NATSUSHIMA Cruise Report

NT13-11

The Spawning Ecology of Eels
Along the West Mariana Ridge

26 MAY – 11 June 2013

Japan Agency for Marine-Earth Science and Technology
(JAMSTEC)
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   ● Cruise ID
     NT13-11
   ● Name of vessel
     R/V NATSUSHIMA (JAMSTEC)
   ● Title of the cruise
     Spawning Ecology of Eels Along the West Mariana Ridge
   ● Title of proposal
     Spawning Ecology of Eels
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   ● Ports of call
     Yokohama and Saipan
   ● Research area
     Western North Pacific along the West Mariana Ridge
   ● Research Maps: Figure 1 (hydrographic stations) and Figure 2 (operation area)
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Oiler: Keiya Taniguchi
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Steward: Tatsunari Onoue
Steward: Toru Wada
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Figure 1. Map of the study area of the NT13-11 cruise showing the locations of X-CTD stations (X15–X24) made from the R/V NATSUSHIMA (black circles) along the east side of the West Mariana Ridge (numbered according to proposed stations, with western transect not being conducted). Hydrographic stations on the west side of the ridge made by Kaiyo Maru are shown with blue circles. The three shallow seamounts within the West Mariana Ridge are shown with red triangles (Pathfinder, Arakane, Suruga).
Figure 2. Map of the region that was surveyed during NT13-11 cruise of the R/V NATSUSHIMA (JAMSTEC) in May and June 2013 using the ROV Hyper-Dolphin and Una-Cam systems. Red circles show the deployment locations of the Una-Cam drifting camera systems (N = 3 systems) and the green circles show the starting points of the Hyper-Dolphin deployments. Dates of the deployment lines stations are shown and the station numbers are inside the circles. Dotted blue lines show the angles of possible frontal features indicated from the three hydrographic sections (two are shown in Figure 5). The front was distinct in the westernmost transect, but was more variable and spread out near the surface in the eastern transects. The Una-Cam system was tested on 1 June (second red circle in the second line of stations). The 4th scheduled deployment of the Hyper-Dolphin was canceled due to wave height on 5 June.

3. Observations
3.1 Purpose, Objectives, Background
The primary objective of the NT13-11 cruise was to help understand what factors are
used by the Japanese eel, *Anguilla japonica*, to determine where it will form spawning aggregations and to film the spawning behavior of adults in the midwater of the ocean for the first time using the high-definition (HD) cameras of the Hyper-Dolphin and to try to photograph spawning-condition eels that were attracted to artificially matured Japanese eels used as an odor source in the drifting camera systems referred to as Una-Cams. This was attempted by first determining the likely location of spawning by finding the position of the salinity front as it crosses the seamount chain of the West Mariana Ridge in conjunction with hydrographic data also collected by the Kaiyo Maru of the Fisheries Research Agency Japan (Figure 1).

Research conducted previously to collect recently hatched larvae, called preleptocephali, had shown that Japanese eels were spawning along the west side of the seamount ridge, and more recent collections of newly spawned eggs and spawning condition adult Japanese eels as well as giant mottled eels, *Anguilla marmorata*, had provided further clues about where spawning may have been occurring. A salinity front that crosses the ridge had appeared to influence the latitude of spawning (Kimura and Tsukamoto, 2006). At the southern end of the ridge, the range of longitudes over which eggs (2009, 2011, 2012) (Tsukamoto et al., 2011; Aoyama et al., unpublished manuscript), preleptocephali (2007-2012) (Tsukamoto, 2006; Tsukamoto et al., 2011) and adults of both anguillid species (2008, 2009) (Chow et al., 2009; Tsukamoto et al. 2011; Kurogi et al., 2011) had been collected by large midwater trawls along the ridge, varied greatly. The salinity front was often variable in structure, intensity, and location (Aoyama et al., Unpubl. manuscr.), so what determines spawning location has remained uncertain. It had been discovered that the Japanese eel only spawns during new moon periods during its spawning season (Ishikawa et al., 2001; Tsukamoto et al., 2003, 2011), so the timing of collection efforts for all life history stages had been determined by the lunar cycle. These various factors influencing the possible location and timing of spawning provided the basis for new efforts to search for spawning eels using camera systems in their spawning area.

After eggs had been collected during four new moon periods in three different years, a cruise was conducted to use the Shinkai 6500 and Yokosuka Deep-Tow to attempt to observe anguillid eels in their ocean spawning area for the first time in July 2012 (Tsukamoto et al., 2013). That cruise also included a deep dive to the bottom to attempt to detect recent carcasses of spawned-out eels and their remains that could have fallen to the bottom after spawning. The Shinkai 6500 surveyed in the deeper layers during the daytime (200–800 m) and the Yokosuka Deep-Tow was deployed in the more shallower layers at night (130–250 m) (Tsukamoto et al., 2013).
The present cruise was designed to use the Hyper-Dolphin to survey for eels at night and to use the Una-Cam systems to survey at various times of day automatically while drifting. Both were deployed in the depth layers where eels would be expected to be, based on information obtained from satellite data transfer pop-up tags that have been attached on migrating Japanese eels and other anguillid species (Manabe et al., 2011). Each Una-Cam had a chamber containing artificially matured female Japanese eels to emit reproductive pheromones to attract other eels to the drifting camera systems so they could be photographed.

Figure 3. Images of the deployment of the Hyper-Dolphin (A,C), the Hyper-Dolphin HD camera (E) and the observation areas in the control room while observing a long siphonophore (B) and the laboratory below deck (F), and the retrieval at night (D,G).
Figure 4. Images of the Una-Cam system being deployed (A,C) with live artificially matured Japanese eels from the IRAGO Institute (F), one of the HD cameras, HID light, and sphere camera and light systems (E), and an anesthetized eel being prepared for injection with hormones after being removed from the holding tank onboard (D).
3.2 Observations, Activities
The NT13-11 cruise included several types of observation activities that were conducted in a sequential order: (1) Examination of the salinity structure of along the West Mariana Ridge using X-CTD probes deployed from NATSUSHIMA to plot salinity sections (Figure 5), (2) Conduct a test deployment of one of the Una-Cam systems (Figure 2, 5), 3) Deployment of the Una-Cam system with eels in 7 lines of stations in 7 days (Figure 2), (4) a single deployment of the Hyper-Dolphin in each line of stations for 7 consecutive days (the 5 June station was cancelled due to wave height) (Figure 2).

![Figure 5. Salinity section across the North Equatorial Current in the western North Pacific on the west side of the West Mariana Ridge (top panel; from Kaiyo Maru) and on the east side of the ridge during the NT13-11 cruise (bottom panel; data used from Kaiyo Maru for the southernmost stations) at the stations shown in Figure 1.](image-url)
3.3 Methods, Instruments

The salinity structure of the study area along the West Mariana Ridge was examined by making a transect of expendable conductivity, temperature and depth profiler (X-CTD) probes (Tsurumi-Seiki Co., LTD) on the east side of the West Mariana Ridge during the NT13-11 cruise (Figure 1). These data were combined with hydrographic data obtained by Kaiyo Maru in two transects to the west of the ridge. In the NT13-11 transect a single X-CTD probe was deployed at each station and data was recorded to a depth of about 1000 m. The 2 southernmost stations in the transect were not occupied, because the Kaiyo Maru measured the hydrography at those stations (Figure 1).

At each observation line, single Una-Cam systems were deployed at 3 different stations in the morning before noon, which would be recovered the next day starting at 13:00 (only two systems in the first line). The Una-Cam systems were made of a rectangular metal frame with the cameras and lights on the top of the frame (Figure 4A). Each system had a transparent tubular chamber to hold artificially matured eels (2–7 eels; maximum 3 females, 4 males) in the middle of the bottom of the frame (Figure 4A,F). Just before deployment of each Una-Cam, the eels were given a final injection of human chorionic hormone and 17 alpha hydroxy progesterone to stimulate reproductive maturation (Figure 4D); after having had 2 months of injections including before the cruise at the IRAGO Institute in Japan.

The Una-Cams were suspended by rope from a set of buoys and floats at the surface. Each Una-Cam system had a depth recording system, to see how close to the target depth of 200 m they were drifting. Attached to the buoy set (Figure 4C), was a ARGOS satellite transmitter system (transmitting every 30 seconds) that relayed the position of the buoy about every one hour depending on the location of the satellites, with the data then being transmitted back to the ship. The ship was also equipped with direction finder system that could detect the every 30 sec ARGOS signal emitted from the transmitter attached to the buoy. At the final stage, the ship’s radars could be used to detect the radar reflector on the buoy. Also attached to the radar reflector buoy was a GPS position recorder that recorded the buoy position every 10 sec, which could be downloaded later.

Once the ship had detected the buoy and moved close by, the zodiac boat was deployed to take a rope from the NATSUSHIMA to the buoy system to enable the Una-Cam system to be retrieved (Figure 4B). After each system was onboard, the eel tube was removed and the eels returned to their holding tank. Then the camera systems were removed and the video files downloaded to a computer systems.
The camera systems of each Una-Cam system consisted of 2 HD video cameras in their own underwater housings, each pointing downward at an oblique angle towards one end of the eel tube (Figure 4A,E). An HID light source (Goto-Aquatics Inc., Japan) was attached to the top of the frame at an angle facing downwards within the frame. Each Una-Cam system also had a camera sphere in the center of the top of the frame containing cameras and lights (Figure 4E). The spheres were made of a prototype underwater seismometer glass sphere that were equipped with two GoPro cameras, each angled to view to the sides of the eel tubes. The spheres also contained two white LED light sources. The LED lights and the HID light were powered by rechargeable batteries.

The Una-Cam systems were programmed to have a specific pattern of lights on and off and cameras on and off during their approximately 20 hour deployments. Six min HD video camera movies were recorded every 30 min with the HID lights turning on 2 min after the cameras turned on and then the light stayed on for 3 m. Then every hour the HD system was also on, plus the GoPro cameras recorded for 6 min with the LED coming on after 2 min staying on for 2 min, but the HID light was still on for 1 min longer. Then all lights were off for the remaining 1 min of video recording during both the 30 min and 1 hour scheduled periods. The MP4 (GoPro) and AVCHD (HD) video files were then downloaded after each deployment. Frame capture images were made from the video files.

After the Una-Cam systems were re-deployed in the afternoon each day, the Hyper-Dolphin ROV was deployed each evening at about 18:00. At the beginning of each dive, the ROV went to deeper layers (~500–800 m). As the submersible was coming back up to shallower depths, it usually stopped every 100 m and turned off the lights after agitating a mesh bag containing Japanese eel ovaries from the IRAGO Institute. This was intended to release a possible pheromone odor to attract eels. The lights were then turned back on to see if any eels had been attracted. After the 100 m depth intervals were surveyed in that way, the ROV was towed by the ship at speeds of usually < 1 knt in an oblique pattern of moving up and down from a maximum depth of 300 m to a minimum depth of about 180 m.

The forward facing HD video camera on the front of the Hyper-Dolphin (Figure 3E) was recording during each deployment and a lower-resolution CC camera recorded video at an angle from the side looking across the area in front of the ROV. During the deployment of the Hyper-Dolphin, frame captures were made using each camera, but primarily from the HD camera. Only the images from the HD camera of the ROV are included in this report.
4) Research results

4.1 Survey to find the salinity front
The salinity structure along the ridge was similar to its usual pattern with lower surface salinity in the southern region and a subsurface tongue of higher salinity water above the deeper lower salinity water (Figure 5). The sections to the west of the ridge showed a distinct salinity front just south of 13.5°N. The section along the east side of the ridge did not show a very distinct salinity front because a shallow layer of low salinity water extended to the north up to about 14–15°N.

4.2 Hyper-Dolphin surveys
There were 6 deployments of the Hyper-Dolphin ROV along the eastern or central part of the southern part of the West Mariana Ridge (Figure 2). The ROV then surveyed to the east of the starting points. No possible eels were seen in the video imagery from the two cameras, but a wide range of plankton and nekton could be seen moving past the ROV as it moved through the water, as well as marine snow. Only a few types of
fishes were seen during the deployments (Figure 6). However, these included a likely yellowfin tuna (Figure 6C) and small scombrids were present in the shallow layers. A few mesopelagic fishes were also seen, mostly in deeper layers (Figure 6A,B,D), which included an apparent *Idiacanthus*, with a chin barble (bioluminescent organ). The *Idiacanthus* held a very ridged and straight body form (Figure 6A), with only the tip of the tail moving to hold the body position. This is similar to the body form used by an *Avocettina* eel seen during the cruise in the previous year from the Shinkai 6500 (Miller et al., In press), which also only used the tip of its tail to maintain its position. The ROV was able to see various types of gelatinous zooplankton and other types of organisms (Figure 7,8). Some could be seen clearly even if the ROV did not stop moving, but others were photographed more clearly by stopping the ROV. The types of invertebrates seen included siphonophores, cnidarians (a few jellyfish or hydroids), ctenophores, polychaetes, crustaceans, including a type of shrimp with long antennae, chaetognaths, and pyrosomes. Pteropods with their webs extended out were seen, as well as a few heteropods. A chaetognath was observed attempting to curl its body into a half-circle shape (Figure 7A), which is a shape-change behavior that has been reported to be used by leptocephali (Miller et al., 2013) as well as other marine organisms (Robison, 1999), apparently to reduce predation. No leptocephali were detected in the ROV video, although they may have been hard to distinguish if moving fast.

Small squid were abundant and would enter the lighted area in front of the ROV. They would often discharge their ink when startled by some aspect of the ROV. An excellent video recording and frame captures were made of one squid discharging its ink and then remaining inside the ink, in an apparent attempt to escape from view (Figure 9). Another different squid was also recorded to do this, with one frame capture being made (not shown). At times diffuse groups of up to about 10 squid would move into view at various distances from the ROV. At least 2 species of squid were present based on basic morphological features, but only one type was common.

The various observations of fishes and invertebrates by the HD camera of the Hyper-Dolphin suggested that if spawning aggregations of eels were approached and the eels did not move away immediately, the eels would have been seen by the scientists and Hyper-Dolphin pilots that were watching the real-time video feeds. Although eels were not seen, the observations that were made will be used in a study to evaluate the various methodologies for camera system deployment in the pelagic layers for observing fishes and other nekton and plankton that will include the present cruise (Hyper-Dolphin and Una-Cam system) and the previous cruise last year (Shinkai 6500 and Yokosuka Deep-Tow).
Figure 7. Frame-capture images of gelatinous-type zooplankton that appear to be siphonophores (B,C), polychaetes (A,D), a pyrosome (E), and a chaegognath showing a shape-change curling behavior, likely designed to mimic round gelatinous zooplankton like jellyfish or ctenophores to avoid predation (Robison, 1999; Miller et al., 2013).

4.3 Una-Cam deployments
There were 3 Una-Cam systems deployed each day (except for the first day with 2 systems deployed) for 7 days resulting in 20 deployments that included live eels. The 20 deployments were at different stations located at intervals to the north of the ROV deployment points (Figure 2). The video files obtained from the 4 cameras of each UC system were then viewed onboard during the cruise. About 60% of the video files have been viewed at the time of this report being written at the end of the cruise, but the proximity of the last station to the ending port in Saipan did not provide enough time to view all files during the cruise.
Figure 8. Frame-capture images of gelatinous zooplankton and a pelagic shrimp with long antennae (F), showing examples of a siphonophore with its stinging cells mostly retrieved (A) or partially being released (B), and another siphonophore with retracted stinging cells (E).

Observations of the video files from the Una-Cam systems showed that the camera systems were effective to see the areas around the eel tubes. The eels in the tubes could be seen clearly, and when fishes or invertebrates came into view they could be clearly seen in the video files. One interesting observation was that a set of eels was actually observed to release eggs and sperm while inside the tubes. They were prepared to reach the final stage of maturation according to the IRAGO Institute protocols, so this is not unexpected. Various small fishes and invertebrates could also been seen by the Una-Cam cameras as they swam around nearby or with the frame of the Una-Cam (Figure 10). Gelatinous zooplankton, such as siphonophores were also frequently seen as they drifted past the field of view.
Figure 9. Frame-capture images of a squid that was hiding within its ink trail. The squid first ejected an ink trail and then it maintained its position within the ink in each photo. Another squid was also observed moving through its ink trail (not shown). If this type of behavior has not been well-documented with images and video footage, this will be an interesting observation to report.

Figure 10. Examples frame-capture images of fishes seen in the HD cameras of the Una-Cam drifting camera systems deployed during the NT13-11 cruise. Many invertebrates such as siphonophores could be clearly seen in the Una-Cam cameras that included two types of cameras for a total of 4 cameras per deployed system. A deep-bodied type of fish was frequently seen (A), which is likely a common species that has a tendency to hide under floating structure in the ocean. A more shallow bodied fish was also seen in at least one Una-Cam deployment (B).
Figure 11. Frame captures of two eels that were apparently attracted to one of the Una-Cam systems. A larger eel entered the field of view first (eel 1, panel A,B), and then moved directly to the opening of the eel tube (C), before moving out of view to the bottom-left. Then a second eel (eel 2, panel D) suddenly appeared and then moved back out of view. This is hypothesized to be a female *Derichthys serpentinus* that was displaying a form or mate attraction or reproductive related behavior, possibly to attract a male *Derichthys* eel. The extremely high frequency undulations could emit a mechanical signal detected by the male eel. The pheromone stimulus from the mature anguillid eels may have attracted these eels and triggered their behaviors.

The ability to observe eels that would be attracted to the pheromone odor from the maturing eels was verified by an observation of two small eels that approached one of the Una-Cam systems. A larger eel (eel 1 in Figure 11) entered the view (appearing at a large relative size) and approached directly to the front opening of the eel tube (appearing at a small relative size). It was undulating its body extremely rapidly in a way apparently not related to locomotion, but may have been a mechanism to produce a vibration signal. The undulations were anguilliform in structure, but with very high frequency and low amplitude. The eel then moved over the top and down the side of the front of the tube before moving out of the field of view. Immediately after the first eel disappeared, a second smaller eel possibly a male (eel 2 in Figure 10), appeared to the upper right, outside of the UC frame. This eel moved around nearby and then disappeared from view. Another possible small eel was seen drifting by the UC and
turning back towards the frame, but there were no distinguishing features to determine if it was an eel or something else capable of locomotion and turning behavior.

4.4 Future plans
The deployments of the Una-Cam systems showed good results and performed well, so these systems appear to be one of the best future methods for observing spawning eels. These systems drift free of the research vessel and do not produce sound like ROV or submersible systems. Conducting a future cruise using 5 Una-Cam systems instead of 3, was found to be possible based on the present cruise, if time was not used for an ROV or other major camera deployment systems. However, more detailed hydrographic measurements using both X-CTD and CTD observations in higher resolution grids are also needed to better position the observation area in relation to salinity structure. Another possibility is the development of automated underwater vehicle (AUV) systems to detect and observe eels in their spawning area. If an AUV could be equipped with an acoustic detection system, then once eels were detected, the AUV could stop and turn on its lights and film the eels or move around to detect them more clearly. The use of low-noise or low-light impact camera systems seem to be the best way to advance the methodology for observing spawning eels for the first time and for learning about how they determine where they will form spawning aggregations.

5. Acknowledgements
We sincerely thank Captain Takafumi Aoki and his crew of the R/V NATSUSHIMA, and the Hyper-Dolphin Team for their excellent support and assistance during the cruise. It was a challenging task to find and retrieve the 3 UC systems in the morning and then redeploy them at the next 3 stations in the afternoon. This was done with excellent efficiency and skill, for which we are very grateful. The Hyper-Dolphin was also deployed and retrieved in an impressive way and it performed very well. We also thank the JAMSTEC Cruise Management Division for assistance with this cruise.

6. References
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