u>	<u>S.S.(m2)</u>	<u>Date</u>
12° 00'	(1)	0	0	(○)	×	×	○	St. 94	29.7	28.6	06:00-	5043	29
12° 30'	5	1	4	○	○	×	×	St. 95	29.7	30.6	09:20-	5031	29
13° 00'	2	2	0	○	×	×	×	St. 96	30.2	30.3	13:35-	5281	29
13° 30'	5	4	1	○	○	×	○	St. 97	30.1	30.5	17:20-	5519	29
14° 00'	5	2	3	○	○	×	×	St. 98	29.5	29.1	21:25-	5076	29
14° 30'	2	0	2	×	×	○	○	St. 99	29.8	28.8	0:15-	5059	30
14° 30'	1	1	0	○	×	×	×	St.18	29.5	28.1	22:35-	5368	18
15° 00'	4	3	1	○	×	×	×	St.19	29.4	27.7	03:15-	5191	19
15° 00'	7	7	0	○	○	×	×	St. 100	29.7	28.3	03:09-	5781	30
15° 00'	3	1	2	×	×	○	○	St.105-1	29.7	29.0	06:36-	3866	31
15° 00'	2	1	1	×	○	×	×	St.105-2	29.7	29.0	08:40-	6950	31
15° 00'	1	1	0	×	○	×	○	St.105-3	29.9	30.4	10:30-	4920	31
15° 00'	2	2	0	×	○	○	×	St.105-4	30.1	30.3	12:08-	5460	31
15° 00'	7	3	4	×	○	○	×	St.105-5	30.4	30.4	14:53-	6758	31
15° 00'	2	2	0	×	○	×	×	St.105-6	30.2	29.3	17:13-	5460	31
15° 00'	2	0	2	×	○	×	×	St.105-7	30.0	29.8	18:52-	5953	31
15° 00'	2	1	1	×	○	×	×	St.105-8	29.9	29.8	20:15-	6109	31
15° 00'	5	2	3	○	○	×	×	St.105-9	29.7	29.6	21:30-	5916	31
15° 00'	1	1	0	×	○	×	×	St.105-10	29.8	30.0	22:20-	6191	31
15° 30'	4	3	1	○	×	×	×	St. 20	29.5	28.6	07:50-	5346	19
15° 30'	3	2	1	○	×	○	×	St. 101	29.8	29.3	08:03-	4945	30
16° 00'	18	6	12	○	×	○	○	St. 102	30.8	32.0	12:25-	5293	30
16° 00'	4	3	1	○	○	×	○	St. 21	30.0	29.4	12:45-	5412	19
16° 30'	1	0	1	×	○	×	×	St. 22	29.8	28.7	16:00-	4875	19
16° 30'	16	7	9	×	○	○	○	St. 104	30.3	29.3	23:59-	978	30
17° 00'	12	10	2	○	○	×	○	St. 103	30.3	29.6	21:10-	5329	30

Table 2. Results of “heat-paralysis” experiments performed on 5<sup>th</sup> instars and adults of *Halobates micans* (H.m.), *H. germanus*(H.g.) and *H. sericeus*(H.s.). TA: temp. at which specimen adapted, THP: temp. at which heat-paralysis occurred. “Date and Time of day” when experiments were performed

TA (°C)	THP(°C)	Species	Stage	Date	Time of day
28	33	H.m.	Adult (female)	Sep. 1	8:55-
28	33	H.m.	Adult (female)	Sep. 1	8:55-
28	33	H.m.	Adult (female)	Sep. 1	8:55-
28	33	H.m.	Adult (female)	Sep. 1	8:55-
28	29	H.m.	Adult (female)	Aug. 29	15:00-
28	29	H.m.	Adult (male)	Aug. 29	13:00-
28	29	H.m.	Adult (female)	Aug. 30	0:00-
28	29	H.m.	Adult (female)	Aug. 30	0:00-
29	>35	H.m.	5 <sup>th</sup> instar	Sep. 2	14:00-
28	>35	H.s.	Adult (female)	Aug. 31	0:15-
28	>35	H.s.	Adult (male)	Aug. 31	0:15-
28	34	H.s.	Adult (female)	Aug. 31	13:00-
28	>35	H.s.	Adult (male)	Aug. 31	13:00-
28	32	H.g.	5 <sup>th</sup> instar	Aug. 30	8:25-
28	32	H.g.	5 <sup>th</sup> instar	Aug. 30	8:25-
28	32	H.g.	5 <sup>th</sup> instar	Aug. 30	8:25-
29	31	H.g.	Adult (female)	Sep. 1	20:50-
29	32	H.g.	Adult (female)	Sep. 1	20:50-
29	33	H.g.	Adult (female)	Sep. 1	20:50-
29	34	H.g.	Adult (female)	Sep. 1	20:50-
29	35	H.g.	5 <sup>th</sup> instar	Sep. 2	14:25-
29	>35	H.g.	Adult (female)	Sep. 2	14:25-
29	>35	H.g.	Adult (female)	Sep. 2	14:25-
28	29	H.g.	5 <sup>th</sup> instar	Sep. 2	20:35

Table 1. Sample data.

Sample No.	Date	St.	TL (mm)
311	22 Aug 06	43	3.3
312	22 Aug 06	43	6.8
313	22 Aug 06	43	7.0
314	22 Aug 06	43	8.8
320	22 Aug 06	44	6.3
324	22 Aug 06	45	3.0
360	23 Aug 06	59	6.2
361	23 Aug 06	59	7.8

Table 2. Reaction liquid.

Components of the experiments	The conventional method Volume (concentration)	The new method Volume (concentration)
2x TaqMan Universal PCR Master Mix	10 $\mu$ L (1x)	10 $\mu$ L (1x)
36 $\mu$ M Forward primer	0.5 $\mu$ L (0.9 $\mu$ M)	0.5 $\mu$ L (0.9 $\mu$ M)
36 $\mu$ M Reverse primer	0.5 $\mu$ L (0.9 $\mu$ M)	0.5 $\mu$ L (0.9 $\mu$ M)
Aj TaqMan Probe	0.32 $\mu$ L (0.2 $\mu$ M)	
Aj-Se 1 TaqMan Probe		0.31 $\mu$ L (0.2 $\mu$ M)
Aj-Se 2 TaqMan Probe		0.30 $\mu$ L (0.2 $\mu$ M)
Sample DNA	3 $\mu$ L (12-15 ng/ $\mu$ L)	3 $\mu$ L (12-15 ng/ $\mu$ L)
Milli Q water	to 20 $\mu$ L	to 20 $\mu$ L

## **Deep, cold- water coral survey on NW Pacific seamounts**

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Cold- water corals are known to be essential habitat-forming organisms in deep, cold waters and make unique environments for the other benthic animals especially on the seamounts in the middle of ocean. However, there is no information of these corals on seamounts of North Equatorial Current region of Mariana area in NW Pacific Ocean. By analysing the recorded videos of manned- submersible JAGO, Max-Planck Institute, Germany in 1998 Hakuho-maru cruise (KH98-2) and of Deep-tow camera system in 2001 JAMSTEC Yokosuka cruise (YK01-08), it was firstly found the several images of deep, cold- water corals on the seamounts of this area (Matsumoto, A.K. unpublished data). However, in Invertebrate collections of Marine Laboratory of University of Guam and National Museum of Natural History, Smithsonian Institution, there is only one deep- water specimen of corals (Cnidaria, Octocorallia). Therefore, the detail of species biodiversity of cold- water corals in the area is completely unknown. To collect the specimens of these deep, cold- water corals and identify species were the main purpose of this KH06-2, leg. 5.

For the sampling tool, ORI-TI Type Chain Bag Dredge which has been designed for geological survey to collect rock samples at the bottom was used (Fig.1). It is already known the difficulty to use biological dredge and beam trawl for the survey on rocky bottom such as seamounts where cold- water corals normally inhabit and suggested the efficiency to use geological dredge at such rocky steep environments. This ORI-TI type Chain Bag Dredge have experience of collecting deep- water animals attached to the rock in 2005 Hakuho-maru cruise, KH05-1 Leg 5.

The area for the survey were described by Sea beam (Fig.2) and selected target sites as follows, Station A: 13- 59.94 N, 137- 46.25 E, 4200m, Station B-1: 13-22.54 N, 140- 39.37 E, 2200m and Station B-2: 13- 22.66 N, 140- 39.17 E, 2300m. Sites are basically close to the top of seamounts and each deployment has 25-50 min tows of gear



Dredge & Lander site St.A

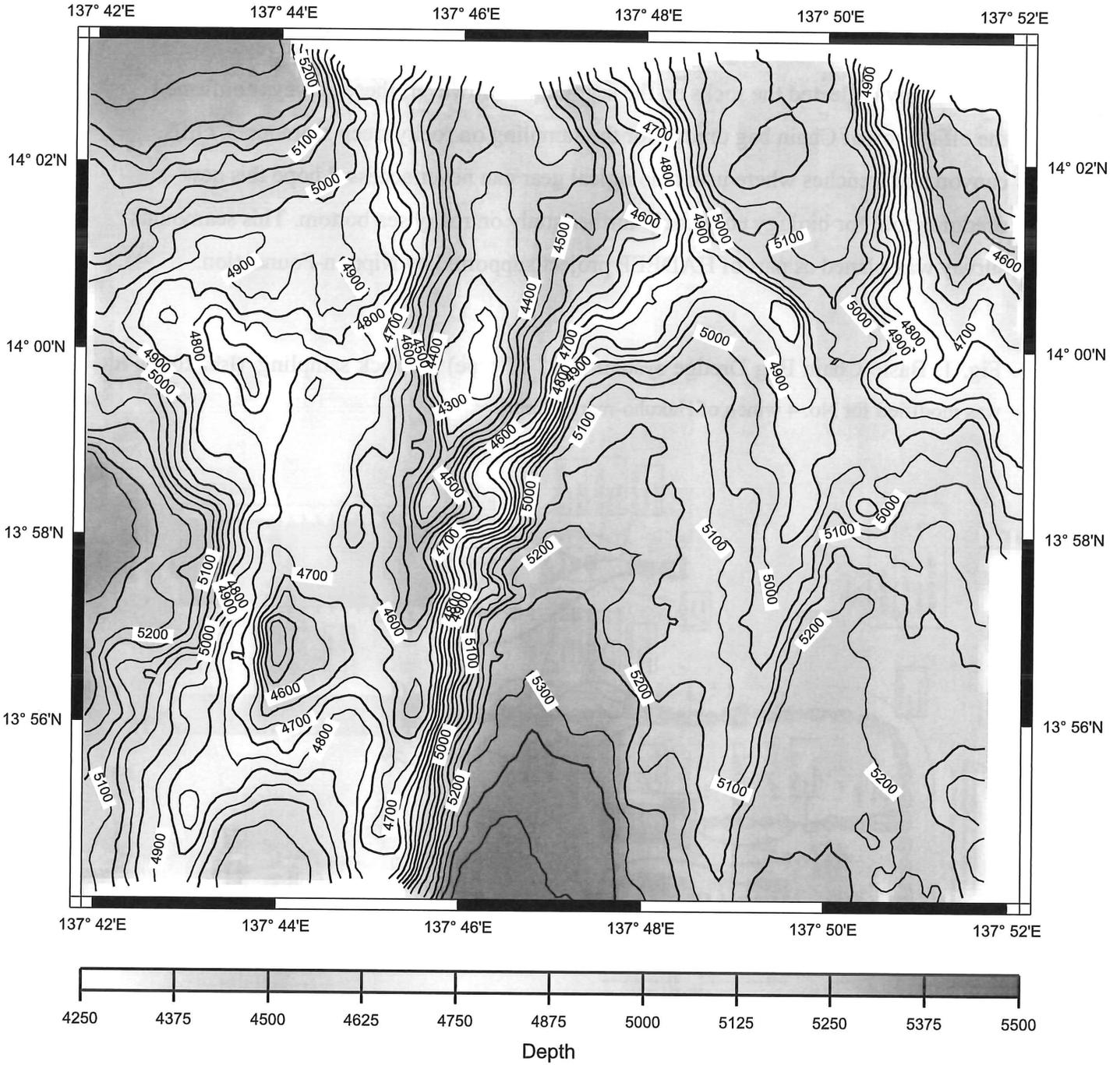
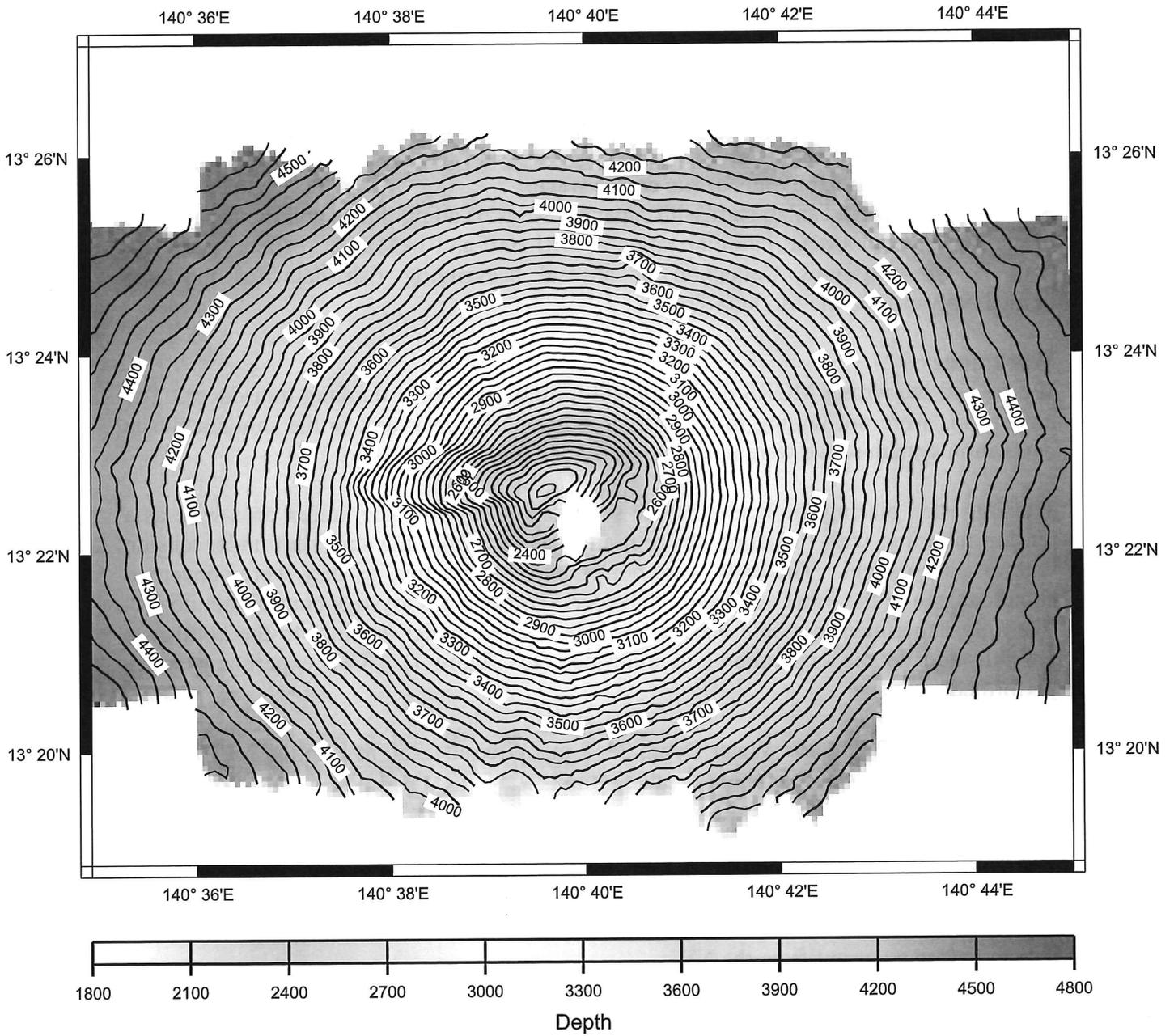


Fig. 2 Sea beam map of Dredge and Lander deployments .

Dredge & Lander site St.B



## HADEEP Lander Trials During the KH-06-2 Cruise

**Dr. David Bailey and Dr. Camila Henriques, Oceanlab  
Seishi Hagihara, Dr. Asako Matsumoto and Professor Katsumi Tsukamoto, ORI**

### **Introduction**

The Hadal Environmental Education Partnership (HADEEP) project has been in discussion for several years and this cruise was intended to be the first attempt at doing some science. The project is a partnership between Oceanlab and the University of Tokyo's Ocean Research Institute (ORI). Some funding has been provided by the Nippon Foundation. In the absence of hadal depth rated gear the Sprint video lander was moved from San Diego to Guam to join the ship there. Floatation etc. was sent from Oceanlab. The current plan is for Oceanlab to come on a *Hakuho Maru* cruise next year (2007), and another in 2008, subject to funding becoming available.

### **For the lander team the aims of the cruise were:**

Familiarization of Oceanlab staff with Japanese ways of doing things and the capabilities of the RV *Hakuho Maru*. Familiarization of ORI staff and JAMSTEC officers and crew with lander operations Collection of abyssal scavenging fish data for the area immediately around the Mariana Trench.

### **Work done**

We packed the wrong MORS deck unit and it didn't contain a dunking transducer. The technicians were not willing to allow us to connect the deck unit to the ship's acoustic navigation system so we were unable to do lander deployments

We suggested placing the lander on the seafloor by lowering the lander on a wire as a tension mooring, with floatation above it and the wire from the ship kept under tension with another weight. The crew were not prepared to try this, as they were concerned about damage to the trawl warp and the lander. The ship has joystick control, but no DP. The only experiment possible was to lower the baited lander as close to the bottom as possible and do the experiments in midwater (see figure 1). Two experiments were performed.

Deployment #1 27/08/06. Water Depth 2449 m, Wire out 2250 m Position N13<sup>o</sup> 23.23' E140<sup>o</sup> 39.41'. Time at maximum depth 57 min.

This experiment was immediately NW of a seamount, the peak of which was at 2000 m. The experiment was performed at this depth in order to allow any odour plume to impinge on the seamount, and be as shallow as possible in order to maximize the biomass of fishes available. The only available current data was for 400 m water depth and these data indicated a current from the NW.

The bait and reference cross were clearly visible and centered in the field of view. Camera system functioned correctly but no fish or other animals were seen at the lander.

Deployment #2 28/08/06. Depth 4807 m Wire out 4670 Position N13° 59.97' E137° 47.75' Time at maximum depth 120 min.

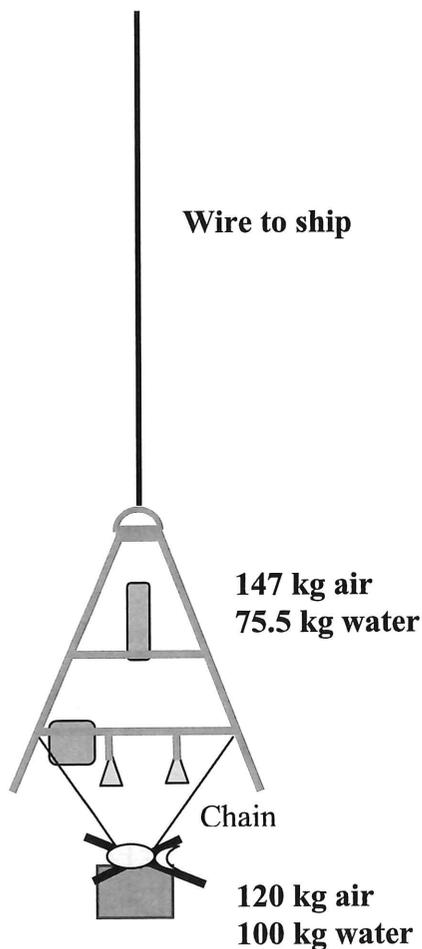
This experiment was performed in a slight depression surrounded by ridges 100 m higher than the flat area we deployed above. This was the only location available. Time on bottom was increased to 120 minutes in the hope of attracting scavengers. Camera functioned correctly but no fish or other animals were seen at the lander.

### Conclusions

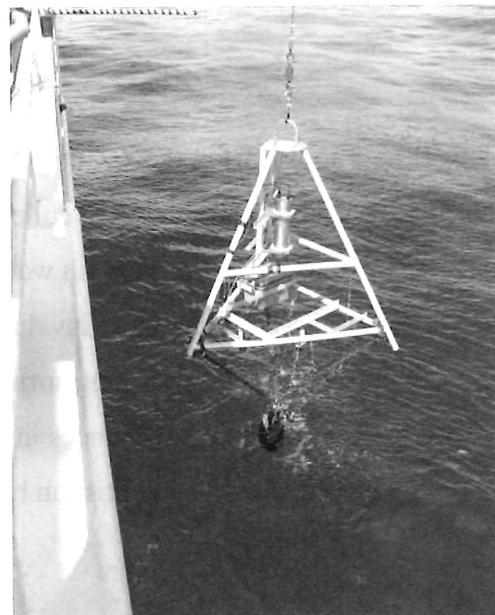
Given the very low productivity of the area it's difficult to know whether the lack of animals seen in the 1-2 hour deployments was real. It appears most likely that the bait hanging >100 m above the seafloor was not detected by the benthopelagic fishes, and/or that they were unable to catch up with the movements (up to 1 knot) of the ship.

Additional funding is required to build the equipment necessary for hadal work and to pay for Oceanlab staff for future projects. An ORI ship time proposal may also be required and if required will be written by Dr AK Matsumoto based on Oceanlab's NERC proposal. The planned cruise track and deployment opportunities in the Marianas Trench in 2007/8 will be subject to future discussion.

**Figure 1.** Wire mode deployment setup of Sprint lander.



Three anchor chain links were slung below the frame (without legs) using thinner chain. A 60 cm reference cross was fitted to the top of the ballast and it was baited with approx 1 kg of Pacific mackerel.



## Impression of the cruise

In this cruise, we have collected many leptocephali and some eggs of Derichthyidae, Nemichthyidae and especially Serrivomeridae to analyze in my study. I examined specimens of Serrivomeridae which were collected during the research cruises of KH-98-2, KH-02-2, KH-04-2 for my bachelor's thesis. But I have never joined for those cruises, the KH-06-2 cruise was my first cruise. I found that it was very hard to collect those specimens. Therefore, I will treat those valuable specimens carefully. I spent precious time during the KH-06-2 cruise.

M1 Etsuko SAWADA  
Ocean Research Institute  
The University of Tokyo

I participated on the KH-06-2 expedition aboard the R/V Hakuho-Maru for the first time. I am very pleased to have the opportunity of joining this research cruise and coming to know many persons. We reaped a rich harvest from the cruise, and it will become honorable achievement. I wish, finally, to thank you all.

M1 YASUO TOMIDA  
Ocean Research Institute  
The University of Tokyo

This time, I took part in the scientific cruise, KH-06-02-Leg.5 on the R/V Hakuho-Maru. During the cruise, I supported to collect sea skater. Usually, I am studying on water striders inhabiting terrestrial fresh waters in Japan. This cruise is my first one for scientific research I could have invaluable experience to take part in various investigations of marine life, egg sorting leptocephals of eels and their relatives. I am very happy to get valuable chances in this cruise. I would like to thank, sincerely, every crew member and every scientist on board for kind supports and helps.

M1 Takamasa Ishibashi  
Graduate School of Education  
Kochi University

This cruise on Hakuhomaru is the first one in my life. In the first several days of the cruise, I was in seasick. However, as the days go on, my physical and mental conditions adapted to the cruising life and have completely recovered from the seasick. Then, I could enjoy the watch work calmly. I was very interested in equipments and their operation for several measurements and samplings in the sea, i.e. the ORI-net, the CTD, the NEUSTON-net, etc. which I have seen for the first time that attracted me. I have learned a lot of things during this cruise. Such experience is exclusive in the cruise and I want to make such invaluable experience link effectively to my scientific work in the graduate school.

M1 Takao Inoue  
Graduate School of Education  
Kochi University

This time I embark on Hakuho for the first time. I was enjoy for everything. I do want to embark on Hakuho next year. I wish, finally, to thank you all.

M1 Munehiro Takami  
Graduate School of Oceanography  
Tokai University

I enjoyed the cruise on the R/V Hakuho Maru as an amazing experience. I thank the captain and the crew for safely navigating and compassionate support. I thank the senior researchers for good administration of the research cruise program and good advice for me. I thank Dr. Bailey and Dr. Henriques for teaching me about deep-sea biology. I thank the other same grade students for sharing delightful days.

Unfortunately, we could not find eggs of the Japanese eel, *Anguilla japonica*, in this cruise, but I found precious treasure! Arigato, everyone!

M1 Seishi Hagihara  
Ocean Research Institute  
The University of Tokyo

Last summer I participated in this “Unagi Cruise” as a first-year graduate student. During the cruise my colleagues taught me a number of procedures including handling of seawater samples, setting up the CTD, etc. This summer there were opportunities to utilize my experience from the last cruise. As my research is focused on abalones and the coastal water, my knowledge in eels and open sea is yet to be enhanced. However, what my colleagues taught me last summer gave me something to offer to my new colleagues who have recently joined our research team just like me a year ago.

M2 Yoichi Miyake  
Ocean Research Institute  
The University of Tokyo

KH-06-2 by R/V Hakuho-Marui was my first cruise. I learned how to collect Japanese eel (*Anguilla japonica*) and how to investigate the oceanic conditions. However, we could not collect eggs and newly hatched larvae of the Japanese eel, which was an absolute purpose of this cruise. I want to collect them and understand their behavior more in detail next time.

M1 Kei Zenimoto  
Ocean Research Institute  
The University of Tokyo

This was my first cruise, which is a good experience for me. I am going to study ocean environmental conditions related to Japanese eel, but I have not still so many scientific information about that and about getting on ship. Therefore every time on the ship was precious for me. I have experienced many various tools and gears for observation and learned how to operate them. Moreover, as many scientists joined us on this cruise, I had a good chance to hear their research interest. However I could not understand well their researches because of the deficiency of my knowledge. I will study much more until the next cruise.

M1 Sachie Miyazaki  
Ocean Research Institute  
The University of Tokyo

It was my first time to participate in such a long time and large-scale cruise, so not only the operation but the life on the vessel were fresh to me. Throughout the contact with many scientists who have many different research interests, I could make progress in scientific mind of thinking and in knowledge and skills about using observation equipments. If there will be a chance, I want to participate in next eel cruise or other research cruises.

M1 Naoki Yamaoka  
Ocean Research Institute  
The University of Tokyo