Preliminary Report
of
The Hakuho Maru Cruise
KH-08-1

Leg 1: May 21, 2008 - June 15, 2008
Leg 2: June 19, 2008 - July 14, 2008
(Eel Cruise XV)

Atmosphere and Ocean Research Institute
The University of Tokyo
2012

Preliminary Report of The Hakuho Maru Cruise KH-08-1 (EEL XV)

Leg 1: May 21, 2008 - June 15, 2008
Leg 2: June 19, 2008 - July 14, 2008

Atmosphere and Ocean Research Institute
The University of Tokyo
2012

By
The Scientific Members of the Expeditions

Edited by
Shun Watanabe, Kazuki Yokouchi
and Katsumi Tsukamoto

Contents

01 Introduction Katsumi Tsukamoto and Shingo Kimura 1

02 Station and working log 2

03 List of participants 4

04 Track chart 5

05 Studies on oceanic conditions of larval transport of the Japanese eel in North Equatorial Current 10
Kei Zenimoto, Sachie Miyazaki, Youichi Miyake, Hiroshi Morioka, Nao Mizorogi, Takashi Kitagawa and Shingo Kimura

06 Preleptocephali and leptocephali of *Anguilla japonica* collected during the KH-08-1 cruise 14
All Scientists on Board

07 Leptocephali of tropical anguillid species (*Anguilla marmorata* and *A. bicolor pacifica*) collected during the KH-08-1 cruise 18
All Scientists on Board

08 Notes on some fish eggs collected west of the Mariana Islands 20
Akihiro Okamura, Jun Aoyama, and Katsumi Tsukamoto

09 Leptocephali collected during the KH-08-1 cruise 26
Michael J. Miller, Tsuguo Otake, Jun Aoyama, Seishi Hagihara, Hiroaki Okazawa, Tatsuki, Yoshinaga, Akira Shinoda, Ryusuke Sudo, and Katsumi Tsukamoto

10 Genetic characterization of the assemblage of leptocephali around the Suruga seamount of the West Mariana Ridge 30
Tatsuki Yoshinaga, Michael J. Miller, Jun Aoyama, Akira Shinoda, Seishi Hagihara, Hiroaki Okazawa, Shun Watanabe and Katsumi Tsukamoto

11 **Offshore transport of small marine eel larvae from the Ryukyu Ridge by a rare branch of the Kuroshio Current** 33
Michael J. Miller, Takashi Kitagawa, Jun Aoyama, Tatsuki Yoshinaga, Akira Shinoda, Seishi Hagihara, Hiroaki Okazawa, Shun Watanabe, and Katsumi Tsukamoto

12 **Functional morphology of the telescopic eye of leptocephali of the family Synaphobranchidae** 37
Seishi Hagihara, Michael J. Miller, and Katsumi Tsukamoto

13 **Identification and distribution of open ocean midwater eels of the Nemichthyidae** 38
Hiroaki Okazawa and Katsumi Tsukamoto

14 **Ariosoma leptocephali collected during the KH-08-1 cruise and the ecology of the *Ariosoma* sp. 5 leptocephali in the Central Indo-Pacific** 39
Mikiya Yamaguchi, Michael J. Miller, Tatsuki Yoshinaga and Katsumi Tsukamoto

15 **Studies on larval transport of Bluefin tuna from the spawning area to nursery grounds** 40
Takashi Kitagawa, Nao Mizorogi, Tatsuki Yoshinaga and Shingo Kimura

16 **Occurrence of bothid larvae collected during Leg.1 of the KH-08-01 cruise** 41
Kazuyuki Ban

17 **Cookie-cutter shark Isistinus brasiliensis and snake mackerel Gempylidae collected during KH-08-1 Hakuho Maru cruise** 43
Yosuke Amano, Sho Tanaka and Tsuguo Otake

18 **Studies on feeding ecology and species identification of phyllosoma larvae of palinurid and scyllarid lobsters** 45
Masamichi Machida and Shuhei Nishida

19 **Identification of Hox gene complement of a Salpa species** 51
Hiroaki Okazawa and Hidetoshi Saiga

20 Genetic species identification of Anguilla japonica eggs and larvae: Leg 1 53
Tatsuki Yoshinaga

21 Genetic species identification of Anguilla japonica eggs and larvae: Leg 2 58
Ryusuke Sudo, Jun Aoyama and Tatsuki Yoshinaga

22 Studies on diet environment for the Japanese eel larvae using stable isotope analyses 61
Sachie Miyazaki, Hiroshi Morioka,
Takashi Kitagawa and Shingo Kimura

23 Natural iron stable isotope variation in fishes 62
Takafumi Hirata and Tsuguo Otake

24 Endocrinorogical analysis of growth retardation and malformation in captive-bred eel larvae 63
Ryota Tosaka and Shinji Adachi

25 Net records 64

26 CTD data table 83

27 XCTD data table 110

28 Working log 114

Introduction

Living resources in the sea are drastically declining in recent decades, and some of them are even at the edge of extinction. To conserve marine organisms properly and to aim for sustainable use of the resources in future generations, we need to understand the mechanism of population fluctuation based on accurate knowledge about each life history event such as growth, reproduction, mortality, distribution, migration etc. Among the biological resources in the sea, freshwater eels and tunas are highly exploited marine resources that show large-scale migrations and are vulnerable species that need urgent and prudent care.

We planned the KH-08-1 research cruise to increase our understanding of the spawning ecology and migration behavior of the Japanese eel and tunas. The cruise was separated into two legs, Leg 1 (21 May - 15 June) and Leg 2 (19 June - 14 July), with a port call at Naha, Okinawa in between. The main objectives of this cruise were (1) to confirm spawning events, and their timing and location by collecting eggs or small larvae of these two species, and (2) to determine the role of oceanic environmental factors in the formation of spawning aggregations in the ocean. A characteristic of the cruise this year was a combination survey together with the Kaiyo Maru of the Fishery Research Agency, Japan, by exchanging real-time oceanographic data and sampling information during the cruise, and the objective of the Kaiyo Maru was to collect adult eels using a big mid-water otter trawl.

Because of the calm sea during the cruise, we made almost 100% of the sampling and observations that were scheduled. As a result, we could successfully collect and genetically identify *A. japonica* preleptocephali to the south of the Suruga Seamount, confirming a spawning event of the Japanese eel at the southern West Mariana Ridge around the day of new moon (June 4) of Leg 1, as was observed during the new moon of June 2005 and August 2007. During Leg 2, however, we collected only a few possible anguillid preleptocephali in the same survey area with that of Leg 1, and this discrepancy in the abundance of larvae is of importance and will be analyzed in detail later. In the study area for tunas near Okinawa, we successfully collected about 100 specimens in combination with interesting oceanographic current data, all of which are to be analyzed later.

On behalf of all the scientists on board, we acknowledge the captain and his crew of the R/V Hakuho Maru for their heartful cooperation during the intensive sampling.

July 11, 2008 In the cabin of Hakuho Maru

K. Tsukamoto

Katsumi Tsukamoto
Shingo Kimura*
Chief Scientists of KH-08-1

*Could not be on board because of urgent and unavoidable matters on land

Station and working log. KH-08-1_Leg1

No.1

Station and working log. KH-08-1_Leg2

No.2

Scientists on board HAKUHO-MARU (KH-08-1)

Name	Affiliation
TSUKAMOTO Katsumi	Ocean Research Institute, The University of Tokyo
AOYAMA Jun	Ocean Research Institute, The University of Tokyo
Oya Machiko	Ocean Research Institute, The University of Tokyo
MILLER Michael J.	Ocean Research Institute, The University of Tokyo
WATANABE Shun	Ocean Research Institute, The University of Tokyo
SHINODA Akira	Ocean Research Institute, The University of Tokyo
YOKOUCHI Kazuki	Ocean Research Institute, The University of Tokyo
SUDO Ryusuke	Ocean Research Institute, The University of Tokyo
HAGIHARA Seishi	Ocean Research Institute, The University of Tokyo
OKAZAWA Hiroaki	Ocean Research Institute, The University of Tokyo
MANABE Ryotaro	Ocean Research Institute, The University of Tokyo
KITAGAWA Takashi	Ocean Research Institute, The University of Tokyo
MIYAKE Yoichi	Ocean Research Institute, The University of Tokyo
MIYAZAKI Sachie	Ocean Research Institute, The University of Tokyo
MORIOKA Hiroshi	Ocean Research Institute, The University of Tokyo
MIZOROGI Nao	Ocean Research Institute, The University of Tokyo
MACHIDA MASAMICHI	Ocean Research Institute, The University of Tokyo
OTAKE Tsuguo	Ocean Research Institute, The University of Tokyo
HATA Masayoshi	Ocean Research Institute, The University of Tokyo
AMANO Hironori	Ocean Research Institute, The University of Tokyo
IMAI Keiri	Ocean Research Institute, The University of Tokyo
ISHIGAKI Hideo	Ocean Research Institute, The University of Tokyo
OGUMA Kenji	Ocean Research Institute, The University of Tokyo
TOSAKA Ryota	Graduate School of Fisheries Science, Hokkaido University
YOSHINAGA Tatsuki	School of Marine Biosciences, Kitasato University
TAKANASHI Kazumasa	School of Marine Biosciences, Kitasato University
YAMAGUCHI Mikiya	School of Marine Biosciences, Kitasato University
SATO Manabu	Graduate School of Comprehensive Human Sciences,University of Tsukuba
BAN Kazuyuki	School of Marine Science and Technology, Tokai University
YOSHIDA Yuho	Gaduate School of Biosphere Science, Hiroshima University

Track Chart [KH-08-1_leg1]

Track Chart [KH-08-1_leg1]

Track Chart [KH-08-1_leg1]

Track Chart [KH-08-1_leg2]

Track Chart [KH-08-1_leg2]

Studies on oceanic conditions of larval transport of the Japanese eel in North Equatorial Current

Kei Zenimoto, Sachie Miyazaki, Youichi Miyake, Hiroshi Morioka, Nao Mizorogi, Takashi Kitagawa and Shingo Kimura

Ocean Research Institute, The University of Tokyo

The spawning area of Japanese eel (*Anguilla japonica*) was identified in the North Equatorial Current (henceforth NEC) to the west of the Mariana Islands. Since small larvae of the species were collected at and on the south side of the salinity front, the front considered as a landmark for detection of the spawning area. In this cruise, the CTD observation was carried out along 130, 134, 137 and 140°E (Fig 1). The vertical temperature and salinity structures along the each line were investigated by using there data (Fig 2). The salinity front with a large gradient wasn't identified along 140°E line.

Additionally, three zenilight buoys were released at 13°, 14°, 15°N along 142°E (Fig 1) with 150m depth. The buoys released at 14° and 15°N drifted generally westward for the first 5d after release. On the other hand, the buoy released at 13°N drifted southwestward (Fig 3). We will investigate latitudinal difference in drift process between three in the NEC.

Moreover, using OFES (OGCM for the Earth Simulator) velocity data, a numerical simulation for particle-tracking was conducted in case of fixed spawning site (13°N, 142°E) where the small larvae below 10mm in TL were distributed. Duration of the simulation was five months from May, and the larval inhabiting depth was fixed at 100m. The $100m^2s^{-1}$ was adopted as a horizontal diffusivity coefficient. As a result, transported rates into the Kuroshio fluctuated from 0.0% to 36.9% from 1999 to 2004 (Fig 4). We will investigate the flow structure, and recalculate a transported rate in this year.

Fig 1. Locations of CTD observation lines (a) 130, (b) 134, (c) 137, (d) 140° E (solid line with circles), release point of zenilight bouys(squares), and release point used to numerical simulation for particle tracking (red stars). Red triangles indicate three seamount (i.e. Passfinder, Arakane, Suruga).

Fig. 2. Profiles of temperature (left:°C) and salinity (right:psu) along the each line.

Fig. 3. Detailed trajectory of the zenilight buoys for the first 5d after release. Location .
Location of the buoys is plotted every day and shoown by the circles. Stars indicate release points.

Fig 4. Numerical Simulation for particle-tracking from 1999 to 2004. Dots indicate the release points of particle. The figures indicate the percentages of particle transported to the Kuroshio during each year.

Preleptocephali and leptocephali of *Anguilla japonica* collected during the KH-08-1 cruise

All Scientists on Board

During the KH-08-1 cruise, the larvae of the Japanese eel, *Anguilla japonica*, were the most abundant species of leptocephali that was collected. There were 393 preleptocephali or leptocephali that were morphologically or genetically identified as being the Japanese eel during the cruise. These leptocephali were collected with 2 kinds of nets and in latitudinal transects or in station grids (Fig. 1-3). During Leg 1 in the 140°E transect, daytime IKMT tows with 2000 m of wire out (w.o.) and nighttime tows with 900 m of w.o. were made for a total of 9 tows. Around the Suruga Seamount, 135 IKMT tows were made with a maximum net depth 300 m (see Yoshinaga et al., Report). In the Leg 1 preleptocephalus grid, the ORI-Big Fish net was used in 29 tows with a net depth of 300 m. Then a latitudinal transect of stations was sampled with 12 IKMT tows along 134°E with daytime tows with 900 m w.o. and nighttime tows having a net depth of 120 m. During Leg 2, sampling was made with 12 IKMT tows along 130°E with the same method as along 134°E. There were also 12 tows made with this method in the 137°E transect. During the egg survey of Leg 2, the ORI-Big Fish net was towed to a depth of 250 m in a total of 56 tows. In the preleptocephalus grid, 79 tows were made with the same maximum depth of 250 m. Many of the *A. japonica* specimens were identified on board with Real-Time PCR (RTPCR).

Figure 1. Map showing the locations that were sampled with the IKMT (transects and Surgua Seamount) and the ORI net (shaded box), and the catches of *Anguilla japonica* larvae (colored circles) during Leg 1 and Leg 2 of KH-08-1.

Figure 2. Map showing the stations that were sampled around the Suruga Seamount (inset), and the locations where *Anguilla japonica* preleptocephali were collected during Leg 1 of the KH-08-1 cruise. The numbers in the circles indicate the number of larvae collected at each station and the star shows the Kaiyo Maru point.

During Leg 1, there were 15 *A. japonica* leptocephali collected in the 140°E transect that were 9.3 - 12.1 mm TL and were born in May (Fig. 1). No *A. japonica* leptocephali were collected around Suruga Seamount, but in the preleptocephalus grid, 241 preleptocephali were collected that were about 5 days old with a spawning date of 2 June, 2 days before new moon. None were collected in the 134°E line of Leg 1.

Figure 3. Map showing the stations that were sampled in the egg grid (shaded circles) and in the preleptocephalus grid (open circles), and the locations were *Anguilla japonica* preleptocephali were collected during Leg 2 of the KH-08-1 cruise. The numbers in the circles indicate the number of larvae collected at each station, but the western specimen was not confirmed genetically yet (not shown in Fig. 1).

During Leg 2 of the cruise, 2 larger *A. japonica* leptocephali were collected in the 130°E transect that were 24.5 and 24.7mm TL (Fig. 1). A large number of June born leptocephali 11.6-19.2 mm TL (N = 132) were collected in the 137°E transect, along with one May born leptocephalus 28 mm TL (Fig. 1). No Japanese eel larvae were collected in the egg survey, but one 4.7 mm TL preleptocephalus about 5 days old that would have been spawned on 1 July, 2 days before new moon was collected. Another morphologically identified 5.1 mm TL *A. japonica* larva was also collected to the west, but was not confirmed using RTPCR (Fig. 3). All specimens will be genetically identified later in the laboratory.

Figure 4. Photographs of different stages of *Anguilla japonica* preleptocephali that were collected during Leg 1 of the KH-08-1 cruise that range in size from 4.7 to 5.1 mm TL.

The preleptocephali that were collected during Leg 1 were at a variety of different developmental stages that ranged from having no teeth and a large oil globule (Fig. 4, top), to having no oil globule and teeth and jaws (Fig. 4, bottom). These different stages were also collected during the 2005 cruise (Tsukamoto, 2006).

The results of the catch data showed that spawning could have been occurring at a range of latitudes at various times May, June and July of 2008, because at least a few leptocephali were collected at latitudes from 11°N to 16°N, which is consistent with previous reports of *A. japonica* leptocephali being caught at various latitudes (Ishikawa et al., 2001; Tsukamoto et al., 2003; Kimura and Tsukamoto, 2006; Tsukamoto, 2006). Further analysis will evaluate the distribution of leptocephali in 2008 in relation to ocean conditions.

References

Ishikawa, S., et al. 2001. Spawning time and place of the Japanese eel, *Anguilla japonica*, in the North Equatorial Current of the western North Pacific Ocean. Fisheries Sci. 67: 1097-1103.

Kimura, S., and K. Tsukamoto. 2006. The salinity front in the North Equatorial Current: A landmark for the spawning migration of the Japanese eel (*Anguilla japonica*) related to the stock recruitment. Deep-Sea Res. II 53:315–325.

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

Tsukamoto, K., et al. 2003. Seamounts, new moon and eel spawning: the search for the spawning site of the Japanese eel. Environ. Biol. Fish. 66: 221-229.

Yoshinaga, T, M. J. Miller, J. Aoyama, A. Shinoda, S. Hagihara, H. Okazawa, S. Watanabe, and K. Tsukamoto. 2008. Genetic characterization of the assemblage of leptocephali around the Suruga Seamount of the West Mariana Ridge. KH-08-1 Cruise Report, This volume.

Leptocephali of tropical anguillid species (*Anguilla marmorata* and *A. bicolor pacifica*) collected during KH-08-1 cruise.

All scientists on board

A total of 21 specimens of tropical anguillid leptocephali were collected using IKMT or ORI-Big Fish with 0.5 mm mesh sized plankton net during KH-08-1 cruise. These specimens were morphologically identified as 17 *A. marmorata* and 4 *A. bicolor pacifica* on board, although subsequent genetic analysis will be carried out later to confirm these morphological species identification.

Total length of the specimens ranged from 12.4 – 50.1 mm and 36.6 – 50.8 mm for *A. marmorata* and *A. bicolor pacifica*, respectively. *Anguilla marmorata* leptocephali were widely found throughout the North Equatorial current region within the study area from 10°00' – 16°00' N and 130°00' – 141°43' E whereas *A. bicolor pacifica* leptocephali were collected at a limited locations of 11° 00' and 12°00' N on 134°00' E (Fig.1).

Larval distribution of *A. marmorata* obtained from this cruise was consistent with the previous report of the estimated spawning area of this species that was located to the south of the Japanese eel spawning site in the North Equatorial current (Miller et al. 2002). However, recent population genetic analyses found that *A. mamorata* in the North Pacific has at least two different genetic populations, the one being distributed from Sulawesi Island north through the Philippines to southern Japan, and the other specifically around Mariana region (Minegishi et al. in press). This was also supported morphologically with the eels from Guam showing a significantly higher number of vertebrae than in any other areas in the North Pacific (Watanabe et al. in press). These findings suggested that specimens collected during this cruise probably included leptocephali derived from different populations. Population genetic analysis of leptocephali which will be conducted later and their distributional analyses in fine scale will give new insight into the migratory ecology of *A. marmorata*.

In the case of *A. bicolor pacifica*, no small leptocephali have ever been collected, so its spawning area is presently not known. The geographic range of this species extends across northern Indonesia, the Philippines and New Guinea, so it may also spawn somewhere offshore in the Western North Pacific. The lack of small leptocephali of *A. bicolor pacifica* requires more extensive research cruise in the tropical Pacific areas.

References

Miller MJ, Mochioka N, Otake T, Tsukamoto K. Evidence of a spawning area of *Anguilla marmorata* in the western North Pacific. Mar. Biol. 2002;140: 809-814.

Minegishi Y, Aoyama J, Tsukamoto K. Multiple population structure of the giant mottled eel *Anguilla marmorata*. Mol. Ecol. In press.

Watanabe S, Aoyama J, Miller MJ, Ishikawa S, Feunteun E, Tsukamoto K. Evidence of population structure in the giant mottled eel, *Anguilla marmorata*, using total number of vertebrae. Copeia. In press.

Fig.1 Collections of the tropical anguillid leptocephali during KH-08-1 cruise. Blue circles indicate stations where *A. marmorata* leptocephali were collected, and red circle, *A. bicolor pacifica*. Numbers inside the circle showed number of specimens, and outside, total length of specimens.

Notes on some fish eggs collected west of the Mariana Islands

Akihiro Okamura[1], Jun Aoyama[2] and Katsumi Tsukamoto[2]
[1]*IRAGO Institute Co., Ltd.*
[2]*Ocean Research Institute, The University of Tokyo*

Hundreds of fish eggs were collected by plankton nets (IKMT or ORI nets) in waters west of the Mariana Islands during the R/V Hakuho-Maru cruise (KH-08-1), from May to July 2008. Out of all eggs collected, 310 eggs were selected for further detailed observation based on their morphological resemblance to the eggs of Japanese eels Anguilla japonica that were obtained through artificial maturation and fertilization technique. These eggs were preserved in 99% ethanol for subsequent genetic species identification in a laboratory (Table 1).

Here, we briefly describe the morphological characteristics of these eggs. We observed the embryogenesis and the morphology of larvae hatched from some eggs for species identification by incubating eggs on board.

Morphological characteristics of several egg specimens were similar to those of A. japonica eggs (Fig. 1). Some characteristics such as egg diameter (ca. 1.3 mm) and oil-drop size (ca. 0.3 mm) of type A eggs (n=5) collected at St. 137 (Fig. 1a) were mostly the same with those of A. japonica eggs, although the specimens had a handling damage. Type B eggs collected at St. 258 were relatively common in these areas (Fig. 1b). Shape of the larval body was similar, but egg diameter (ca. 2.0 mm) was apparently larger than those of A. japonica eggs (1.2-1.6 mm) (Fig. 1e-g). Type C eggs had also some similar characteristics to A. japonica, but previtellin space was somewhat narrower (Fig. 1c). Type D eggs (Fig. 1d) appeared to be the early stage of the type B eggs (Fig. 1b), because egg diameter and oil droplet size were similar, but final decision will be made by genetic species identification in a laboratory.

More than 20 eggs were collected at St. 73 (Fig. 2a) and out of these, two types (E, F) of eggs were incubated in a small glass dish (2.5 cm in diameter) at about 26°C to observe the morphology of hatched larvae. Type E eggs had somewhat similar to A. japonica eggs (Fig. 2b). However, the morphology of hatched larvae was totally different from that of A. japonica larvae, with many lateral pigments and posteriorly localized oil-drop (Fig. 2b). Type F eggs and their hatched larvae had yellow colored oil-drop (Fig. 2c). The morphology of these larvae suggests that these were not anguilliform fishes.

Type G eggs collected at St. 113 were also common in these area (Fig. 3). This type had a unique characteristic with "double egg envelope" during the early developmental stage, although exact nature was unclear (Fig. 3a,b). Hatched larvae had a relatively long and thin body (fig. 3d).

Type H and I eggs collected at St. 307 and 309, respectively, frequently occurred in these area (Fig. 4). Overall morphology of these eggs at the early developmental stage was similar to that of A. japonica eggs except its smaller egg diameter (about 1 mm). However, various different characters appeared at the later stage during the incubation experiment. In

type H eggs, the previtellin space became narrow and a number of pigment spots appeared on the embryo and the yolk sac membrane (Fig. 4b,c). In type I eggs, oil-drop were localized to the posterior part of yolk sac at the later developmental stage and in hatched larvae.

A total of 310 eggs in Table 1 will be subjected to genetic analyses for exact species identification in a laboratory.

Table 1. Number of eggs sampled for genetic species identification at each station.

St.	No. eggs collected	St.	No. eggs collected	St.	No. eggs collected	St.	No. eggs collected
25	1	113	1	199	6	303	1
32	1	116	1	200	1	307	10
33	1	119	1	205	3	309	38
42	4	121	1	207	1	314	3
46	5	122	1	221	9	318	1
47	1	137	6	226	3	320	1
48	1	140	8	249	1	328	1
62	1	154	1	258	1	333	1
73	148	154?	1	275	16	337	1
75	1	159	3	276	2	341	2
82	3	164	1	288	3	348	1
92	2	176	1	289	1		
106	1	181	1	299	1	Total	310
108	2	191	2	302	1		

Figure 1. Photographs of collected eggs (type A-D) during the cruise KH08-1 (a-d) and *Anguilla japonica* eggs obtained by artificial reproduction technique in laboratory (e-g).

Figure 2. Photographs of two types of eggs (type E, F) collected at St. 73 during the cruise KH08-1. (a) A large sample collected at this station. (b) Type E eggs and its hatched larva after incubation. (c) Type F eggs and its hatched larva.

Figure 3. Photographs of eggs (type G) collected at St. 113 during the cruise KH08-1. (a) before incubation. (b) about 6h after incubation. (c) about 16h after incubation. (d) a hatched larva about 24 h after incubation.

Figure 4. Photographs of eggs (type H,I) collected at St. 307 (a-c) and 309 (d-f) during the cruise KH08-1. (a) A type H egg before incubation. (b) A type H egg about 24h after incubation. (c) A Type H egg 36h after incubation. (d) A type I egg before incubation. (e) A type I egg 24h after incubation. (f) a hatched larva from a type I egg.

Leptocephali collected during the KH-08-1 cruise

Michael J. Miller[1], Tsuguo Otake[1], Jun Aoyama[1], Seishi Hagihara[1], Hiroaki Okazawa[1], Tatsuki Yoshinaga[2], Akira Shinoda[1], Ryusuke Sudo[1], and Katsumi Tsukamoto[1]

1*Ocean Research Institute, the University of Tokyo*

2*School of Marine Biosciences, Kitasato University*

A total of 1,479 leptocephali and 23 juvenile eels of at least of 17 families of eels and their close relatives were collected during the KH-08-1 cruise (Table 1). These leptocephali were collected using either the Isaacs Kidd Midwater Trawl (IKMT) or the large ORI ring net. The IKMT has an 8.7 m2 mouth opening and 0.5 mm mesh, and the large ORI ring net has a 7.1 m2 mouth opening and 0.5 mm mesh, and both nets were fished in oblique tows in the upper 300 m during both day and night. Sampling was conducted in several regions with different objectives, so the data are separated by region and net type in Table 1.

A continuous grid around Suruga Seamount was sampled with the IKMT that collected 292 leptocephali and 5 juvenile eels. North-south transects with the IKMT were also made between 8 and 20°N during both legs that collected 416 leptocephali including 147 Anguilla japonica, 15 A. marmorata, and 4 Anguilla bicolor (See other reports). Sampling was also conducted in shallow tows with the IKMT in the bluefin tuna spawning area southeast of Okinawa Island that collected 175 leptocephali. The large ORI ring net was used in the sampling grids for Japanese eel eggs and preleptocephali and collected 239 leptocephali during Leg 1 and 311 during Leg 2, including 241 likely Anguilla japonica preleptocephali, as well as 13 juvenile eels (Table 1).

Combining all catch data from KH-08-1, the leptocephali of Anguilla japonica (N = 390) were the most abundant species, followed by the leptocephali of the Congridae (N = 301) whose adults live in shallow water or on the upper continental shelf, with those of the genus Ariosoma being collected in the greatest numbers (N = 197). The leptocephali of the mesopelagic eels of the Serrivomeridae were also abundant (N = 295) and were collected at a wide range of sizes (Fig. 1). 20 juvenile serrivomerid eels were also collected. Many of the unidentified small preleptocephali ("UI" in Table 1) that were collected may also be of the Serrivomeridae. The leptocephali of the shallow water eels of the Muraenidae were the fourth most abundant family (N = 143) and were collected at a wide range of sizes (Fig. 2). Various species of the other shelf and slope eel families such as Chlopsidae (N = 21), Moringuidae (N = 1), Ophichthidae (N = 26), Nettastomatidae (N = 23), Synaphobranchidae (N = 10) were also collected. Leptocephali and a few juveniles of mesopelagic, bathypelagic, or deep-sea eels or their close relatives were collected, such as Nemichthyidae (N = 92), the Derichthyidae (N = 91), the Cyematidae (N = 2), the Eurypharyngidae (N = 2), and the Notacanthiformes (N = 8). Some leptocephali of the coastal fishes, the tarpons and ladyfishes of the Elopiformes, were also collected (N = 6), with all but one being caught near Okinawa (Table 1).

A wide variety of small leptocephali were collected that are useful to learn about the spawning

areas of eels (Fig. 3,4,5). Many of these were collected around the Suruga Seamount, and others were collected in the tuna larvae sampling area near Okinawa. Small larvae of the mesopelagic eels were collected near the seamount and in the open ocean. Genetic identification techniques will be used to attempt to match these small leptocephali with their adult taxa.

Table 1. Catch data of leptocephali and juvenile eels that were collected in each of the sampling regions or transects (Trans.) using the two types of nets that were used during the KH-08-1 cruise.

Elopomorph Taxa	Suruga SM IKMT	Tuna Area IKMT	Leg 1 Trans. IKMT	Leg 1 ORI Net	Leg 2 Trans. IKMT	Leg 2 ORI Net	Cruise Total
Anguillidae							
Anguilla japonica	-	-	15	241*	132	2	390
Anguilla marmorata	-	-	8	3	7	-	18
Anguilla bicolor	-	-	4	-	-	-	4
Congridae							
Ariosoma sp. 7	37	6	8	13	8	76	148
Ariosoma sp. 4	9	16	2	2	1	3	33
Ariosoma spp.	5	3	2	-	2	4	16
Conger	10	2	16	1	2	2	33
Congrinae	8	50	1	-	2	4	65
Heterocongrinae	-	2	-	-	1	3	6
Chlopsidae	4	8	1	2	-	6	21
Muraenidae	28	44	3	6	5	57	143
Moringuidae	-	-	-	-	-	1	1
Ophichthidae	9	13	-	1	-	3	26
Muraenesocidae	-	3	-	-	-	-	3
Nettastomatidae	6	2	1	-	7	7	23
Synaphobranchidae							
Synaphobranchinae	5	-	-	-	-	-	5
Dysomma	-	-	1	-	1	2	4
Ilyophinae	-	-	-	-	-	1	1
Derichthyidae	47	7	13	8	4	11	90
Juvenile	1	-	-	-	-	1	2
Nemichthyidae							
Avocettina	16	1	5	5	2	11	40
Nemichthys	6	10	8	-	22	6	52
Juvenile	1		-	-	-	-	1
Serrivomeridae	92	2	33	53	38	77	295
Juvenile	4	-	2	4	2	8	20
Eurypharyngidae	-	-	-	1	-	1	2
Cyematidae	1	-	-	1	-		2
Elopiformes	-	5	-	-	-	1	6
Notacanthiformes	2	-	-	-	1	5	8
Unidentified/prelepto	6	1	1	2	6	28	44
Total	297	175	124	343	243	320	1502

* 121 not yet genetically confirmed

Figure 1. Length frequency distribution of the leptocephali of mesopelagic eels and of the outer shelf and deep slope eels (Synaphobranchidae) collected in all sampling during the KH-08-1 cruise.

Figure 2. Length frequency distribution of the leptocephali species of the Congridae (left panels) and other shelf and slope eels collected in all sampling during the KH-08-1 cruise.

Figure 3. A photograph of a 10.9 mm *Anguilla japonica* leptocephalus collected in the 140°E transect during Leg 1 of the KH-08-1 cruise.

Figure 4. Examples of photos of small leptocephali collected in various areas during the KH-08-1 cruise. A small congrid leptocephalus collected around the Suruga Seamount (top panel), a unidentified leptocephalus collected offshore (middle panel), and a small ophichthid preleptocephalus collected in the tuna sampling area (bottom panel).

Figure 5. Small leptocephali of the Ophichthidae (left) and Nettastomatidae (right) collected near Okinawa.

Figure 6. Anterior regions of *Muraenesox* ("hamo", left) and *Conger* (right) leptocephali during KH-08-1.

Genetic characterization of the assemblage of leptocephali around the Suruga seamount of the West Mariana Ridge

Tatsuki Yoshinaga[1], Michael J. Miller[2], Jun Aoyama[2], Akira Shinoda[2], Seishi Hagihara[2], Hiroaki Okazawa[2], Shun Watanabe[2], Katsumi Tsukamoto[2]
[1]*School of Marine Biosciences, Kitasato University.*
[2]*Ocean Research Institute, The University of Tokyo.*

Seamounts are interesting features of the worlds oceans that provide habitat for various benthic, epipelagic, and pelagic organisms in the deep ocean at depths more than 1000 m shallower than the surrounding environment (Rogers, 1994). Little has been known historically about the ecology of seamounts until commercially exploitable fishery resources were discovered in 1967 at deep seamounts at depths of about 300 - 500 m (Uchida et al., 1987). The discovery and rapid exploitation of these large schools of fishes then stimulated scientific research on seamounts in various locations of the world. Although only a tiny fraction of the many seamounts around the world have been studied, researchers quickly realized that seamounts are unique hotspots of biodiversity that may have endemic species and also serve as feeding or aggregation areas for migratory species such as tuna (e.g. de Forges et al., 2000; Morato and Pauly, 2004; Worm et al., 2004). However, because the deeper seamounts had the new unexploited fishery species, it has been the deeper seamounts that were studied, and not the shallower seamounts whose faunas were likely more similar to islands or atolls, which have many widely exploited coral reef or migratory fish species.

Figure 1. Map showing the location of the Suruga Seamount and the grid of sampling stations around the seamount during the KH-08-1 cruise.

Three seamounts of the West Mariana Ridge that reach very shallow depths of less than 50 m from the surface have been investigated as possible aggregation or spawning sites of the Japanese eel, *Anguilla japonica* (Tsukamoto et al., 2003), because small leptocephali or preleptocephali were captured near these seamounts or in the region to the west (Ishikawa et al., 2001; Tsukamoto et al., 2003; Tsukamoto, 2006). Various types of observations have also resulted in the confirmation of pelagic predators such as yellowfin tuna, *Thunnus albacares*, or bigeye tuna, *Thunnus obesus,* skipjack tuna, *Katsuwonus pelamis*, wahoo, *Acanthocybium solandri*, dogtooth tuna, *Gymnosarda unicolor*, tille trevaly, *Caranx tille*, the rainbow runner, *Elagatis bipinnulata*, and the roudi escolar, *Promethichthys prometheus*, around these 3 seamounts (Miller et al., 2008). A study using genetic identification of fish eggs around these seamounts and the region to the west has also confirmed that a variety of fish species spawn at the seamounts including skipjack tuna (Kawakami et al., In review). However, although some eel species have been collected in trawling surveys of deeper

seamounts and synaphobranchids have been observed in an apparent spawning aggregation at a seamount (Fujikura et al., 2001), there have been no studies of the fauna of marine eels that use seamounts. The present study will examine the possible species composition of marine eels at the Suruga Seamount by genetically identifying the taxa of eels that may have been spawning at the seamount based on collections of their small recently spawned leptocephali during the KH-08-1 cruise.

A square grid consisting of 8 stations was sampled continuously with each station being occupied repeatedly as the ship moved around the grid from 27 May - 6 June 2008 during Leg 1 of the KH-08-1 cruise. Sampling was conducted using the Isaacs Kidd Midwater Trawl (IKMT) with an 8.7 m^2. mouth opening and 0.5 mm mesh that was fished in oblique tows to a depth of 300 m during both night and day in a total of 135 tows.

Figure 2. Length frequency distribution of leptocephali of shallow water eels (*Ariosoma*, Muraenidae, *Conger*, Ophichthidae) and the mesopelagic nemichthyid genus *Avocettina* collected around the Suruga Seamount during the KH-08-1 cruise. Gray shading indicates the size range of small leptocephali < 15 mm that likely would have been spawned at Suruga Seamount.

During the sampling around Suruga Seamount 292 leptocephali, and 5 pelagic juvenile eels, of at least 41 species were collected that included 11 families of marine eels or their close relatives. The leptocephali collected ranged from tiny preleptocephali as small as 5 mm to very big *Ariosoma* leptocephali as large as 285 mm (Fig. 2, 3). The leptocephali of the mesopelagic eels of the family Serrivomeridae were the most abundant and were caught at a wide range of sizes up to about their maximum larval size of around 60 mm, but the greatest number was of the small recently spawned larvae (Fig. 3). Many small leptocephali of the other mesopelagic familes Derichthyidae and Nemichthyidae (*Avocettina*) were also collected along with larger size classes (Fig. 3).

In addition to the mesopelagic eel families that spawn and recruit to the open ocean, small leptocephali of several families of shallow water or slope eels were collected that based on their small size, must have been spawned at the Suruga Seamount. These included small ophichthids (snake eels), *Conger*, muraenids (moray eels), and nettastomatids. Almost all of these taxa of eels spawn close to the shelf or slope without long offshore migrations, so the Suruga Seamount is the only logical source of these small recently spawned leptocephali of shallow water eel species. Genetic sequences will be used to determine exactly how many marine eel species may have been spawned around the seamount, and how many total species were collected in the sampling grid conducted there. Because the Suruga Seamount is located within the typical latitude of the

westward flowing North Equatorial Current, the larger size leptocephali could have been transported to near the seamount from the east, and some of the large sized shallow water eel leptocephali such as muraenids or ophichthids, could have come from the coral reef or shallow habitats of the West Mariana Ridge, which includes islands such as Guam and Saipan (Fig. 1).

Figure 3. Length frequency distribution of leptocephali of shallow water eels (*Ariosoma*, Muraenidae, *Conger*, Ophichthidae) and the mesopelagic nemichthyid genus *Avocettina* collected around the Suruga Seamount during the KH-08-1 cruise. Gray shading indicates the size range of small leptocephali < 15 mm that likely would have been spawned at Suruga Seamount.

References

de Forges, B. R., J. A. Koslow, and G. C. B. Poore. 2000. Diversity and endemism of the benthic seamount fauna in the southwest Pacific. Nature 405: 944-947.

Fujikura, K., et al. 2001. Search for spawning areas of the Japanese eel *Anguilla japonica* using a deep-sea survey system. pp. 237–239. In: K. Aida, K. Tsukamoto & K. Yamauchi (ed.) Proc. Int. Symp.: Advances in Eel Biology. The University of Tokyo, Tokyo.

Ishikawa, S., et al. 2001. Spawning time and place of the Japanese eel, *Anguilla japonica*, in the North Equatorial Current of the western North Pacific Ocean. Fisheries Sci. 67: 1097-1103.

Kawakami, T., J. Aoyama, and K. Tsukamoto. In review. An evaluation of the species identification and distribution of fish eggs west of the Mariana Islands, using mitochondrial DNA sequences. Aquatic Biology.

Miller, M. J., T. Inagaki, J. Aoyama, K. Zenimoto, A. Shinoda, and K. Tsukamoto. 2008. Ichthyofauna at shallow seamounts: Unseen islands of biodiversity in the deep ocean. Abstract of the 5.th. World Fisheries Congress 2008, Yokohama Japan.

Morato, T., and D. Pauly, eds. 2004. Seamounts: Biodiversity and fisheries. Fisheries Centre Research Reports 12(5), 78 pp.

Rogers, A. D. 1994. The biology of seamounts. Advances in Marine Biology 30: 305-350.

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

Tsukamoto, K., et al. 2003. Seamounts, new moon and eel spawning: the search for the spawning site of the Japanese eel. Environ. Biol. Fish. 66: 221-229.

Uchida, R.N., S. Hayasi, and G. W. Boehlert, eds. 1986. Environment and resources of seamounts in the North Pacific. NOAA Technical Report, NMFS 43.

Worm B., H. K. Lotze, and R. A. Myers. 2004. Predator diversity hotspots in the blue ocean. Proceedings of the National Academy of Science 100: 9884-9888.

Offshore transport of small marine eel larvae from the Ryukyu Ridge by a rare branch of the Kuroshio Current

Michael J. Miller[1], Takashi Kitagawa[1], Jun Aoyama[1], Tatsuki Yoshinaga[2], Akira Shinoda[1], Seishi Hagihara[1], Hiroaki Okazawa[1], Shun Watanabe[1], Katsumi Tsukamoto[1]
[1]*Ocean Research Institute, The University of Tokyo.*
[2]*School of Marine Biosciences, Kitasato University.*

The Kuroshio Current flows northward along the edge of the continental shelf in the East China Sea (ECS) and is very strong and narrow in this region (Ichikawa and Beardsley, 2002; Lie and Cho, 2002). Some minor curvature or eddies occur as it flows through the ECS, but compared to when the Kuroshio flows offshore into the Kuroshio Extension and has many large meanders, the flow of the current in the ECS is generally straight to the northeast (Fig. 1).

Figure 1. Map of current speeds in June 2007 and July 2006 showing the typical flow pattern of the Kuroshio Current. Yellow and orange show current speeds of 40-78 cm/sec. Maps were generated from the 1/32° NLOM data assimilative real-time model using SST and SSH data gathered continuously.

However, in June 2008 there appears to have been a large branch of current flow transporting Kuroshio water to the east and then southward across the Ryukyu Ridge into the Philippine Sea of the western North Pacific (Fig. 2). This flow also appeared to be associated with a large persistent eddy to the east of Okinawa Island (Fig. 2). Large eddies do occur in this region (Takikawa et al., 2005; Nagano et al., 2007), but they are likely more short lived features than this eddy that remained stationary for at least a week (Fig. 2) and longer.

Figure 2. Map of current speeds in June 2008 during the KH-08-1 Hakuho Maru Cruise showing the sampling stations in the tuna larvae sampling area (bottom 2 panels) and current speeds that show an unusual branch off the Kuroshio Current that flows from the ECS into the western Philippine Sea.

This unusual pattern of surface circulation is interesting in relation to leptocephali, which have long larval duration and can be transported long distances by currents, because both the branch of the Kuroshio and the large eddy transported water over shallow water and coral reef habitats of some parts of the Ryukyu Ridge where many species of marine eels live and probably spawn. During the last several days of Leg 1 of the KH-08-1 cruise, sampling for bluefin tuna larvae was carried out southeast of Okinawa Island that collected many leptocephali including a variety of small sized leptocephali of shallow water and coral reef associated marine eels. This study will examine the species composition and size of these leptocephali in relation to this unusual pattern of Kuroshio Current flow and the large eddy off Okinawa Island.

17 stations were sampled in the area southeast of Okinawa Island on 13-15 June 2008 using the Isaacs Kidd Midwater Trawl (IKMT) with an 8.7 m^2 mouth opening and 0.5 mm mesh that was fished in oblique tows with 250 m of trawl wire out during both night and day. This resulted in 9 night tows and 8 daytime or crepuscular period tows. The maximum depth of these tows was about 50 m with some reaching as deep as 60 m.

Figure 3. Length frequency distributions of leptocephali of shallow water and outer shelf and slope eels that were collected southeast of Okinawa Island. Shaded areas show the size range of leptocephali likely spawned near Okinawa Island.

These 17 shallow tows of the IKMT collected 176 leptocephali of at least 44 species and 11 families. Leptocephali of moray eels of the family Muraenidae were the most abundant, and those of the congrid genus *Gnathophis* were second most abundant. There were many small muraenid leptocephali collected including 9 that were ≤ 10 mm that were caught at 5 different stations, but a wide range of sizes of ≥ 11 muraenid species were also among those collected (Fig. 3). A wide range of sizes of *Gnathophis* leptocephali were also collected (Fig. 4), and most of these were *Gnathophis nystromi nystromi*, which spawns at the shelfbreak of the ECS (Miller et al., 2002) and at other locations along the Pacific coast of Japan (Kimura et al., 2006), and is abundant offshore in the Kuroshio Extension (Takahashi et al., 2008; Miller et al., unpublished data). A high proportion of the other specimens of the Congridae, including *Ariosoma*, which reach very larges sizes of 250 - 300 mm, Ophichthidae, and Chlopsidae were small size leptocephali (Fig. 3, 4). Small *Ariosoma* sp. 7 (11 - 15 mm) were collected that provide an important clue to where this species spawns before their leptocephali become widely distributed offshore in the western North Pacific subtropical gyre, including the Japanese eel spawning region far offshore. Only small sized *Gorgasia* (garden eels), and nettastomatids were collected. Five specimens of leptocephali of tarpon and ladyfishes (Elopiformes) were also collected, which are not present in offshore collections away from coastal areas. This, and the collection of many small size leptocephali of muraenids, ophichthids, and chlopsids whose adults do not appear to migrate offshore offshore to

Figure 4. Length frequency distributions of leptocephali of shallow water congrid eels (*Ariosoma*, Congrinae, *Gorgasia*) and the mesopelagic nemichthyid genus *Nemichthys* collected southeast of Okinawa Island. Shaded areas show the size range of leptocephali likely spawned near Okinawa Island.

spawn (Miller, 2002), as well as small congrids that likely are also mostly spawned near the outer continental shelf, indicates that the water mass that was sampled had been transported offshore after passing by the islands and shelf areas in the Okinawa Island region (Fig. 2).

Intrusions of the Kuroshio Current onto the continental shelf of the ECS have been reported (e.g. Guo et al., 2006), but there is no mention of branches of the Kuroshio Current extending to the east towards the Ryukyu Ridge in reviews describing this region (Ichikawa and Beardsley, 2002; Lie and Cho, 2002). Therefore, the event observed in the 1/32 NLOM model output data (Fig. 2) seems to be a rare occurrence. The 1/32 NLOM model output data appears to be a very accurate representation of actual ocean conditions (Shriver et al., 2007), and the ADCP current data collected during the tuna larvae sampling confirmed strong southward flow in the western region of the sampling area (Fig. 5), as was shown in the current map for 14 June 2008 in Figure 2 (lower left). Surface flow in this direction is rare, but at least one drifter moved from the Kuroshio across the Ryukyu Ridge as indicated in the mean current field derived from all surface drifters in the region from 1989 - 1996 (Nakamura et al., 2007).

Because leptocephali have a long larval duration, they can serve as useful indicators of the history of the water mass in which they are collected. In this case, the assemblage of leptocephali indicates that the water that was sampled had passed by the coastal regions of Okinawa Island and the nearby shelf areas. This may also typically occur with other large eddies in the region, but the strong flow of a eastward branch of the Kuroshio may be unusual. Oceanographic studies have indicated there is typically a northeast current close to the eastern edge of the Ryukyu Ridge from about Okinawa Island and to the north, and a southwest current further offshore (Zhu et al., 2005; Nagano et al., 2007), but these currents may be stronger at depths below 200 m (Ishikawa et al., 2004) and may not affect leptocephali as much as currents near the surface that may be more determined by eddy structure.

Further analyses can genetically identify some of the small leptocephali collected in this area to better understand the species composition of the eels that had been spawning in the region. The oceanographic literature will also be examined to determine what is known about this type of unusual eastward branch of the powerful Kuroshio Current in the ECS.

Figure 5. Map showing the ADCP current vectors during the last part of the line of IKMT stations in the tuna larvae sampling area, showing strong southward flow of the apparent branch of the Kuroshio.

References

Guo, X., Y. Miyazawa, and T. Yamagata. 2006. The Kuroshio onshore intrusion along the shelf break of the East China Sea: The origin of the Tsushima Warm Current. J. Phys. Oceanogr. 36: 2205-2231.

Ichikawa, H., R. C. Beardsley. 2002. The current system in the Yellow and East China Sea. J. Oceanogr. 58: 77-92.

Ichikawa, H., H. Nakamura, A. Nishina, and M. Higashi. 2004. Variability of northeastward current southeast of northern Ryukyu Islands. J. Oceanogr. 60: 351-363.

Kimura, Y., M. J. Miller, G. Minagawa, S. Watanabe, A. Shinoda, J. Aoyama, and K. Tsukamoto. 2006. Evidence of a local spawning site of marine eels along northeastern Japan, based on the distribution of small leptocephali. Fish. Oceanogr.15: 183-190.

Lie, H. J., C. H. Cho. 2002. Recent advances in understanding the circulation and hydrography of the East China Sea. Fish. Oceanog. 11: 318-328.

Miller, M. J. 2002. Contrasting migratory strategies of marine and freshwater eels. Fish. Sci. 68 (Suppl. I): 37-40.

Miller, M. J., T. Otake, G. Minagawa, T. Inagaki, and K. Tsukamoto. 2002. Distribution of leptocephali in the Kuroshio Current and East China Sea. Mar. Ecol. Progr. Ser. 235: 279-288.

Nagano, A., H. Ichikawa, T. Miura, K. Ichikawa, M. Konda, Y. Yoshikawa, K. Obama, and K. Murakami. 2007. Current system east of the Ryukyu Islands. J. Geophys. Res. 112: 06009.

Nakamura, H., H. Ichikawa, and A. Nishina. 2007. Numerical study of the dynamics of the Ryukyu Current system. J. Geophys. Res. 112: C04016.

Shriver, J. F. H. E. Hurlburt, O. M. Smedstad, A. J. Wallcraft, R. C. Rhodes. 2007. 1/32° real-time global ocean prediction and value-added of 1/16° resolution. J. Mar. Systems 65: 3-26.

Takahashi, M., N. Mochioka, S. Shinagawa, A. Yatsu, and A. Nakazono. 2008. Distribution patterns of leptocephali in the Kuroshio–Oyashio transitional region of the western North Pacific. Fish. Oceanogr. 17: 165-177.

Takikawa, T., H. Ichikawa, K. Ichikawa, and S. Kawae. 2005. Extraordinary subsurface mesoscale eddy detected in the southeast of Okinawa in February 2002. Geophys. Res. Lett. 32: L17602.

Zhu, X.-H., J.-H. Park, and I. Kaneko. 2005. The northeastward current southeast of the Ryukyu Islands in late fall of 2000 estimated by an inverse technique. Geophys. Res. Lett. 32: L05608.

Functional morphology of the telescopic eye of leptocephali of the family Synaphobranchidae

Seishi Hagihara, Michael J. Miller, and Katsumi Tsukamoto
Ocean Research Institute, The University of Tokyo

The deep-sea eels of the family Synaphobranchidae live in relatively deep waters mostly between 200 m to 3000 m and are one of dominant species on the upper continental slopes of the world oceans. The collection of synaphobranchid leptocephali has been rare despite their adults having a wide distribution and high densities. Therefore knowledge about the ecology of Synaphobranchid leptocephali is scarce. Synaphobranchid leptocephali are distinguished from all other families of anguilliform leptocephali by having a so-called telescopic eye (Fig. 1). The telescopic eye is somewhat elongate in an anterodorsal-posteroventral direction, with the lens at the anterodorsal end. This character seems related to the depth of their habitat. However, there is no knowledge about the function of the telescopic eye of Synaphobranchid leptocephali. Understanding the function of the telescopic eye of synaphobranchid leptocephali will likely give some clue about their depth distribution and feeding ecology, and this will be discussed in relation to the evolution of synaphobranchid leptocephali. The purpose of this study will be to describe the function of the telescopic eye of synaphobranchid leptocephali using histological observations.

During the KH-08-1 cruise, 10 individuals of leptocephali of the family Synaphobranchidae were collected with the Isaacs Kidd Midwater Trawl (IKMT) or the ORI ring net. These included 5 individuals of subfamily Synaphobranchinae (46.8 – 98.2 mm TL) and included 5 individuals of subfamily Ilyophinae (28.0 – 59.0 mm TL). Specimens were sorted fresh from the plankton and decapitated expeditiously. Then the heads were fixed in 10% formalin for histological observations and their bodies were preserved in 99% ethanol for genetic identification. After the cruise, the visual axis and the visual acuity of synaphobranchid leptocephali will be estimated based on the density of the photoreceptor cells that will be analyzed using histological observations.

Figure 1. The head region of a 58.5 mm Ilyophinae leptocephalus collected during the KH-08-1 cruise.

Identification and distribution of open ocean midwater eels of the Nemichthyidae

Hiroaki Okazawa and Katsumi Tsukamoto
Ocean Research Institute, The University of Tokyo.

Pelagic eels of the family Nemichthyidae include 3 genera with about 9 species (Nelson, 1994) that are widely distributed from tropical to temperate regions in the Pacific, Atlantic and Indian Oceans (Castle, 1984). Some species of Nemichthyidae juveniles can be identified to the species level. However their larvae, called "leptocephali", are much less morphologically developed than the adults and are more difficult to exactly identify to the species level. In the Atlantic Ocean, 4 species have been identified by their leptocephalus morphology (Böhlke, 1989). But in the Pacific Ocean, their species composition is not known.

Thus, we will examine the genetic characters of the Nemichthyidae specimens collected during the KH-08-1 cruise (Table 1) to determine their species composition using molecular genetic characters within a portion of about 500 bp of their mitochondrial 16SrRNA sequences. At total of 52 *Nemichthys* and 40 *Avocettina* leptocephali were collected during the cruise. After the cruise, these genetic data will be compared with juveniles that have been identified to species by their morphology. These data will enable us to genetically identify Nemichthyidae leptocephali. Furthermore, their distribution will provide important information about their life history and spawning ecology.

Table 1. Number of Nemichthyidae leptocephali collected during the KH-08-1 cruise.

Genera	Leg 1	Leg 2	Total
Nemichthys	25	27	52
Avocettina	27	13	40
Labichthys	0	0	0

References

Böhlke, E. B. 1989. Fishes of the Western North Atlantic. Volume 2. Sears Foundation Marine Research, Yale University Pages 925-932.

Castle, P. H. J. 1984. Notacanthiformes and Anguilliformes: Development, in ontogeny and systematics of fish. Moser H. G., Richards W. J. (eds) American Society of Ichthyology and Herpetology Special Publication No.1. Allen Press, Kansas.

Nelson, J. G. 1994. Fishes of the world. 3rd ed.

Ariosoma leptocephali collected during the KH-08-1 cruise and the ecology of the *Ariosoma* sp. 5 leptocephali in the Central Indo-Pacific

Mikiya Yamaguchi[1], Michael J. Miller[2], Tatsuki Yoshinaga[1], and Katsumi Tsukamoto[2]

[1]*School of Marine Biosciences, Kitasato University.*

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

Eight species of leptocephali of the genus *Ariosoma* that have a normal-type gut have been described from the western North Pacific. During the KH-08-1 cruise, *Ariosoma* sp. 7 was the most abundant species of the genus and we collected 148 specimens of with total length between 11.5 to 346.8 mm (Table 1). So far neither egg nor small leptocephali of *Ariosoma* sp. 7 have been collected in this region though, but the smaller size leptocephali were collected near Okinawa, suggesting that spawning may occur in that region. This species has recently been genetically identified as being *Ariosoma major*, which is abundant in the East China Sea (Ma et al., 2008). Based on the distribution pattern of relatively large specimens, many larvae of this species are transported back to the west by the North Equatorial Current, after being transported offshore by countercurrents or recirculation from the Kuroshio Extension region. *Ariosoma* sp. 4 was the next most abundant during the cruise, and may have a similar spawning and distribution pattern, since they are also typically present in the North Equatorial Current.

Species	Leg 1	Leg 2	Leg 1+Leg 2
Ariosoma sp. 7	64	84	148
Ariosoma sp. 3	2	0	2
Ariosoma sp. 4	27	4	31
Ariosoma sp. 5	1	1	2
Ariosoma sp. 6	3	0	3
Ariosoma sp. 8	2	0	2
Total	99	89	188

Table 1. Number of *Ariosoma* specimens collected during the KH-08-1 cruise.

The other species of *Ariosoma* leptocephali were much less abundant and only 2 specimens of sp. 5 were collected during the cruise (Table 1). Although sp. 7 is the most abundant species in the North Equatorial Current region, *Ariosoma* sp. 5 is the most abundant species in the Indonesian Seas (Miller et al., 2006). These are likely the larvae of *Ariosoma scheelei*, which is a tropical species. This study will focus on the distribution and ecology of sp. 5 leptocephali in several sampling surveys in the Indonesian Seas region. These data will then be compared to the distribution and abundance of sp. 5 leptocephali in the western North Pacific during KH-08-1 and previous cruises, and will use genetic techniques to compare the species composition of the larvae.

References

Ma, T., M. J. Miller, J. Aoyama, G. Minagawa, J. G. Inoue, S. Watanabe, K. Tsukamoto. 2008. Genetic identification of two species of *Ariosoma* leptocephali. Coastal Marine Science, In press.

Miller, M. J., S. Wouthuyzen, G. Minagawa, J. Aoyama, and K. Tsukamoto. 2006. Distribution and ecology of leptocephali of the congrid eel, *Ariosoma scheelei*, around Sulawesi Island, Indonesia. Marine Biology 148: 1101–1111.

Studies on larval transport of Bluefin tuna from the spawning area to nursery grounds

Takashi Kitagawa[1], Nao Mizorogi[1], Tatsuki Yoshinaga[2], Shingo Kimura[1]
[1]*Ocean Research Institute, The University of Tokyo,*
[2]*School of Marine Biosciences, Kitasato University.*

The main spawning area of Paciific bluefin tuna *Thunnus orientalis,* Scombridae is located in subtropical warm waters on the east side of the Kuroshio Current, between the south of Japan and the Philippines. The Kuroshio Current probably plays an important role in transporting the larvae, postlarvae, and juveniles northward from the spawning area. Larval transport is governed by oceanic conditions, and the objectives of our study were therefore to investigate the hydrographic structures linked to the transport of bluefin tuna larvae.

17 stations were sampled in the area southeast of Okinawa Island on 13-15 June 2008 using CTD (+CMS) (or XCTD) and/or the Issacs Kidd Midwater Trawl (IKMT) with an 8.7 m^2 mouth opening and 0.5 mm mesh that was fished in oblique tows with 250 m of trawl wire out during both night and day. The maximum depth of these tows was about 50 m with some reaching as deep as 60 m.

Scombrid larvae were caught at stations of St. 195, 196, 197, and 198. All of the samples were used for DNA analysis for species identification. Though analysis of detailed hydrographic structures is now in progress, but in June 2008 there appears to have been a large branch of current flow transporting Kuroshio water to the east (Miller et al. in this report). This branch may play a role of offshore larval transport.

Figure 1. Map of sampling stations in the tuna larvae sampling area during the KH08-1.

Occurrence of bothid larvae collected during Leg.1 of the KH-08-01 cruise

Kazuyuki Ban
School of Marine Science and Technology, Tokai University

Fishes of the family Bothidae, suborder Pleuronectoidei, are comprised of about 140 species of 20 genera in two subfamilies, Taeniopsettinae and Bothinae (Nelson, 2006). Among them, larvae of 17 genera have already been reported by previous studies (Fukui, 1997). They are characterized by an extremely compressed transparent body, dorsal fin that begins anterior to the eye, elongated second ray of dorsal fin in preflexion and flexion larvae. Furthermore, their metamorphic size are the largest among Pleuronectoid fishes (more than 15 mm in standard length), being known 120 mm SL in *Chascanopsetta lugubris lugubris* (Amaoka, 1970). Adults of bothid are distributed in shallower than 200 m depth except several deeper genera, e.g. *Chascanopsetta*, their larvae widely dispersed from shallower waters (spawning ground) to oceanic waters (Fukui and Ozawa 1988). Taxonomic and ontogenetic studies on bothid fishes were few in the South Pacific Ocean until 1990s. However, the former studies has been recently advanced (Amaoka and Arai, 1998; Amaoka and Mihara, 2001; Amaoka and Bernard, 2005). Based on these newly studies, I will review the ontogeny of bothid larvae in the western South Pacific Ocean collected by R/V Hakuho Maru (KH-04-5 cruise). In this (KH-08-01) cruise, I aimed to make clear the fauna of bothid larvae in the western North Pacific as comparative data in the western South Pacific Ocean.

Methods

A total of 186 specimens (13 species of 6 genera) collected at Leg.1 of the KH-08-01 cruise, using IKMT net (mesh size 0.55 mm) and ORI net (mesh size 0.55 mm) (Table 1). They were fixed in approximately 10% seawater formalin. The larval periods and species identification followed those of Fukui and Ozawa (1988): stage Ⅰ, preflexion stage; stage Ⅱ, flexion stage; stage Ⅲa, postflexion stage, until disappearance of the air bladder; stage Ⅲb, metamorphic stage, until completion of right-eye migration to the left side; stage Ⅳ, after metamorphosis. This study area were divided into three locations: St.1 -St.181 "Sea Mount area"; St.182 -193 "134°E line"; St.194 -St. 211 "East coast to Okinawa ".

Results

Sea Mount area

A total of 27 specimens [6 species of 5 genera (*is not identification type)], collected at "Sea Mount area" (Table 1). *Crossorhombus kobensis* (16 specimens) was dominant in this area. The developmental stage is only stage IIIa, the standard length ranged from 7.8 to 12.2 mm (mean± SD, 10.0 ± 1.39 mm). Developmental stages of other bothid larvae are IIIa or IIIb.

134°E line

A total of 2 specimens (only *Asterorhombus* sp.1*, stage IIIa), collected at "134°E line" (Table 1).

East coast to Okinawa

A total of 157 specimens [13 species of 6 genera (*is not identification type)], collected at "East coast to Okinawa " (Table 1).

Asterorhombus sp.1* (30 specimens) and *Engyprosopon* sp.2* (30 specimens) were dominant in this area. *Asterorhombus* sp.1*, the developmental stage is stage I and IIIa, the standard length ranged from 4.1 to 10.3 mm (mean± SD, 7.2 ± 1.22 mm). *Engyprosopon* sp.2*, the developmental stage is stage I to IIIa, the standard length ranged from 4.8 to 8.9 mm (mean± SD, 7.2 ± 2.68 mm). Developmental stages of other bothid larvae are I -IIIa.

Table 1. Bothid sorted out during Leg.1 of the KH-08-01 cruise

Location	Taxon	N	Standard length (mm)		Developmental stage *
			Range	Mean± SD	
"Sea Mount area"	*Crossorhombus kobensis*	16	7.4-12.2	10± 1.39	III a
	Bothus myriaster	5	17.2-26.5	17.3± 4.10	III b
	Arnoglossus sp.**	1	—	28.2	III b
	Engyprosopon sp.2*	3	8.4-11.6	9.9± 1.62	III a-III b
	Engyprosopon sp.**	1	—	8.5	III b
	Chascanopsetta lugubris lugubris	1	—	33.6	III b
"140 °E line'	*Asterorhombus* sp.1*	2	10.6-13.5	12.1± 2.05	III a
"East coast to Okinawa'	*Arnoglossus* sp.**	1	—	6.8	II
	Asterorhombus sp.1*	30	4.1-10.3	7.2± 1.22	I ,IIIa
	Asterorhombus sp.2*	17	6.2-12.0	8.1± 1.82	II-IIIa
	Bothus myriaster	5	5.3-13.4	7.5± 3.38	IIIa
	Chascanopsetta lugubris lugubris	3	11.5-15.9	13.3± 2.32	IIIa
	Crossorhombus kanekonis	1	—	5.7	I
	Crossorhombus kobensis	21	4.0-12.4	7.1± 2.60	I -IIIa
	Crossorhombus sp.**	16	4.2-7.9	5.2± 0.89	I – II
	Engyprosopon grandisquama	8	5.9-9.4	7.2± 1.13	IIIa
	Engyprosopon sp.**	15	4.9-16.8	7.8± 2.82	IIIa
	Engyprosopon sp.2*	30	3.9-13.2	7.2± 2.68	II – IIIa
	Engyprosopon sp.3*	1	—	5.1	II
	Psettina tosana	9	5.1-10.2	6.4± 2.10	I -IIIa

*Ozawa and Fukui (1988) *not identification

Cookie-cutter shark *Isistinus brasiliensis* and snake mackerel Gempylidae collected during KH-08-1 Hakuho Maru cruise

Yosuke Amano[1], Sho Tanaka[2] and Tsuguo Otake[1]

[1]*Ocean Research Institute, The University of Tokyo.* [2]*School of Marine Biology, Tokai University*

Cookie-cutter shark *Isistinus brasiliensis* is known to be an undersized species with the maximum length of only 500 mm (Nakabo, 2000). Although this species distributes in a depth range from 85 to 3500 m of worldwide tropical and subtropical waters, the knowledge about the life history is quite limited.

Snake mackerels *Promethichthys prometheus* and *Thyrsitoides marley* (Gempylidae), tropical and subtropical species, distribute in various sea area including continental shelf and sea mount. These species are both rich in wax within the muscle and are prohibited to trade as a commercial product.

Those three species are not commercially important. However, since they are all top predator in marine ecosystem, it is indispensable to study on their life history and behavior for understanding the structure and function of marine ecosystem and for optimizing the conservation strategy.

The objective of this study is to accumulate biological information about such fishes of top predator in marine ecosystem. During the KH-08-1 Hakuho Maru cruise (Leg.1 and Leg.2), a total of four cookie-cutter sharks *I.brasiliensis* (202-312mm TL), and two snake mackerels (a *P. prometheus,* 78 mm TL and a *T. marley,* 70 mm TL) were sampled (Table 1, Figure 1-3). We will examine age, growth, stomach contents and maturity of each individual. Collection data as well as such biological information will also contribute to further understanding of the distribution of those species.

Table 1. Collection data

Station No.	Species	Date	Latitude	Longitude	Total length (mm)
42	*Isistinus brasiliensis*	2008.5.29	14.03N	142.42E	245
85	*Promethichthy prometheus*	2008.6.1	14.23N	142.52E	78
198	*Isistinus brasiliensis*	2008.6.14	26.00N	129.00E	202
239	*Isistinus brasiliensis*	2008.6.29	13.35N	142.37E	312
329	*Thyrsitoides marley*	2008.7.7	14.45N	141.45E	70
354	*Isistinus brasiliensis*	2008.7.8	13.30N	141.40E	250

Figure 1. A: Cookie-cutter shark *Isistinus brasiliensis* (St. No.198, 202mm TL), B: Head part, and C: teeth of lower jaw

Figure 2. *Promethichthys prometheus* (St. No.85, 78mm TL)

Figure 3. *Thyrsitoides marley* (St. No.329, 70mm TL)

References

Nakabo,T. 2000. Fishes of Japan with pictorial keys to the species second edition. Tokai university press: 150, 1338-1340.

Studies on feeding ecology and species identification of phyllosoma larvae of palinurid and scyllarid lobsters

Masamichi Machida and Shuhei Nishida
Ocean Research Institute, The University of Tokyo

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. A large number of phyllosomas has been collected in previous research cruises (KH91-4, KH98-2) around West Mariana Ridge. The main purpose of the present cruise is to collect phyllosoma larvae for analyses of stable isotope ratios and gut contents, and for species identification using genetic markers.

Phyllosoma and zooplankton sampling

A total of 189 phyllosomas was corrected using a 3-m ORI ring net (7.1 m^2 mouth opening and 0.5 mm mesh aperture) and an Isaacs-kidd Midwater Trawl (IKMT, 8.7 m^2 mouth opening and 0.5 mm mesh aperture) both fished in oblique tows during Leg 1 and 2 (Table.1). Phyllosomas were sorted from the original zooplankton samples immediately after capture. Of these phyllosomas, undamaged specimens were kept alive for observations of swimming and/or feeding behavior, while dead, inactive, or damaged specimens were fixed or frozen for the respective analyses as described below.

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) and net zooplankton mixture (random sampling) for stable isotope analysis to estimate trophic levesl of phyllosoma (Table 2). These zooplankton samples and sorted phyllosomas were identified to genera, families, or higher taxonomic groups and frozen at -80°C for the analyses of stable-isotope ratios 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. Active phyllosomas were kept alive in aquaria, to which various zooplankton were provided, and the swimming and feeding behavior of the phyllosomas were observed and recorded.

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 KH08-1 cruise.

	Leg. 1	Leg. 2	KH 08-1
Panulirus spp.	38	42	80
Scyllarus spp.	53	39	92
Parribacus or *Scyllarides* spp.	2	5	7
Palinurid larvae *	8	2	10
Total	101	88	189

* Not identified to the genus level owing to damaged body structure.

Table 2. List of all samples during KH08-1 Leg.2 cruise. Circle: sorted out samples each station. Numerals in the table: number of collected phyllosoma larvae.

	St.212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237
Cnidaria		○	○	○					○	○		○				○	○	○	○	○	○	○	○	○	○	
Ctenophore				○														○								
Mollusca																										
Heteropoda																										
Atlantidae		○	○	○		○		○	○						○	○				○		○	○	○	○	
Carinariidae					○		○											○		○	○		○	○	○	
Pterotracheidae				○		○			○										○		○	○	○	○	○	
Thecosomata		○	○	○			○	○					○	○		○	○	○	○	○	○	○	○	○	○	
Gymnosomata		○			○		○	○	○	○	○	○	○	○	○	○	○	○			○	○	○			
Cephalopoda															○					○				○	○	
Annelida		○																		○						
Arthropoda																										
Amphipoda											○			○	○						○			○		
Decapoda											○	○	○					○						○		
phyllosoma	1				2					1			1				5	3			1					5
Chaetognatha		○	○	○		○	○	○		○	○	○	○	○	○	○	○	○	○	○		○	○	○	○	
Chordata																										
Urochordata																										
Thaliacea									○				○	○			○	○			○	○			○	
Salpida					○	○	○		○	○	○	○	○		○	○	○	○	○		○	○		○	○	
Vertebrata																										
Carangidae			○																							
Myctophidae				○					○			○					○					○	○			
Molidae						○																				
Psenes sp																								○		
Fish larva																○										

	St.239	247	248	250	252	261	262	265	270	276	283	284	285	286	292	295	296	297	300	306	307	309	310	311	316	317
Cnidaria																										
Ctenophore																										
Mollusca																										
Heteropoda																										
Atlantidae																										
Carinariidae																										
Pterotracheidae																										○
Thecosomata																										
Gymnosomata																										
Cephalopoda																										○
Annelida																										
Arthropoda																										
Amphipoda																										
Decapoda																										
phyllosoma	2	3	1	3	2	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1	2	1	1	1	2	
Chaetognatha																									○	
Chordata																										
Urochordata																										
Thaliacea																									○	
Salpida																										
Vertebrata																										
Carangidae																										
Myctophidae																										
Molidae																										
Psenes sp																										
Fish larva																										

	St.318	319	322	323	325	326	329	330	332	333	335	336	337	339	341	343	345	348	350	352	354	355	356	357	358	359
Cnidaria	○		○	○		○	○	○		○	○	○	○				○					○	○			
Ctenophore						○		○		○			○		○								○			
Mollusca																										
Heteropoda																										
Atlantidae																						○				
Carinariidae				○		○	○	○															○			
Pterotracheidae			○			○	○	○		○	○	○	○					○				○	○			
Thecosomata	○			○											○		○	○				○	○			
Gymnosomata						○																				
Cephalopoda						○							○				○					○	○			
Annelida	○																						○			
Arthropoda																										
Amphipoda																						○				
Decapoda																										
phyllosoma		1			1	2	1	1	1				2	2	3	1			2	2	2	1		1	2	1
Chaetognatha	○		○	○		○	○	○		○	○	○	○				○	○				○	○			
Chordata																										
Urochordata																										
Thaliacea						○	○	○									○	○				○	○			
Salpida	○			○		○	○				○	○	○					○				○	○			
Vertebrata																										
Carangidae																										
Myctophidae																										
Molidae																										
Psenes sp																										
Fish larva																										

	St.360	361	362	364	365	366	369	370	371
Cnidaria	○		○	○					
Ctenophore									
Mollusca									
Heteropoda									
Atlantidae	○		○	○	○				○
Carinariidae	○								
Pterotracheidae	○								
Thecosomata	○		○	○	○				○
Gymnosomata			○						
Cephalopoda									
Annelida			○						
Arthropoda									
Amphipoda			○	○	○			○	
Decapoda									
phyllosoma	1	1	1	2		1	1	1	
Chaetognatha	○							○	○
Chordata									
Urochordata									
Thaliacea			○	○					
Salpida	○		○	○					○
Vertebrata									
Carangidae									
Myctophidae			○						
Molidae									
Psenes sp									
Fish larva									

Identification of Hox gene complement of a Salpa species

Hiroaki Okazawa[1] and Hidetoshi Saiga[2]

[1]*Ocean Research Institute, The University of Tokyo.* [2]*Tokyo Metropolitan University*

Salpae are pelagic marine invertebrates, belonging to the class Thaliacea, one of the three classes of the subphylum Urochordata, phylum Chordata. Previously, we have identified nine Hox genes, which constitute the Hox gene complement of the ascidian *Ciona intestinalis*, are localized on two chromosomes (1) unlike most of animal species. In Oikopleura dioica, belonging to Larvacea, the third class of Urochordata, it is known that Hox gene cluster is likely present under degraded conditions, missing some members from its genome. Considering these observations, we have hypothesized that in urochordates, some of the Hox gene members might have lost upon their divergence from the lineage to vertebrates during evolution (2). To test this hypothesis, we are planning to examine Hox gene complement in Salpa species, and have to collect Sapla preferably of single species. In this cruise, we caught many Salpae in 27 stations.

Table 1 List of Salpae collected stations during the KH-08-1 Cruise

St.	Location	
	Net in	Net out
18	N14-03.4	N14-04.1
	E142-42.3	E142-43.7
19	N14-13.9	N14-14.8
	E142-42.6	E142-41.9
25	N14-03.7	N14-03.8
	E142-52.6	E142-50.7
53	N14-23.7	N14-23.2
	E142-52.9	E142-54.3
67	N14-13.6	N14-12.4
	E142-42.7	E142-43.3
80	N14-03.7	N14-03.6
	E143-02.9	E143-00.7
107	N14-13.6	N14-12.7
	E142-42.8	E142-44.3
154	N12-52.5	N12-52.5
	E141-45.0	E141-45.8
193	N20-00.1	N20-00.7
	E133-59.6	E134-01.1
195	N25-00.4	N25-01.6
	E128-59.9	E129-00.3
201	N26-11.5	N26-10.5

	E129-00.6	E129-00.4
206	N25-29.4	N25-28.2
	E128-00.0	E128-00.2
207	N25-22.1	N25-20.6
	E127-45.3	E127-45.5
265	N14-29.1	N14-28.9
	E142-37.7	E142-38.8
294	N14-15.1	N14-15.7
	E142-45.0	E142-46.1
307	N12-45.4	N12-46.5
	E142-29.8	E142-28.8
308	N13-00.5	N13-01.6
	E142-30.0	E142-30.0
320	N13-30.0	N13-29.4
	E141-44.8	E141-43.8
321	N13-44.9	N13-44.3
	E141-44.8	E141-43.6
327	N15-15.3	N15-16.5
	E141-45.0	E141-45.2
328	N15-30.1	N15-30.8
	E141-44.8	E141-43.4
340	N13-45.0	N13-44.8
	E142-00.0	E141-58.8
341	N13-29.9	N13-29.3
	E141-59.9	E141-59.0
363	N12-44.7	N12-43.8
	E140-59.5	E140-58.2

References

(1) Ikuta, T., Yoshida, N., Satoh, N. and Saiga, H. (2004)
Ciona intestinalis Hox gene cluster: its dispersed structure and residual colinear expression in development. Proc. Natl. Acad. Sci. U.S.A. 101, 15118-15123.

(2) Ikuta T. and Saiga, H. (2005)
Organization of Hox genes in ascidians: present, past and future. Dev. Dyn. 233, 382-389

Genetic species identification of *Anguilla japonica* eggs and larvae: Leg 1

Tatsuki Yoshinaga
School of Marine Biosciences, Kitasato University

Background

The Japanese eel *Anguilla japonica* at early developmental stages such as the egg and early larva (pre-leptochephalus) lack key morphological features to enable identification. Indeed, some species of *Serrivomer* possess morphological characteristics that resemble those of *A. japonica*, making it difficult to precisely identify the target species. Accordingly, a polymerase chain reaction (PCR) based genetic identification method (*A. japonica* sequence detection system; *Aj*-SDS) has been employed onboard Hakuho Maru since 2004 (Sudo et al, 2007), and contributed to the discovery of a precise spawning location of *A. japonica* in 2005 (Tsukamoto 2006). Therefore, combined use of PCR with morphological examination is currently the most reliable strategy to identify *A. japonica* specimens onboard (Sudo et al, 2007).

Materials and methods

Selected specimens, sorted out as to possibly be *A. japonica* from plankton samples collected by the IKMT and ORI Big Fish nets, were examined (Table 1). Eggs were incubated for a certain period to obtain enough number of cells for analysis (Minegishi et al., in prep). For larvae, a small portion of the posterior body was dissected, and homogenized in 50 !L of 5% (w/v) Chelex-100 resin solution (Bio-Rad). The homogenate was boiled at 98°C for 15 minutes, followed by centrifugation at 8,000*g* at room temperature for 10 minutes. Twenty !L of PCR mixture consisted of 10 !L of 2x TaqMan universal PCR master mix (Applied Biosystems), 900 nM each of Aja16S-L3 (F3) and Aja16S-H3 (R3) PCR primers (5'-AAT CAG TAA TAA GAG GGC CCA AGC-3' and 5'-TGT TGG GTT AAC GGT TTG TGG TA-3', respectively), 200 nM *A. japonica*-specific TaqMan probe (Eel-2; 5'-CAC ATG TGT AAG TCA GAA CGG ACC GAC C-3'), and 3 !L of crude DNA solution (Watanabe et al, 2004). The reactions were carried out with an ABI PRISM 7300 Sequence Detection System (Applied Biosystems). The template DNA solutions were not diluted except in some initial tests (tests 1-3; Table 1). Positive and negative controls were also examined in each test, and any results with possible experimental errors were discarded. Results were obtained as a Ct value that is a number of PCR cycles at the threshold, and the threshold was determined with a positive control sample (*A. japonica*) at the Ct of 30.

Results and discussion

A total of 13 tests have been performed in Leg 1, and 3 eggs and 97 larvae specimens were tested (Table 1). No contamination, shown as an absence of negative control amplification, has occurred, and thus all the tests were confirmed to be successful. No egg examined showed a positive signal, suggesting that they were not of *A. japonica* (tests 4-6). On the other hand, 82 larvae specimens showed a positive signal with the Ct values from 20-30 (Table 1). Thus these specimens were strongly

suggested to be *A. japonica*. Specimens #383 and #384 showed ambiguous results, miss matching with morphological data and inconsistent results from multiple *Aj*-SDS analyses (tests 8-10), suggesting that mishandling of the samples might have occurred. The results will be further confirmed by determining the DNA nucleotide sequence of each specimen immediately after the cruise.

The *Aj*-SDS also revealed that steamed and baked eel (kabayaki), served for dinner on 3 June, was made of *A. japonica* (test 13).

Acknowledgements

I thank Dr MJ Miller for a grammatical correction of the manuscript, and all the scientists for their enthusiastic effort to sort out tiny eggs and larvae.

References

Sudo R, Sawada E, Kuroki M, Miller MJ, Yoshinaga T. Genetic species identification of *Anguilla japonica* preleptocephali using realtime PCR sequence detection system: matching with morphological characteristics, prevention of contamination, and a trial for identification of other anguillid species. *KH-07-2 Leg 1 & 3 Cruise Report* Pp 57-62.

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

Minegishi Y, Yoshinaga T, Aoyama J, Tsukamoto K. Species identification of *Anguilla japonica* eggs by real-time PCR based on a sequence detection system. *In preparation.*

Watanabe S, Minegishi Y, Yoshinaga T, Aoyama J, Tsukamoto K. 2004. A quick method for species identification of the Japanese eel *Anguilla japonica* using real-time PCR: an onboard application for use during sampling surveys. *Marine Biotechnology* **6**, 566-574.

Table 1. Results of *Aj*-SDS in Leg 1 of KH-08-1

Test ID	Test date	Leg	Station	Specimen	Ct	Note
01	26-May	Leg 1	7	17	28	x50 dil.
01	26-May	Leg 1	7	17	29	x100 dil.
01	26-May	Leg 1	7	17	30	x200 dil.
01	26-May	Leg 1	7	24	29	x50 dil.
01	26-May	Leg 1	7	24	30	x100 dil.
01	26-May	Leg 1	7	24	31	x200 dil.
01	26-May	Leg 1		jap CNTL	30	
01	26-May	Leg 1		NTC	undet.	
02	26-May	Leg 1	7	17	32	x50 dil.
02	26-May	Leg 1	7	24	30	x50 dil.
02	26-May	Leg 1	10	32	32	x50 dil.
02	26-May	Leg 1	10	34	33	x50 dil.
02	26-May	Leg 1	10	35	33	x50 dil.
02	26-May	Leg 1	10	36	32	x50 dil.
02	26-May	Leg 1	10	37	36	x50 dil.
02	26-May	Leg 1	11	43	30	x50 dil.
02	26-May	Leg 1	11	44	29	x50 dil.
02	26-May	Leg 1	11	45	31	x50 dil.
02	26-May	Leg 1	11	46	30	x50 dil.
02	26-May	Leg 1	11	47	32	x50 dil.
02	26-May	Leg 1		jap CNTL	30	
02	26-May	Leg 1		mar CNTL	undet.	
02	26-May	Leg 1		NTC	undet.	
03	28-May	Leg 1	9	29	undet.	x10 dil.
03	28-May	Leg 1	9	29	undet.	x50 dil.
03	28-May	Leg 1	13	49	undet.	x10 dil.
03	28-May	Leg 1	13	49	undet.	x50 dil.
03	28-May	Leg 1	13	50	undet.	x10 dil.
03	28-May	Leg 1	13	50	undet.	x50 dil.
03	28-May	Leg 1		jap CNTL	30	
03	28-May	Leg 1		NTC	undet.	
04	30-May	Leg 1	46	egg 05	undet.	16 hrs incubation
04	30-May	Leg 1		jap CNTL	30	
04	30-May	Leg 1		mar CNTL	undet.	
04	30-May	Leg 1		ser CNTL	undet.	
04	30-May	Leg 1		NTC	undet.	
05	31-May	Leg 1	75	egg 13	undet.	1 pc
05	31-May	Leg 1	75	egg 13	undet.	2 pcs
05	31-May	Leg 1		jap CNTL	30	
05	31-May	Leg 1		NTC	undet.	
06	3-Jun	Leg 1	108	egg 19	undet.	
06	3-Jun	Leg 1		jap CNTL	30	
06	3-Jun	Leg 1		NTC	undet.	

CNTL, control; NTC, no template control, undet., undetectable; dil., dilution

Red and blue characters indicate positive and negative, respectively, inferred from Ct values.

Test ID	Test date	Leg	Station	Specimen	Ct	Note
07	6-Jun	Leg 1	154	351	20	
07	6-Jun	Leg 1	154	352	20	
07	6-Jun	Leg 1	154	353	21	
07	6-Jun	Leg 1	154	354	20	
07	6-Jun	Leg 1	154	355	21	
07	6-Jun	Leg 1	154	356	22	
07	6-Jun	Leg 1	154	357	21	
07	6-Jun	Leg 1	154	358	21	
07	6-Jun	Leg 1	154	359	21	
07	6-Jun	Leg 1	154	360	21	
07	6-Jun	Leg 1	154	361	22	
07	6-Jun	Leg 1	154	362	21	
07	6-Jun	Leg 1	154	363	21	
07	6-Jun	Leg 1	154	364	21	
07	6-Jun	Leg 1		jap CNTL	30	
07	6-Jun	Leg 1		NTC	undet.	
08	6-Jun	Leg 1	155	367	23	
08	6-Jun	Leg 1	156	376	24	
08	6-Jun	Leg 1	156	377	24	
08	6-Jun	Leg 1	156	378	30	
08	6-Jun	Leg 1	156	379	25	
08	6-Jun	Leg 1	156	380	24	
08	6-Jun	Leg 1	156	381	23	
08	6-Jun	Leg 1	156	382	32	
08	6-Jun	Leg 1	156	384	24	sample mishandling?
08	6-Jun	Leg 1		jap CNTL	30	
08	6-Jun	Leg 1		NTC	undet.	
09	6-Jun	Leg 1	155	373	undet.	
09	6-Jun	Leg 1	155	374	undet.	
09	6-Jun	Leg 1	156	383	undet.	sample mishandling?
09	6-Jun	Leg 1	157	386	25	
09	6-Jun	Leg 1	157	387	22	
09	6-Jun	Leg 1	157	388	24	
09	6-Jun	Leg 1	158	390	22	
09	6-Jun	Leg 1	158	391	23	
09	6-Jun	Leg 1	158	392	23	
09	6-Jun	Leg 1	158	393	22	
09	6-Jun	Leg 1	158	394	22	
09	6-Jun	Leg 1	158	395	21	
09	6-Jun	Leg 1	158	396	23	
09	6-Jun	Leg 1	158	397	23	
09	6-Jun	Leg 1	158	398	24	
09	6-Jun	Leg 1	158	399	22	
09	6-Jun	Leg 1	158	400	25	
09	6-Jun	Leg 1	158	401	23	
09	6-Jun	Leg 1	158	402	23	
09	6-Jun	Leg 1	158	403	24	
09	6-Jun	Leg 1	158	404	23	
09	6-Jun	Leg 1	158	405	23	

Test ID	Test date	Leg	Station	Specimen	Ct	Note
09	6-Jun	Leg 1	158	406	23	
09	6-Jun	Leg 1	158	407	23	
09	6-Jun	Leg 1	158	408	22	
09	6-Jun	Leg 1		jap CNTL	30	
09	6-Jun	Leg 1		NTC	undet.	
10	7-Jun	Leg 1	156	384	undet.	sample mishandling?
10	7-Jun	Leg 1	159	441	22	
10	7-Jun	Leg 1	159	445	undet.	
10	7-Jun	Leg 1	159	446	undet.	
10	7-Jun	Leg 1	160	447	22	
10	7-Jun	Leg 1	160	448	23	
10	7-Jun	Leg 1	161	473	22	
10	7-Jun	Leg 1	161	474	37	
10	7-Jun	Leg 1	161	475	22	
10	7-Jun	Leg 1	161	476	22	
10	7-Jun	Leg 1	161	477	23	
10	7-Jun	Leg 1		jap CNTL	30	
10	7-Jun	Leg 1		NTC	undet.	
11	7-Jun	Leg 1	162	597	24	
11	7-Jun	Leg 1	162	598	undet.	
11	7-Jun	Leg 1	162	599	23	
11	7-Jun	Leg 1	162	600	31	
11	7-Jun	Leg 1	162	601	undet.	
11	7-Jun	Leg 1	162	641	undet.	
11	7-Jun	Leg 1		jap CNTL	30	
11	7-Jun	Leg 1		NTC	undet.	
12	8-Jun	Leg 1	163	623	25	
12	8-Jun	Leg 1	163	624	24	
12	8-Jun	Leg 1	163	625	30	
12	8-Jun	Leg 1	163	626	25	
12	8-Jun	Leg 1	163	627	23	
12	8-Jun	Leg 1	169	644	23	
12	8-Jun	Leg 1	170	652	24	
12	8-Jun	Leg 1	170	653	26	
12	8-Jun	Leg 1	170	654	26	
12	8-Jun	Leg 1	170	655	25	
12	8-Jun	Leg 1	170	656	undet.	
12	8-Jun	Leg 1		jap CNTL	30	
12	8-Jun	Leg 1		NTC	undet.	
13	8-Jun	Leg 1	171	661	23	
13	8-Jun	Leg 1	171	662	22	
13	8-Jun	Leg 1	171	663	22	
13	8-Jun	Leg 1	171	664	25	
13	8-Jun	Leg 1	171	665	undet.	
13	8-Jun	Leg 1	181	691	23	
13	8-Jun	Leg 1	181	695	undet.	
13	8-Jun	Leg 1		kabayaki	30	dinner on 3 June
13	8-Jun	Leg 1		jap CNTL	30	
13	8-Jun	Leg 1		NTC	undet.	

Genetic species identification of *Anguilla japonica* eggs and larvae: Leg2

Ryusuke Sudo[1], Jun Aoyama[1], and Tatsuki Yoshinaga[2]
[1]*Ocean Research Institute, The University of Tokyo.*
[2]*School of Marine Bioscience Kitasato University*

Background

Large well developed leptocephali of the Japanese eel (*A. japonica*) can be identified by only morphological characteristics such as their body shape and total number of myomeres. However, *A. japonica* at early developmental stages such as the egg and preleptocephalus lack key morphological characteristics to enable identification. For example, some species of species of *Serrivomer* have morphological features that is closely resembled those of *A. japonica*. Accordingly, onboard genetic species identification of eggs and larvae (leptocephali) using a realtime PCR *A. japonica* sequence detection system (Aj-SDS) has been employed since 2004 (Sudo et al. 2007), and contributed to a discovery of pre-leptocephali of the species in 2005 (Tsukamoto, 2006). Therefore, combined use of PCR with morphological examination is currently the most reliable strategy to identify *A. japonica* specimens onboard.

Materials and methods

Larvae and eggs were collected by either the Isaacs Kidd Midwater Trawl (IKMT) with 5 mm mesh or the ORI BigFish Trawl with 0.5 mm 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 have been analyzed by the Aj-SDS onboard, if they were thought to be potentially *A. japonica* inferred from their morphological characters. Posterior body (tail) tissue was excised, and homogenized in 50 μL of 5% (w/v) Chelex-resin solution (Bio-Rad) with a disposable plastic pestle. The remaining anterior part was preserved in 99.5% (v/v) ethanol for later otolith aging analysis. The homogenized tissue was boiled at 95°C for 15 minutes to heat-denature proteins, and thus inactivate nucleases. Crude DNA fraction was separated by centrifugation at 2000*g* for 1 minute with a small table-top centrifuge, and supernatant was used for the subsequent PCR. The Aj-SDS was carried out according to Yoshinaga (2008). Result were obtained as a Ct value that is a number of PCR cycle at the threshold, and the threshold was determined with a positive control sample (*A. japonica*) at the Ct of 28.

Results and discussion

During the cruise, total 7 assays have been carried out on board. All tests were comfirmed to be successful. No eggs examined showed a positive signal, suggesting that they were not of *A.*

japonica (test17, 18). 5 larvae specimens showed a positive signal with the Ct value around 21 (Table 1). Thus these specimens were strongly suggested to be *A. japonica*. Specimen No.1105, No.1448 and No.1463 were not showed a positive signal, so these were suggested not to be *A. japonica*. The result will be further confirmed by determining the DNA nucleotide sequence of each specimen after the cruise.

References

Sudo R, Sawada E, Kuroki M, Miller MJ, Yoshinaga T. Genetic species identification of *Anguilla japonica* preleptociphali using realtime PCR sequence detection system: matching with morphological characteristics, prevention of contamination, and a total trial for identification of other anguillid species. KH-07-2 Leg 1 and Leg 3 Cruise Report

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

Yoshinaga T, Genetic species identification of *Anguilla japonica* eggs and larvae: Leg1 KH-08-1 Cruise report.

Table 1. Results of Aj-sequence detection system in Leg2 of the KH-08-1 cruise
CNTL, control; NTC, no template control; undet., undetectable;
Red and blue characters indicate positive and negative, respetctively, inferred

Test ID	Test date	Leg	Station	Specimen	Ct	Note
14	23-Jun	Leg 2	221	992	20	
14	23-Jun	Leg 2		jap CNTL	28	
14	23-Jun	Leg 2		NTC	undet.	
15	25-Jun	Leg 2	228	1009	21	
15	25-Jun	Leg 2	228	1010	21	
15	25-Jun	Leg 2	228	1011	21	
15	25-Jun	Leg 2		jap CNTL	28	
15	25-Jun	Leg 2		NTC	undet.	
16	26-Jun	Leg 2	228	1105	34	A. mar ?
16	26-Jun	Leg 2		jap CNTL	28	
16	26-Jun	Leg 2		NTC	undet.	
17	2-Jul	Leg 2	275	egg61-1	undet.	
17	2-Jul	Leg 2	275	egg61-2	undet.	
17	2-Jul	Leg 2	275	egg61-3	undet.	
17	2-Jul	Leg 2	275	egg61-4	undet.	
17	2-Jul	Leg 2	275	egg61-5	undet.	
17	2-Jul	Leg 2		jap CNTL	28	
17	2-Jul	Leg 2		NTC	undet.	
18	5-Jul	Leg 2	307	egg80	undet.	
18	5-Jul	Leg 2	307	egg81	undet.	
18	5-Jul	Leg 2		jap CNTL	28	
18	5-Jul	Leg 2		NTC	undet.	
19	8-Jul	Leg 2	343	1424	21	
19	8-Jul	Leg 2		jap CNTL	28	
19	8-Jul	Leg 2		NTC	undet.	
20	9-Jul	Leg 2	349	1448	undet.	
20	9-Jul	Leg 2	354	1463	undet.	
20	9-Jul	Leg 2		jap CNTL	28	
20	9-Jul	Leg 2		NTC	undet.	

Studies on diet environment for the Japanese eel larvae using stable isotope analyses

Sachie Miyazaki, Hiroshi Morioka, Takashi Kitagawa, and Shingo Kimura
Ocean Research Institute, The University of Tokyo

The spawning area of Japanese eel (*Anguilla japonica*) was located in waters of the North Equatorial Current (NEC) (Tsukamoto, 2006). Larvae hatched there are transported to coastal estuarine waters of the East Asia in winter by the Kuroshio following the NEC. During the transport in the NEC, the larvae (referred as leptocephali) grow to more than 40mm in total length. However, the diet of the eel larvae is not clarified yet during the transport.

During the KH-08-1, Leg 1 and 2, a total of 140 Japanese eel larvae, together with 6 Anguilliformes larvae (*Ariosoma* sp) and a number of zooplankton (including copepoda or gelatinous) were collected by oblique tows of an ORI ring net with 3 m in diameter (7.1m^2 mouth open and 0.5 mm mesh) and IKMT (Isaacs Kidd Midwater Trawl, 8.7m^2 mouth opening and 0.5mm mesh). Particulate Organic Matter (POM) were filtered onto precombusted glass-fiber filters from sea water sampled at depths of 5m, 50m, 100m, 150m and 200m at seven stations of 140°E, and at 12 stations of 134°E and 130°E, and at 13 stations of 137°E. These samples will be used for stable isotope analyses to clarify the diet of the Japanese eel larvae.

For clarifying the species composition of zooplankton in the NEC, zooplankton were also collected by vertical tows of a NORPAC twin net (North Pacific Standard Net, mesh sizes of 0.10 and 0.33 mm) at the seven stations of 140°E, the 12 stations of 134°E and 130°E, and at the 13 stations of 137°E. These samples will be used for identification.

Reference

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

Natural iron stable isotope variation in fishes

Takafumi Hirata[1] and Tsuguo Otake [2]
[1]*Department of Earth and Planetary Science, Tokyo Institute of Technology.*
[2]*Ocean Research Institute, The University of Tokyo*

Recent progress in analytical technique has enabled to analyze iron stable isotope ($^{56}Fe/^{54}Fe$) in animal tissues and blood. Iron stable isotope ratio in human tissue and blood was found to be 2.6 ‰ lower than that of food sources (Walczyk & Blanckenburg 2002), suggesting the possibility that the ratio changes with increase in trophic level in a food web.

The objective of this study is to make clear the variation of natural iron stable isotope in fishes of various taxa and trophic levels. We sampled fishes and fish larvae including leptocephali (Table 1) collected from eel spawning area in southern part of West Mariana Ridge of western North Pacific. Those samples will be analyzed iron stable isotope ratios in the tissues by MC-ICP-MS (Multi Collector Induced Coupled Plasma-Mass Spectrometry). We will discuss about relationship between the variation of iron stable isotope ratio and trophic level in fishes.

Table 1. Samples collected during KH-08-1 Hakuho Maru cruise (Leg.2)

Species	Number of fish	Range of total length (mm)
Leptocephalus:		
Ariosoma sp.7	13	126.8 ～ 236.0
Other fish:		
Myctophidae sp.	10	—
Psenes sp.	1	—
Brama sp.	3	—
Caranx sp.	2	—
Scombridae sp.(larvae)	8	—

Endocrinological analysis of growth retardation and malformation in captive-bred eel larvae

Ryota Tosaka and Shinji Adachi
Graduate School of Fisheries Sciences, Hokkaido University

In Japanese eel, *Anguilla japonica*, establishment of the optimal protocol for consistent mass production of glass eel would be required. Recently, Tanaka et al. (2003) succeeded in cultivating captive-bred eel larvae into glass eel by using modified larvae feed and rearing system. However, the techniques for producing glass eels are not yet firmly established. The survival rate is very low and there are many malformations in captive-bred eel larvae. Moreover, the growth rate is nearly half that of wild Japanese eel larvae for the same number of days after hatching. These problems may be partly due to an incomplete diet and environmental factors that differed from those in natural habitat.

It has been accepted that growth retardation (caused in part by incomplete diet) and incomplete environment factor in the early developmental age are related to malformation. Growth in vertebrate animals is regulated integrally by growth hormone (GH)-insulin-like growth factor-I (IGF-I) axis. In general, GH is secreted by the pituitary gland and stimulates hepatic production of IGF-I. Together, GH and IGF-I regulate cell growth and differentiation. GH and IGF-I transcription and release are known to be affected by nutritional status. Recently, we suggested that malformation in captive-bred eel preleptocephalus larvae may be also due to the lack of thyroid hormone, known to play a key-role in metamorphotic event in some vertebrate.

The aim of this investigation is to evaluate normality of GH-IGF-I and thyroid-stimulating hormone-thyroid axis endocrinologically in captive-bred eel larvae by comparing with wild Japanese eel larvae. Additionally, we also aim to identify physiological marker relating with fast-growing trait to evaluate modified larvae feed and rearing system.