SUPPLEMENTARY INFORMATION

Oceanic spawning ecology of freshwater eels in the western North Pacific

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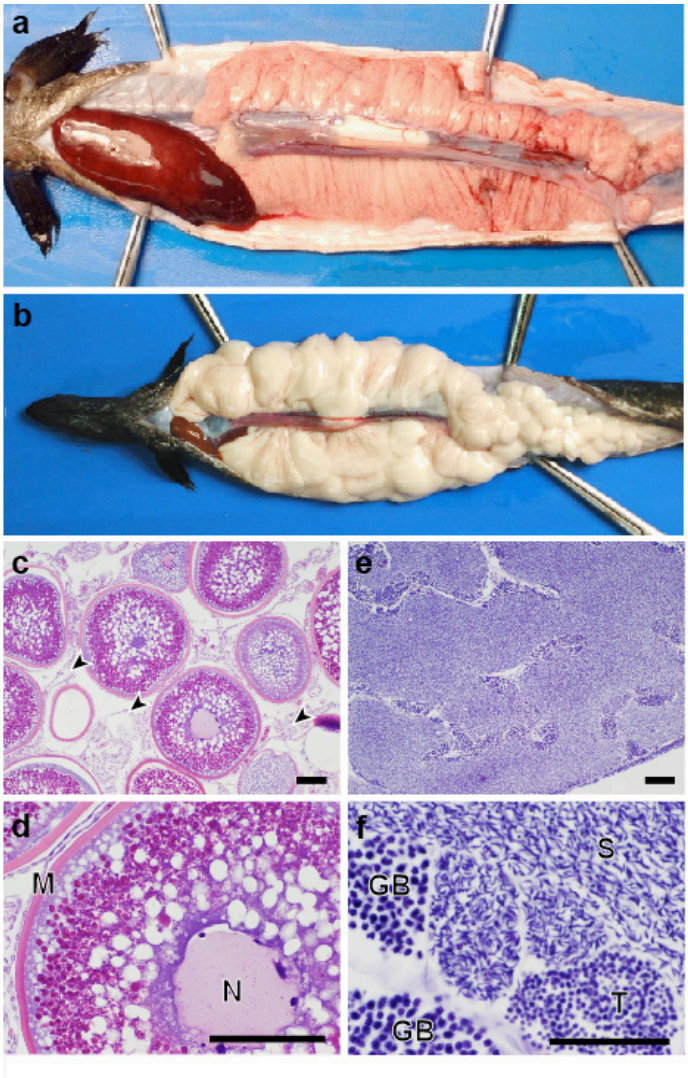
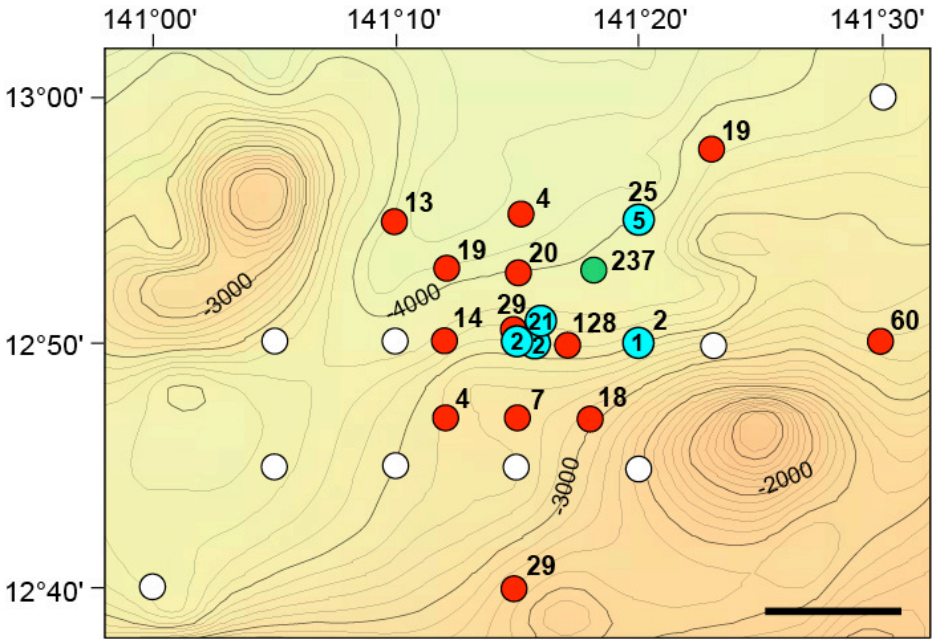
Supplementary Figures



Supplementary Figure S1. Eel gonadal morphology and histology. (**a**) Female *Anguilla japonica* (739 mm, 330 g) collected in June 2009 with gonads in a post-spawning state. (**b**) Male *A. japonica* (447 mm, 129 g) collected in June 2009 (shown in Fig. 1b). (**c**) Histological section of the ovary of the female *Anguilla japonica* in (**a**). Many post-ovulated follicles (arrowheads) were observed in the ovary, suggesting that this eel had already spawned, but secondary batches of oocytes were developing. Oocyte diameter ranged from 300–600 µm with a mode of 500 µm. (**d**) Mid-vitellogenic stage oocyte with a large nucleus (N) and distinct egg membrane (M), within the same ovary in (**a**) and (**c**). (**e**) Testis of the male *A. japonica* in (**b**) and Fig. 1b. (**f**) Sperm (S), spermatatids (T) and late type B spermatogonia (GB) in an enlarged photograph of (**e**). The spermatic duct was filled with sperm in the testis. Scale bars are 100 µm for (**c**), (**d**)**,** (**e**), and 40 µm for (**f**).



Supplementary Figure S2. Egg and preleptocephalus catches in the egg grid. The collection locations of Japanese eel, *Anguilla japonica*, eggs (light blue circles) and preleptocephali (red and green circles) in the grid of 25 stations at the southern part of the West Mariana Ridge in the western North Pacific where the intensive survey for eggs and preleptocephali was made during 22–25 May 2009. The numbers of eggs collected in each tow at 3 stations (3 tows were made at the first station) are shown in circles, and the numbers of preleptocephali at each station to the upper-right of each symbol. White circles show stations with no eggs or larvae. The green circle shows the location where the depth distribution study of newly hatched preleptocephali and CTD observation were conducted (see Fig. 6). Scale bar is 10 km.

Supplementary Table S1. Fishing effort and catches of eel stages in relation to new moon.

Ship/Sampling survey	Stage	Year	Sampling dates	Date of new moon	Number of tows	No. of Positive tows	Number collected	Species/sex	Dates of collection
R/V *Kaiyo Maru*									
KY-08-2	Adult	2008	25 May–9 Jun	4 Jun	21	2	3	2AjM*, 1AmM*	3, 4 Jun
KY-08-4	Adult	2008	25–31 Aug	31 Aug	7	1	2	2AjF**	31 Aug
KY-09-2, Leg 1	-	2009	22–30 May	24 May	10	0	0	-	-
KY-09-2, Leg 2	Adult	2009	17–26 Jun	23 Jun	10	5	8	3AjM, 4AjF, 1AmF	19–25 Jun
R/V *Hokko Maru*									
HK-09-04	Adult	2009	15–22 Jun	23 Jun	14	1	2	1AjM, 1AmM	21 Jun
R/V Hakuho Maru									
KH-05-1, Leg 2	Prelepto	2005	31 May–12 Jun	7 Jun	64	6	110	*A. japonica*	7–10 Jun
KH-05-1, Leg 4	-	2005	30 Jun–14 Jul	6 Jul	114	0	0	-	-
KH-06-2, Leg 3	Prelepto	2006	18-27 Jul	25 Jul	78	3	4	*A. marmorata*	22–23 Jul
KH-06-2, Leg 5	Prelepto	2006	18-31 Aug	24 Aug	100	1	2	*A. marmorata*	29 Aug
KH-07-2, Leg 1	Prelepto	2007	9-18 Aug	13 Aug	63	10	38	*A. japonica*	14–18 Aug
KH-07-2, Leg 1	Prelepto	2007	9-18 Aug	13 Aug	63	7	25	*A. marmorata*	16–18 Aug
KH-07-2, Leg 3	-	2007	3-15 Sep	12 Sep	104	0	0	-	-
KH-08-1, Leg 1	Prelepto	2008	23 May–12 Jun	4 Jun	185	14	249	*A. japonica*	7–9 Jun
KH-08-1, Leg 2	Prelepto	2008	25 Jun–10 Jul	3 Jul	147	2	2	*A. japonica*	8, 10 Jul
KH-09-1	-	2009	18–30 Apr	25 Apr	90	0	0	-	-
KH-09-2	Prelepto	2009	17–31 May	24 May	110	31	753	*A. japonica*	23–26 May
KH-09-2	Egg	2009	17–31 May	24 May	110	5	31	*A. japonica*	22–23 May

*Reported by Chow *et al.*[2], **Kurogi *et al.*[18]. Catches are shown for the number of specimens of the Japanese eel, *Anguilla japonica* (Aj) and giant mottled eel, *Anguilla marmorata* (Am), and male (M) and female (F) eels.

Supplementary Table S2. PCR primers and probes for species identification and parentage analysis using mitochondrial genes.

Primer	Sequence (5'-3')	Target	Purpose	References
Primer A (L2612)	GGTCC(A/T)(A/G)CCTGCCCAGTGA	Part of 16s rRNA gene (forward)	Genetic identification of adult eels	55
H3059 (mod)	CCGGTCTG(A/G)AC(C/T)AGATCACGT	Part of 16s rRNA gene (reverse)	Genetic identification of adult eels	55 (modified H3059)
L15774	ACATGAATTGGAGGAATACCAGT	tRNAThr and tRNAPro genes and part of control region (forward)	Parentage history of preleptocephali	52
H16498	CCTGAAGTAGGAACCAGATG	tRNAThr and tRNAPro genes and part of control region (reverse)	Parentage history of preleptocephali	53
Aja16S-L3	AATCAGTAATAAGAGGGCCCAAGC	Part of 16s rRNA gene (forward)	Onboard genetic identification of eggs and preleptocephali by PCR	34, 56
Aja16S-H3	TGTTGGGTTAACGGTTTGTGGTA	Part of 16s rRNA gene (reverse)	Onboard genetic identification of eggs and preleptocephali by PCR	34, 56
Aja16S-probe	CACATGTGTAAGTCAGAACGGACCGACC	Part of 16s rRNA gene (fluorescent-labeled oligo probe)	Onboard genetic identification of eggs and preleptocephali by PCR	34, 56
L1854	AAACCTCGTACCTTTTGCAT	Part of 16s rRNA gene (forward)	Genetic identification of eggs and preleptocephali	57
H3059	CCGGTCTGAACTCAGATCACGT	Part of 16s rRNA gene (reverse)	Genetic identification of eggs and preleptocephali	57

Supplementary References

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