

## Differences in the maturation level among life histories of the Japanese eel *Anguilla japonica* in the Nagata River, Japan

by

Naoko Chino, Chifumi Imai, Harumi Sakai, Takaomi Arai\*

DOI: [10.1515/ohs-2017-0046](https://doi.org/10.1515/ohs-2017-0046)

Category: **Short communication**

Received: **February 19, 2017**

Accepted: **May 15, 2017**

Environmental and Life Sciences Programme,  
Faculty of Science, Universiti Brunei  
Darussalam, Jalan Tungku Link, Gadong,  
BE 1410, Brunei Darussalam

### Abstract

In order to understand the silver eel migration behavior of the Japanese eel *Anguilla japonica*, the life history of both the yellow stage (immature stage) and the silver stage (early maturing stage) was examined during the silver eel migration period, using otolith microchemical analysis of strontium (Sr) and calcium (Ca) concentrations. The mean Sr:Ca ratios in *A. japonica* from the elver mark to the otolith edge indicate eels of several general categories of migratory histories, including sea eels (average Sr:Ca ratios,  $\geq 6.0 \times 10^{-3}$ ), estuarine eels (average Sr:Ca ratios,  $2.0 \times 10^{-3} \leq \text{Sr:Ca} < 6.0 \times 10^{-3}$ ) and river eels (average Sr:Ca ratios,  $< 2.0 \times 10^{-3}$ ), based on the criteria reported previously in *A. japonica*. A significant negative correlation was observed between the gonadosomatic index (GSI) and the mean Sr:Ca ratios during the growth phase after recruitment. Gonads of river eels were more developed compared to eels with other migratory histories. It thus appears that the maturation level for the silver eel migration to the open ocean varies depending on the life history.

**Key words:** Japanese eel, *Anguilla japonica*, silver eel, gonadosomatic index, otolith, Sr:Ca ratios

\* Corresponding author: [takaomi.arai@ubd.edu.bn](mailto:takaomi.arai@ubd.edu.bn)

## Introduction

The Japanese eel, *Anguilla japonica* Temminck & Schlegel is a catadromous fish (McDowall 1988). The eel is widely distributed in East Asia, from Taiwan in the south, through eastern China, Korea, and up to the Sanriku Coast of northern Honshu Island, Japan (Tesch 1977). The Japanese eel spawns in waters west of the Mariana Islands and their leptocephali drift within the North Equatorial and Kuroshio currents (Tsukamoto 1992). As they approach the continental shelf, the leptocephali metamorphose into glass eels and leave the Kuroshio for coastal waters. Glass eels become elvers in the estuaries (Bertin 1956). After upstream migration, the elvers become yellow eels and live for 5-20 years in freshwater habitats such as rivers and lakes. Then, during the silver eel stage in autumn and winter, their gonads begin to mature and they start the silver eel migration into the ocean and retreat to the spawning area where they spawn and die.

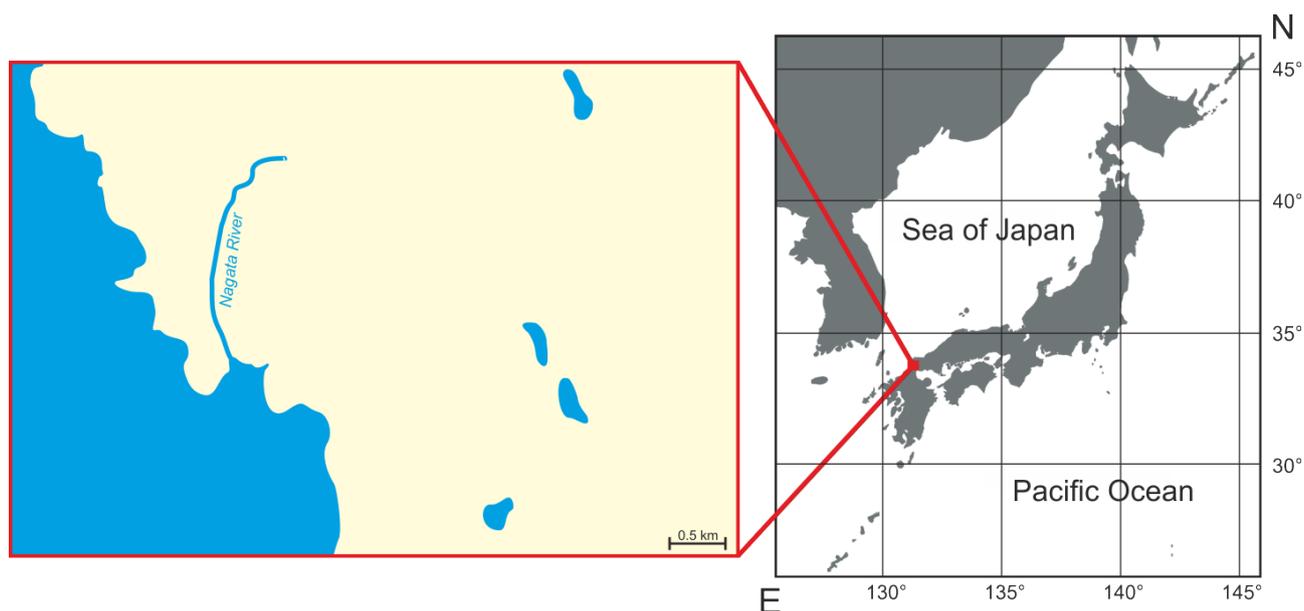
Patterns in the ratio of strontium (Sr) to calcium (Ca) in otoliths have been used to explain the migratory history of anguillid eels such as the habitat use and seasonal migration. The Sr:Ca ratios in otoliths of *A. japonica* are positively correlated with ambient salinity (Tzeng 1996) as they occur in a number of teleost fishes. Accordingly, the Sr:Ca ratios in otoliths of eels are useful to reconstruct their migratory environmental history (Arai & Chino 2012). Recently a number of studies have revealed the diverse migratory behaviors in anguillid eels such as Atlantic (e.g. Arai et al. 2006; Lamson et al. 2006), Japanese (e.g. Tsukamoto

& Arai 2001; Arai et al. 2003a,b; Chino & Arai 2009) and New Zealand (Arai et al. 2004) eels. However, there is little information available on the details of migratory behavior during the silver eel migration period except for the beginning of the oceanic migration (Kotake et al. 2005; 2007). The information regarding the migration of silver eels will contribute to the understanding of their whole life history as well as silver eel migration behavior of anguillid eels.

In this study, we have examined the migratory histories of the Japanese eel *Anguilla japonica* using Sr:Ca ratios in otoliths of both yellow and silver eels collected during their spawning migration season in the Nagata River and the Oji tidal flat, Shimonoseki, Yamaguchi Prefecture. We discuss the implications of these findings in relation to the nature of the silver eel migratory behavior among their migratory types, i.e. sea eels, estuarine eels and river eels of *A. japonica*.

## Materials and methods

Specimens of *Anguilla japonica* were collected by eel pots and fishing in the Nagata River (Fig. 1; 2.2 km long) and the Oji tidal flat, Shimonoseki, Yamaguchi Prefecture in November 2004. These sampling sites were affected by the tidal effect. A total of 50 specimens were collected, and their total length (TL), body weight (BW), sex and the gonadosomatic index (GSI) were measured. The body color was observed to distinguish the developmental stage of male eels, and each individual was classified as a yellow eel after



**Figure 1**

Map showing the location of the Nagata River, Shimonoseki, Yamaguchi Prefecture

Matsui (1972). To distinguish the developmental stage of female eels, each individual was classified as either a yellow (< 1.0) or silver eel (> 1.0) using GSI, following Utoh et al. (2004). Six specimens were identified as female silver eels by GSI of each eel and were further used in otolith analyses. For other forty-four specimens, we randomly selected thirty specimens for further otolith analyses. All eels were identified: 20 females, 14 males and 2 unknown eels.

Sagittal otoliths were extracted from all specimens and the otoliths were embedded in epoxy resin (Struers, Epofix). These otoliths were then ground to expose the core along the anterior-posterior direction in the frontal plane, using a grinding machine equipped with a diamond cup-wheel (Struers, Discoplan-TS), and polished further with oxide polishing suspension on an automated polishing wheel (Struers, PdM-Force-20). Finally, they were cleaned using distilled water and ethanol, and dried at 50°C in an oven prior to examination.

For electron microprobe analyses, all otoliths were Pt-Pd coated by a high vacuum evaporator. Otoliths from all specimens were used for life-history transect analyses of Sr and Ca concentrations, which were measured along a line down the longest axis of each otolith, from the core to the edge, using a wavelength dispersive X-ray electron microprobe (JEOL JXA-8900R), as described in Arai et al. (2003a,b) and Chino and Arai (2009). Wollastonite (CaSiO<sub>3</sub>) and Tausonite (SrTiO<sub>3</sub>) were used as standards. The accelerating voltage and beam current were 15 kV and 1.2 × 10<sup>-8</sup> A, respectively. The electron beam was focused on a point having a diameter of 10 μm, with measurements spaced at 10 μm intervals.

Following the electron microprobe analysis, the otoliths were repolished to remove the coating, etched with 1% HCl and thereafter stained with 1% toluidine blue (Arai et al. 2004). The age of specimens was determined by counting the number of blue-stained transparent zones, following Arai et al. (2004). The positions of transparent zones were then correlated with elemental analysis points. The relative ages at particular elemental analysis points could then be assigned.

The average Sr:Ca ratios were calculated for the values outside the elver mark and specimens were classified as "sea eels" (Sr:Ca ratios, ≥ 6.0 × 10<sup>-3</sup>), "estuarine eels" (Sr:Ca ratios, 2.0-6.0 × 10<sup>-3</sup>) or "river eels" (Sr:Ca ratios, < 2.0 × 10<sup>-3</sup>), according to the criteria defined by Tsukamoto and Arai (2001), Chino and Arai (2010) and Arai and Chino (2017).

Differences in the data were tested using the Mann Whitney *U*-test and the Kruskal-Wallis test. The relationships between GSI and Sr:Ca ratios and

between GSI and age were assessed with Pearson's correlation coefficient (Sokal & Rohlf 1995).

## Results

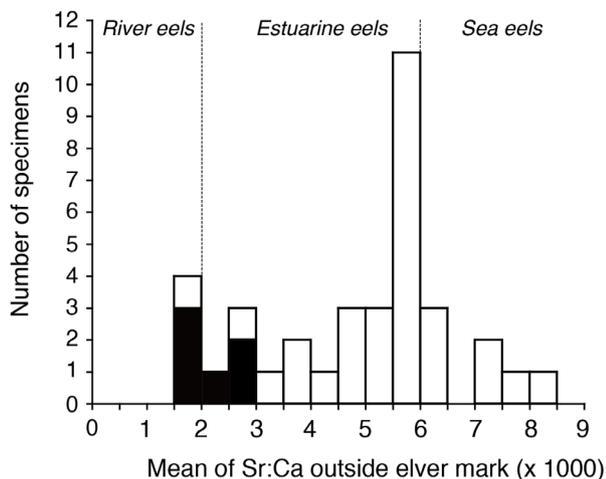
The total length (TL) of females ranged from 41.7 to 68.2 cm, with a mean ± SD of 53.1 ± 6.7 cm (n = 20), while TL of males ranged from 38.6 to 49.9 cm, with a mean of 44.0 ± 3.7 cm (n = 14). The body weight (BW) of females ranged from 94.2 to 407.2 g, with a mean of 227.8 ± 94.5 g (n = 20), and BW of males ranged from 71.4 to 173.7 g, with a mean of 121.0 ± 33.8 g (n = 14). The body size of most females was significantly larger in terms of both TL and BW compared to males (Mann Whitney *U*-test, *p* < 0.005). The age of females ranged from 5 to 13 yr, with a mean ± SD of 8.6 ± 2.2 yr, while the age of males ranged from 4 to 8 yr, with a mean of 6.3 ± 1.2 yr.

The GSI of all females (n = 20) ranged from 0.1 to 2.6 (mean: 0.8 ± 0.77). The GSI of males (n = 14) ranged from 0.0 to 0.4 with a mean of 0.13 ± 0.15. The GSI was larger in females than in males (Mann-Whitney *U*-test, *p* < 0.005).

The Sr:Ca ratios in the transects along the radius of each otolith showed the same common feature in all specimens, but there were generally three different patterns outside the otolith core. All otoliths had a common peak of high values of Sr:Ca ratios at the center of the otolith inside the elver mark (ca 150 μm), which roughly corresponded to the leptocephalus and early glass eel stages during their oceanic life (Arai et al. 1997).

The mean Sr:Ca ratio value outside of 150 μm from the core of all otoliths ranged from 1.81 to 8.03 × 10<sup>-3</sup>, with a mean of 4.84 ± 1.78 × 10<sup>-3</sup> (Fig. 2), and there was no significant difference in the values between males and females (Mann-Whitney *U*-test, *p* > 0.05). Based on those mean values, each specimen was classified as either sea, estuarine, or river eels. Of the specimens examined, sea eels (Sr:Ca ≥ 6.0 × 10<sup>-3</sup>) accounted for 19% (n = 7) of all specimens, river eels (Sr:Ca < 2.0 × 10<sup>-3</sup>) – for 11% (n = 4) and estuarine eels (2.0 × 10<sup>-3</sup> ≤ Sr:Ca < 6.0 × 10<sup>-3</sup>) were the most abundant (69%, n = 25) of the three types (Fig. 2). In silver females, the number of river, estuarine and sea eels was 4, 2 and 0 specimens, respectively (Fig. 2).

A significant negative linear relationship was found between GSI and Sr:Ca ratios during the growth phase after recruitment to the coast as glass eel (Fig. 3) (Pearson correlation, *r* = -0.619, *p* < 0.0001), and there was a significant positive correlation between GSI and age (Fig. 4) (Pearson correlation, *r* = 0.565, *p* < 0.0001).



**Figure 2**  
Frequency distribution of the mean values of the Sr:Ca ratio outside the elver mark (150 μm in radius) in each otolith of specimens of the Japanese eel. Black and white columns suggest silver eels and yellow eels, respectively

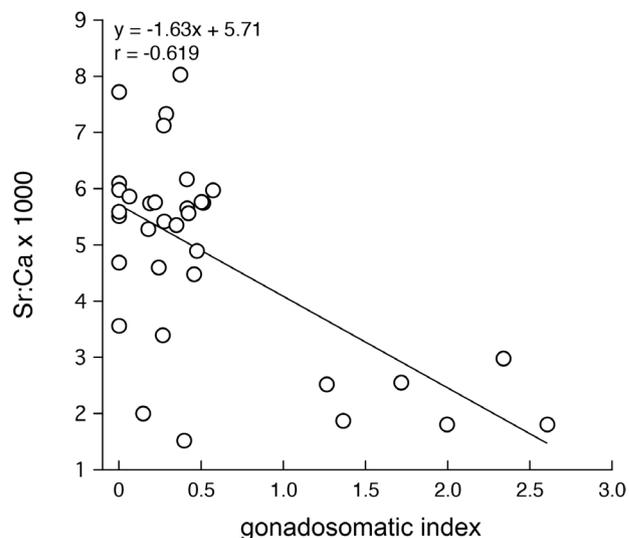
### Discussion

In the present study, all eels were collected in the same period during the spawning migration season. Three migratory types – river, estuarine and sea eels occurred in the study areas (Fig. 2). The GSI values of female river eels were the highest among the three migratory patterns. It is possible that most eels begin their spawning migration in the ocean at about the same maturity level. This suggests that river eels may begin their spawning migration earlier than individuals of the two other migratory types. Morphological changes may occur along with physiological adaptations, which are of critical importance to the survival in the new environment, from fresh and brackish water to seawater. Chino and Arai (2009) also found that female silver eels of river eels collected in the Kii Channel off Shikoku Islands had the highest GSI values compared to other migratory types. These results suggest that river eels may need more time when all eels together start their spawning migration to the spawning ground. For river eels are believed to migrate a longer distance upriver and thus they are adapted to saline and fresh water. The early start of silver eel migration in the case of river eels is probably due to the fact that some of them had higher GSI values compared to the other types. It may also be that estuarine and sea eels start their silver eel migration earlier than the river eel. Furthermore, the river eels may need to acclimatize to higher salinity

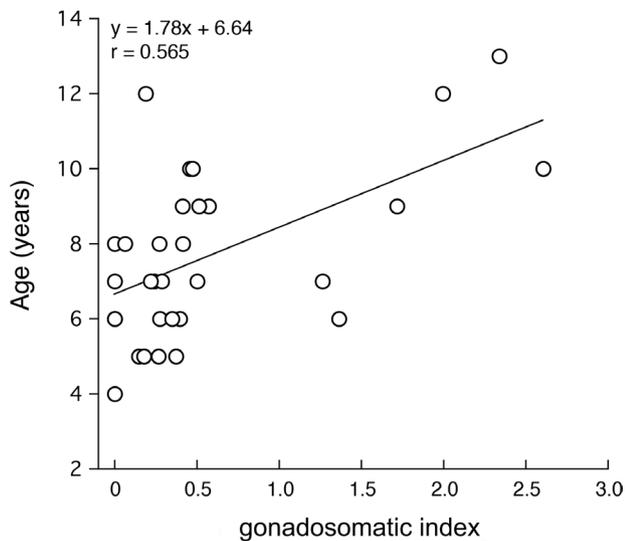
of water before their oceanic migration to the open ocean begins, although the timing of the commencement of the silver eel migration is the same as for estuarine and sea eels. Sudo et al. (2017) suggest that eels collected by set nets in Mikawa Bay are individuals that could have started their migration out of the bay from either freshwater habitats, brackish water areas in estuaries or from the bay. Therefore, further intensive studies during the spawning season would be needed to clarify the important mechanisms of the silver eel migration of the Japanese eel.

The beginning of spawning migration of eels is affected by various intrinsic and extrinsic factors. Intrinsic factors such as sufficient body size, age and lipid content are believed to be prerequisites for starting the migration (Sudo et al. 2017). A positive correlation between the age and the maturation level was found in the present study (Fig. 4). Therefore, age may also be one of the factors determining the silver eel migration. However, the age at the beginning of the spawning migration appear to vary considerably (Svendäng et al. 1996; Poole & Reynolds 1996; Sudo et al. 2017). The age variability of silver eels is probably a reflection of the variability in habitats and growth conditions of individuals.

Due to the lack of research on silver eel migration in Japan and the difficulty in collecting migrating eels in coastal areas, the information on *A. japonica* maturity levels at the beginning of spawning migration is scarce. In the present study, the average maturity



**Figure 3**  
The relationship between the gonadosomatic index (GSI) and the mean values of the Sr:Ca ratio outside the elver mark (150 μm in radius) in each otolith of specimens of the Japanese eel



**Figure 4**

The relationship between the gonadosomatic index (GSI) and the age of the Japanese eel

(GSI) of female silver eels ranged from 1.3 to 2.6. The GSI values of female *A. japonica* silver eels collected in the East China Sea ranged from 1.3 to 3.5 (Sasai et al. 2001), in the Amakusa Islands, Mikawa Bay and Sanriku Coast – from 1.0 to 4.3 (Kotake et al. 2007) and in the Kii Channel (Chino & Arai 2009) – from 1.2 to 4.0. Therefore, the GSI values of female silver eels in this study were comparable to those collected in offshore and coastal areas, and the same values were recorded during silver eel migration in freshwater in Japan (1.1-2.5, Sasai et al. 2001).

In *A. japonica*, the GSI level reaches 40-70 at the final stage of maturation in eels that are artificially matured with hormone treatments (Tsukamoto 2009). Recently, the fully matured eels were collected in the spawning ground and the GSI value of an eel was 18.8% (Chow et al. 2009). These GSI values observed in fully matured eels were much higher than the values in wild eels collected from the offshore and coastal areas of Japan. There have been numerous reports on the maturity of other temperate eels and various GSI values have been found (Tesch 1977; Wenner & Musick 1974; Todd 1981; Jellyman 1987; Krueger & Oliveira 1997), however, all of them were significantly below 9. The low values observed in *A. japonica* silver eels during this study are probably caused by the fact that these eels have to migrate a long distance from the coastal waters of East Asia to their spawning area in the North Equatorial Current, west of the Mariana Islands of the western North Pacific. They start the spawning migration at the beginning of their maturation and continue to mature during the long migration to the

spawning area. Thus, the GSI values of the silver eels collected in coastal areas are much lower than those of the eels that are ready to spawn.

## Acknowledgements

This work was partly supported by Universiti Brunei Darussalam under the Competitive Research Grant Scheme (Vot No. UBD/OVACRI/CRGWG(003)).

## References

- Arai, T. & Chino, N. (2017). Influence of water salinity on the strontium:calcium ratios in otoliths of the giant mottled eel, *Anguilla marmorata*. *Environmental Biology of Fishes* 100(3): 281-286. DOI: 10.1007/s10641-016-0569-7.
- Arai, T., Kotake, A., Lokman, P.M., Miller, M.J. & Tsukamoto, K. (2004). Evidence of different habitat use by New Zealand freshwater eels, *Anguilla australis* and *A. dieffenbachii*, as revealed by otolith microchemistry. *Marine Ecology Progress Series* 266: 213-225. DOI: 10.3354/meps266213.
- Arai, T., Kotake, A. & McCarthy, T.K. (2006). Habitat use by the European eel *Anguilla anguilla* in Irish waters. *Estuarine, Coastal and Shelf Science* 67(4): 569-578. DOI: 10.1016/j.ecss.2006.01.001.
- Arai, T., Kotake, A., Ohji, M., Miyazaki, N. & Tsukamoto, K. (2003a). Migratory history and habitat use of Japanese eel *Anguilla japonica* in the Sanriku Coast of Japan. *Fisheries Science* 69(4): 813-818. DOI: 10.1046/j.1444-2906.2003.00691.x.
- Arai, T., Kotake, A., Ohji, M., Miller, M.J., Tsukamoto, K. et al. (2003b). Occurrence of sea eels of *Anguilla japonica* along the Sanriku Coast of Japan. *Ichthyological Research* 50(1):78-81. DOI: 10.1007/s102280300011.
- Arai, T., Otake, T. & Tsukamoto, K. (1997). Drastic changes in otolith microstructure and microchemistry accompanying the onset of metamorphosis in the Japanese eel, *Anguilla japonica*. *Marine Ecology Progress Series* 161:17-22. DOI: 10.3354/meps161017.
- Bertin, L. (1956). *Eels – a biological study*. London: Cleaver-Hume Press Ltd.
- Chino, N. & Arai, T. (2009). Relative contribution of migratory type on the reproduction of migrating silver eels, *Anguilla japonica*, collected off Shikoku Island, Japan. *Marine Biology* 156(4): 661-668. DOI: 10.1007/s00227-008-1116-7.
- Chino, N. & Arai, T. (2010). Habitat use and habitat transitions in the tropical eel, *Anguilla bicolor bicolor*. *Environmental Biology of Fishes* 89(3): 571-578. DOI: 10.1007/s10641-010-9677-y.
- Chow, S., Kurogi, H., Mochioka, N., Kaji, S., Okazaki, M. et al. (2009). Discovery of mature freshwater eels in the open ocean. *Fisheries Science* 75(1): 257-259. DOI: 10.1007/s12562-008-0017-5.

- Jellyman, D.J. (1987). Review of the marine life history of Australian temperate species of *Anguilla*. *American Fisheries Society Symposium* 1: 276-285.
- Kotake, A., Okamura, A., Yamada, Y., Utoh, T., Arai, T. et al. (2005). Seasonal variation in migratory history of the Japanese eel, *Anguilla japonica*, in Mikawa Bay, Japan. *Marine Ecology Progress Series* 293: 213-221. DOI: 10.3354/meps293213.
- Kotake, A., Arai, T., Okamura, A., Yamada, Y., Utoh, T. et al. (2007). Ecological aspects of Japanese eels, *Anguilla japonica*, collected from coastal areas of Japan. *Zoological Science* 24(12): 1213-1221. DOI: 10.2108/zsj.24.1213.
- Krueger, W.H. & Oliveira, K. (1997). Sex, size and gonad morphology of silver American eel *Anguilla rostrata*. *Copeia* 1997(2): 415-420.
- Lamson, H.M., Shiao, J.C., Iizuka, Y., Tzeng, W.N. & Cairns, D.K. (2006). Movement patterns of American eels (*Anguilla rostrata*) between salt- and freshwater in a coastal watershed, based on otolith microchemistry. *Marine Biology* 149(6): 1567-1576. DOI: 10.1007/s00227-006-0418-x.
- Matsui, I. (1972). *Eel biology – biological study*. Tokyo: Koseisha-Koseikaku.
- McDowall, R.M. (1988). *Diadromy in fishes*. London: Cromm Helm.
- Poole, W.R. & Reynolds, J.D. (1996). Growth rate and age at migration of *Anguilla anguilla*. *Journal of Fish Biology* 48(4): 633-642. DOI: 10.1111/j.1095-8649.1996.tb01458.x.
- Sasai, S., Aoyama, J., Watanabe, S., Kaneko, T., Miller, M.J. et al. (2001). Occurrence of migrating silver eels, *Anguilla japonica*, in the East China Sea. *Marine Ecology Progress Series* 212: 305-310. DOI: 10.3354/meps212305.
- Sokal, R.R. & Rohlf, F.J. (1995). *Biometry. The principles and practice of statistics in biological research*. Third edition. New York: W.H. Freeman.
- Sudo, R., Okamura, A., Fukuda, N., Miller M.J. & Tsukamoto, K. (2017) Environmental factors affecting the onset of spawning migrations of Japanese eels (*Anguilla japonica*) in Mikawa Bay Japan. *Environmental Biology of Fishes* 100(3): 237-249. DOI: 10.1007/s10641-017-0575-4.
- Svendäng, H., Neuman, E. & Wickström, H. (1996) Maturation patterns in female European eel: age and size at the silver eel stage. *Journal of Fish Biology* 48(3): 342-351. DOI: 10.1111/j.1095-8649.1996.tb01432.x.
- Tesch, F.W. (1977). *The eel. Biology and management of anguillid eels*. London: Chapman and Hall.
- Todd, P.R. (1981). Timing and periodicity of migration New Zealand freshwater eels (*Anguilla* spp.). *New Zealand Journal of Marine and Freshwater Research* 15(3): 225-235.
- Tsukamoto, K. (1992). Discovery of the spawning area for the Japanese eel. *Nature* 356: 789-791.
- Tsukamoto, K. (2009). Oceanic migration and spawning of anguillid eels. *Journal of Fish Biology* 74(9): 1833-1852. DOI: 10.1111/j.1095-8649.2009.02242.x.
- Tsukamoto, K. & Arai, T. (2001). Facultative catadromy of the eel, *Anguilla japonica*, between freshwater and seawater habitats. *Marine Ecology Progress Series* 220: 265-276. DOI: 10.3354/meps220265.
- Tzeng, W.N. (1996). Effects of salinity and ontogenetic movements on strontium:calcium ratios in the otoliths of the Japanese eel, *Anguilla japonica* Temminck and Schlegel. *Journal of Experimental Marine Biology and Ecology* 199(1): 111-122. DOI: 10.1016/0022-0981(95)00185-9.
- Utoh, T., Mikawa, N., Okamura, A., Yamada, Y., Tanaka, S. et al. (2004). Ovarian morphology of the Japanese eel in Mikawa Bay. *Journal of Fish Biology* 64(2): 502-513. DOI: 10.1111/j.0022-1112.2004.00317.x.
- Wenner, C.A. & Musick, J.A. (1974). Fecundity and gonad observations of the American eel, *Anguilla rostrata*, migrating from Chesapeake Bay, Virginia. *Journal of the Fisheries Research Board of Canada* 31(8):1387-1391.