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**Influence of environmental parameters on habitat use by sympatric freshwater eels,
Anguilla marmorata and *A. japonica* on Yakushima Island, Japan**

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Abstract

It is known that environmental parameters, such as water depth, size of substrate material, and current velocity influence the organization of stream fish communities. However, few studies have investigated the effects of these parameters on sympatric freshwater eels. Here, the habitat use of two anguillid eels, *Anguilla marmorata* Quoy and Gaimard, 1824 and *A. japonica* Temminck and Schlegel, 1846, coexisting in the same river systems in Japan was investigated. It was found that the tropical species *A. marmorata* exclusively utilized habitats with larger substrate materials, fewer fallen leaves and less leaf detritus, higher current velocity, and lower turbidity than the temperate species *A. japonica*, and the distributions of the two anguillid eels were clearly segregated. Thus, when both species co-occur in the same river systems, clear flowing mainstems and tributaries are preferred habitats for *A. marmorata*, while stagnant muddy estuaries, backwater areas, irrigation channels, and reservoirs are preferred habitats for *A. japonica*. These habitat segregations were consistent through body-size increases and life-stage developments from elver to yellow eel and did not show ontogenetic shifts. The findings indicated that both species had strong habitat preferences, and these environmental parameters must be considered in the conservation of anguillid eels in sympatric zones.

Keywords

Anguillidae, Japanese eel, *Anguilla japonica*, Indo-Pacific eel, *Anguilla marmorata*, habitat preference, interspecific interactions

Introduction

Habitat is a critical factor that can influence the individual growth and survival of animals. Moreover, it is considered one of the major axes of niche partitioning, which reduces competition between species with similar niches and allows them to coexist (Schoener, 1974, 1982). Freshwater habitats are small in area but rich in species diversity (Reid et al. 2019), and many studies have illustrated that congeneric freshwater fishes, such as gobies (e.g., Sone et al. 2001; Allen-Ankins et al. 2017) and salmonids (e.g., Heggenes and Saltveit 2007; Morita et al. 2016), living in the same rivers utilize different habitats. The explanatory factors of these segregations may include interspecific interactions and differences in habitat preferences (e.g., Roper et al. 1994; Sone et al. 2001). It is widely known that these segregations occur according to a set of physical parameters, such as water depth, current velocity, and substrate size (Grossman et al. 1998; Hued and Bistoni 2006), indicating the importance of the integrated influence of these physical parameters on the spatial organization of stream fish communities.

Catadromous anguillid eels, among the top predators in freshwater ecosystems, are another group that segregates their habitats when more than one species occurs in the same river system (e.g., Glova et al. 1998; Jellyman et al. 2003; Hagihara et al. 2018). Several studies have reported the habitat use of allopatric freshwater eels (e.g., Itakura et al. 2018; Itakura and Wakiya 2020; Kume et al. 2020). However, few studies have collected detailed information on the environmental differences in habitats in sympatric freshwater eels, although these habitat features could play key roles in habitat segregation for anguillid species.

One example of habitat segregation that has not yet been well studied is that of the Indo-Pacific eel *Anguilla marmorata* Quoy and Gaimard, 1824 and the Japanese eel *A. japonica* Temminck and Schlegel, 1846. *Anguilla marmorata* is a tropical eel with an extensive geographical distribution ranging from the western Indian Ocean to the Pacific Ocean (Tesch 2003). The species is also one of the largest anguillid eels reaching approximately 1.5 m in total

length (Williamson and Boetius 1993). *Anguilla japonica*, on the other hand, is a temperate species distributed over a wide range of latitudes along the western boundary currents of East Asia (Nguyen et al. 2018; Yoneda et al. 2019; Morita and Kuroki 2021), which is highly exploited commercially, and its protection is a major concern (Kuroki et al. 2014). The leptocephalus larvae of these two species hatch in the ocean, recruit to coastal, estuarine, or freshwater habitats, and grow there for years or decades until they start their spawning migration back to the ocean. Both species are benthic, nocturnally active, and carnivorous, feeding on small fishes and crustaceans during the growth phase. The geographical distributions of *A. marmorata* and *A. japonica* overlap in East Asia, including southeast China, Taiwan, and southern Japan (Han et al. 2012). However, in Taiwanese rivers, the composition of the two species changes along a river continuum, with *A. marmorata* and *A. japonica* mainly utilizing upper and lower reaches, respectively (Shiao et al. 2003; Hsu et al. 2019). In Yakushima Island in southern Japan, it has been reported that *A. japonica* mainly utilizes agricultural irrigation channels and reservoirs, while *A. marmorata* mainly utilizes mainstem and tributary waters (Kumai et al. 2020). Considering that the abiotic parameters, such as water velocity, water quality, depth, and size of substrate materials are likely to differ between the sites inhabited by *A. marmorata* and *A. japonica*, there could be different habitat use between the two species related to these parameters in rivers where they coexist. However, there have been no studies that evaluate the differences quantitatively in such rivers, although the information is indispensable for habitat conservation of the two species in sympatric zones. The present study aimed to investigate the quantitative differences in habitat use associated with these environmental parameters between two anguillid eels, *A. marmorata* and *A. japonica*, in rivers where the species coexist.

Materials and methods

Study location and investigation of species composition

The study area was located on Yakushima Island, a remote island in southern Japan (Fig. 1). This island has a largely mountainous landscape with limited flatland and heavy rainfall with an annual average of about 4.2 m (1938–2020) (Japan Meteorological Agency 2020). Thus, many fast-flowing rivers with steep gradients and limited estuaries have developed around the island. In this island, the number of recruited *A. japonica* glass eels is much higher than that of recruited *A. marmorata* glass eels (Yamamoto et al. 2002; Fig. S1).

The surveys were conducted at nine stations on two rivers, the Isso and Nagata rivers, which run through the northwest part of the island (Fig. 1). The area (width and length) of each survey station was selected to have only homogeneous environments within it, and the width of the station thus did not necessarily correspond with the river width. In the Isso River (ISO), six stations were located; two in the mainstem (ISO-M1 and ISO-M2), one in a tributary (ISO-T), one in an agricultural reservoir with agricultural irrigation water supply (ISO-R), and two in backwater areas of the mainstem (ISO-D1 and ISO-D2) (Fig. 2, Table 1). Stations ISO-M1 and ISO-M2 were located in the middle and lower reaches of the mainstem, respectively. Agricultural drainage from ISO-R and domestic drainage flowed into ISO-D1 and ISO-D2, respectively, and the waters were mixed with mainstem waters in these stations. In the Nagata River (NGT), three stations were located, each in the upper reach of the mainstem (NGT-M), a tributary (NGT-T), and an agricultural irrigation channel (NGT-I) (Fig. 2, Table 1). One side of the riverbank of ISO-M1, ISO-M2, ISO-D1, ISO-D2, and ISO-T, and both sides of the riverbank of NGT-T were revetted with concrete. Station NGT-I had concrete revetments on the sides and bottom and was partly covered by concrete roofs. Mud and sand had accumulated on the river bottom at this station.

To investigate the distributions of *A. japonica* and *A. marmorata*, collections or observations were conducted according to the environmental situation at each station from June

2018 to December 2020 (Table S1). Electrofishing was conducted during both or either the daytime and nighttime at six stations (ISO-T, ISO-D1, ISO-D2, NGT-M, NGT-T, and NGT-I) apart from the two other stations (ISO-M1 and ISO-M2) where the water depths were too deep for electrofishing. Two people carried out the collections; one person operated an electrofishing unit (300–400 V DC; 12B, Smith-Root Inc., WA, USA), and both used a hand net. They moved from the downstream side to the upstream end of the station, zigzagging across rivers, to ensure complete coverage within each station. Visual observations were conducted at three stations located in the deep mainstem (ISO-M1, ISO-M2, and NGT-M). During visual observations, a skilled observer equipped with a snorkel, mask, and hand torches entered from either the downstream or upstream end of each station and crawled slowly to the other end, looking for eels. The observations were conducted at nighttime since freshwater eels are generally nocturnally active. Preliminary visual observations were also conducted at the two stations surveyed by electrofishing (ISO-T and NGT-I). The results of the preliminary observations and electrofishing were identical (only *A. marmorata* ($n = 10$) was observed in ISO-T, and only *A. japonica* ($n = 4$) was observed in NGT-I), indicating that visual observation was effective in determining species composition. At ISO-R, hooks and eel pots baited with the blue mackerel *Scomber australasicus* Cuvier, 1832 were used because this deep reservoir was not transparent enough to allow electrofishing or visual observation. The collection numbers of eels used in this study include the specimens described by Kumai et al. (2020).

The body size, pre-dorsal length (PDL), and pre-anal length (PAL) were measured to the nearest mm in the collected eels after killing; the body size was recorded at 10-cm intervals for visually observed ones. The species was identified by the mottled skin pattern for individuals with a total length of 10 cm or more; smaller specimens without a discernible mottled pattern were identified by the fin positions, according to Watanabe et al. (2004). The collection of eels was approved by the Kumage branch office, Kagoshima Prefecture, Japan, the local

government in charge of the study area.

Habitat measurements

Environmental parameters were measured at all nine collection and observation stations. For stations located in the mainstems and tributaries (ISO-M1, ISO-M2, ISO-T, NGT-M, NGT-T, and NGT-I) longer than 50 m, the measurements were conducted within 50-m lengths that seemed to represent the typical characteristics of each habitat. Depth, substratum, and water velocity were used to choose the measurement areas because these characteristics were easily judged by eye. For the remaining three stations (ISO-R, ISO-D1, and ISO-D2), the measurements were conducted over the entire length of each station. Within the 50-m lengths or over the entire length of stations, ten longitudinal transects were established at even intervals. Along the transects, three spots were set up at even intervals, and, at each spot, the width of the station and the water depth were measured to the nearest 0.1 m and 1 cm, respectively.

At these observation spots, the size of substrate materials and the presence of riverbed cover (leaves and detritus) were recorded, and current velocity was measured. Substrate indices were visually determined by the size of the substrates according to the following rule: 1 = sand or silt; 2 = gravel (2–16 mm diameter in the major axis); 3 = pebble (17–64 mm); 4 = cobble (65–256 mm); 5 = boulder (>256 mm); 6 = bedrock or concrete, a system modified from Bain et al. (1985). Bain et al. (1985) categorized smooth and horizontal bedrock into category 1, but it was categorized into category 6 in the present study. This was because, for anguillid eels, bedrock is considered to differ greatly from sand and silt in that it does not provide shelters for small eels such as glass eels and elvers, whereas sand and silt can provide hiding places (Dou and Tsukamoto 2003). The riverbed cover was also recorded because riverbeds of some stations (ISO-R, ISO-D1, and ISO-D2) were mostly covered by fallen leaves and detritus, and these could contribute to significant environmental differences between stations. Other types of

riverbed cover, such as instream vegetation and debris, were rare and only found in ISO-D2 and ISO-T, where 93% and 10% of the observation spots were covered by algal mats and debris, respectively. The existence probability of riverbed cover was calculated at each station by dividing the number of observation spots where fallen leaves and detritus were present by the total number of observation spots. Current velocity was measured at 60% depth (mean water column velocity, Allan and Castillo 2007) to the nearest 0.1 cm/s using a propeller-type current meter (VR-301; Kenek Co., Tokyo, Japan).

The water temperature at each station was recorded at hourly intervals using data loggers (TidbiT v2; Onset Computer Co., MA, USA) located within the stations for 72 hours from August 14 to August 16, 2020, which corresponds to the period when the activity of the eels increases. In addition, salinity and turbidity were also measured at the surface to the nearest 0.1 ppt and 0.01 NTU, respectively, at three spots set up near each end and the center of the measurement areas.

Statistical analysis

To examine the differences in habitat use between *A. marmorata* and *A. japonica*, the mean environmental parameters (mean depth, substrate index, the occurrence probability of riverbed cover (detritus and fallen leaves), current velocities, water temperatures, salinity, and turbidity) were compared separately for the stations where *A. marmorata* dominated and those where *A. japonica* dominated using Mann–Whitney U tests. In these tests, $p < 0.05$ was considered significant.

A principal component analysis (PCA) was conducted to identify which environmental parameters might influence the composition of the two species. In this analysis, the mean environmental parameters at each station were used as variables after they were normalized, except for salinity. Salinity was excluded from the analysis because it was consistently close to

zero at all nine stations (0.02–0.08 ppt) and was considered not to affect species composition, at least at these stations.

All statistical analyses were conducted using the R 3.6.3 software (R Core Team 2020).

Results

Species composition

A total of 145 anguillid eels were collected or visually observed in the nine stations studied. Both *A. marmorata* ($n = 72$) and *A. japonica* ($n = 73$) were collected or observed (Table 2), but only one or other of the species occurred in all the stations except for ISO-D1. In ISO-D1, even though both species occurred, *A. japonica* ($n = 6$) dominated over *A. marmorata* ($n = 1$) (Table 2). These results show clear habitat segregation between the two species.

Among the eels collected or observed in the two rivers, eels of various sizes were found; the total lengths ranged 6–117 cm for *A. marmorata* and 6–55 cm for *A. japonica* (Fig. 3). In both rivers, eels <10 cm occurred in tributaries (ISO-T and NGT-T) for *A. marmorata*, and in backwater and agricultural irrigation channels (ISO-D1, ISO-D2, and NGT-I) for *A. japonica*, showing that smaller eels segregated their habitats in the same way as the yellow eels of both species.

Environmental differences in habitats

Significant differences in environmental parameters were found between the five stations where *A. marmorata* dominated (ISO-M1, ISO-M2, ISO-T, NGT-M, and NGT-T) and the other four stations where *A. japonica* dominated (ISO-R, ISO-D1, ISO-D2, and NGT-I) (Table 2, Fig. 4). Mean substrate index and current velocity were significantly higher in the stations dominated by *A. marmorata* (mean $\pm$ SD 3.81 ± 0.41 , 15.78 ± 5.20 cm/s, respectively) than in those dominated by *A. japonica* (1.63 ± 0.46 , 5.49 ± 3.68 cm/s, respectively) (Mann–Whitney

U test, both $p = 0.0195$) (Fig. 4). By contrast, the mean occurrence probability of riverbed cover (fallen leaves and detritus) and turbidity were significantly higher in the stations dominated by *A. japonica* (0.39 ± 0.28 , 1.38 ± 1.11 NTU, respectively) than those dominated by *A. marmorata* (0 ± 0 , 0.37 ± 0.078 NTU, respectively) (Mann–Whitney U test, $p = 0.0159$ and 0.0108 , respectively) (Fig. 4). No significant differences were found in mean depth, water temperature, and salinity (Fig. 4). Although not included in the analysis, 93% of the 30 spots measured in ISO-D2 were covered with free-floating algal mats, which may have slightly influenced the electrofishing success.

The PCA also showed environmental differences between the stations where *A. marmorata* dominated and those where *A. japonica* dominated (Fig. 5). The first and second principal components (PCs) accounted for 54.2 and 23.9% of the total variance, respectively. The first PC was positively correlated with substrate index and current velocity and negatively with the occurrence probability of riverbed covers (fallen leaves and detritus) and turbidity, whereas the second PC was positively correlated with water depth and temperature (Table 3, Fig. 5). Along the first PC axis, the stations where *A. marmorata* dominated (ISO-R, ISO-D1, ISO-D2, and NGT-I) and those where *A. japonica* dominated (ISO-M3, ISO-M4, ISO-T, NGT-M1, and NGT-T) were clearly discriminated, with the former having larger PC scores than the latter (Fig. 5). These results indicate that the stations dominated by *A. marmorata* were mainly characterized by higher substrate index, higher current velocity, a lower occurrence probability of riverbed covers (fallen leaves and detritus), and lower turbidity than those dominated by *A. japonica*. However, the stations dominated by *A. marmorata* and those dominated by *A. japonica* were not discriminated along the second PC axis, suggesting water depth and temperature do not influence the composition of the two species in these stations.

Discussion

The present study showed distinct environmental differences in the habitats that *A. marmorata* and *A. japonica* utilize. Both the mean comparison of environmental parameters and the PCA indicated that the habitat of *A. marmorata* is characterized by higher current velocity, larger substrate materials, the lower occurrence probability of fallen leaves or leaf detritus, and lower turbidity than that of *A. japonica*, although the substrate composition, as well as the substrate size, might influence their riverine distribution. These results suggest that when both species occur in the same river, relatively fast-flowing and clear mainstems or tributaries are likely to provide preferred habitats for *A. marmorata*, while relatively stagnant and muddy areas such as estuaries, irrigation channels, ponds, and backwater areas are likely to provide preferred habitats for *A. japonica*. However, the mean comparison showed no significant differences in water depth and temperature, and these two parameters were not related to the composition of the two species in the PCA either. Also, in the present study, *A. japonica* only occurred in stations affected by agricultural or domestic drainage, while only one individual of *A. marmorata* was present in such environments, suggesting that these water types could influence their riverine distributions. *Anguilla japonica* could have a stronger preference or greater tolerance for waters influenced by anthropogenic activities than *A. marmorata*.

It has been reported that habitat segregation of the two species occurs along the river continuum in two large Taiwanese rivers (Shiao et al. 2003; Hsu et al. 2019). In these studies, *A. marmorata* utilized the upper reaches, whereas *A. japonica* utilized the lower reaches. Considering that the upper reaches would be characterized by higher current velocity, larger substrate-materials, and lower turbidity, and the lower reaches would be characterized by lower current velocity, smaller substrate material, and higher turbidity, these patterns correspond to the present study results. This correspondence indicates that habitat preferences are likely to play key roles in habitat segregation of the two species because, without habitat preferences, the consistent tendency to use specific types of habitats would not be observed in different

localities. *Anguilla marmorata* also dominated in some downstream reaches (ISO-M2, ISO-T, and NGT-T) in this study, but this could be due to the unique characteristics of the short rivers on steep slopes in Yakushima Island. The length and average gradients of the rivers surveyed in the present study were approximately 7 km and 9% for the Isso River and 8 km and 7% for the Nagata River, respectively. These rivers were considerably shorter and more steeply graded than the two Taiwanese rivers, the Kaopin River (approximately 171 km and 2%) and the Fengshan River (45 km and 3%) (calculated using Google Maps and Google Earth). In the short, high gradient rivers in Yakushima Island, the areas of brackish-water estuaries and lower reaches were limited, and even the downstream areas were characterized by relatively high current velocity, larger substrate materials, and low turbidity, which seemed to be more similar to middle-reach environments than to downstream environments.

However, other factors could also influence the habitat segregation between the two species. For example, allopatric *A. japonica*, with a total length of more than 20 cm, were reported to utilize habitats where the substrate materials were composed of boulders in a river in northern Japan (Kume et al. 2020). Moreover, *A. marmorata* dominated the downstream areas in a gentle river (Poso River) in Indonesia (Hagihara et al. 2018), where no (or only a few) *A. bicolor* McClelland, 1844 and *A. japonica* were present, although the length and the average gradient of the river were approximately 71 km and less than 1% (calculated using Google Maps and Google Earth). These studies indicate that even though distinct habitat preferences exist between the two species, interspecific interactions might play key roles in their habitat selection when both species coexist in the same river systems. It is also possible that the two species segregate their habitats according to the prey type available, as the prey fauna would be similar in similar habitats (Kumai et al. 2020).

It is also noteworthy that habitat segregation of the two species may be completed to a certain extent before the recruited glass eels become yellow eels, considering that, in this study,

eels <10 cm have already segregated their habitats in the same way that yellow eels do (Fig. 4). This result could indicate that the habitat preferences, interspecific interactions, and prey preferences during the glass eel or elver stages could strongly affect the subsequent riverine distributions of the anguillid species. Preferences for substrate size are found in glass eels of some anguillid species (Silberschneider et al. 2004; Nilsson et al. 2020). It is thus possible that substrate preferences might differ between *A. marmorata* and *A. japonica* glass eels, which might lead to habitat segregation between the two species. Fallen leaves and leaf detritus from surrounding ecosystems might also play a vital role in habitat selection of glass eels, given that glass eels of the temperate American eel *A. rostrata* (Lesueur, 1817) were attracted to the odors of leaf-detritus-decomposing microorganisms (Sorensen 1986). Similarly, this odor could also influence migratory behaviors of glass eels in temperate areas, where leaves fall into rivers from deciduous trees in winter. Since stations characterized by fallen leaves and leaf detritus were dominated only by *A. japonica* and not by *A. marmorata*, it is possible that temperate *A. japonica* glass eels are also attracted to such odors (Kumai et al. 2021), while tropical *A. marmorata* glass eels have no preferences for them or may even avoid them.

The relative abundance of the two species changed greatly after recruitment in the study location. The species composition of glass eels at recruitment was highly skewed to *A. japonica*, and only a relatively small number of *A. marmorata* were recruited in Yakushima Island, where the present study was conducted (Yamamoto et al. 2001; Fig. S1). However, the rivers of this island were dominated by *A. marmorata* while *A. japonica* was rare (Kumai et al. 2020). Sampling bias might have affected the composition since only the glass eels that came up to the water surface could be collected by the present study methods; however, the findings indicate that the species composition may change greatly and even be reversed after recruitment. Differences in recruitment season between the two species might be one of the possible causes of this alteration. In the study area, recruitment of *A. marmorata* glass eels peaked in summer,

while that of *A. japonica* glass eels mainly occurred in winter (Yamamoto et al. 2001; Fig. S1). In the glass eels of the European eel *A. anguilla* (Linnaeus, 1758) and *A. japonica*, the migratory propensity and preference for fresh water have been found to be stronger in higher water temperatures (Edeline et al. 2006; Kumai et al. 2021). The glass eels of *A. marmorata* may thus have more opportunity to use habitats associated with upstream characteristics, which are overrepresented in the steep rivers of Yakushima Island, compared to *A. japonica* glass eels. In addition, habitats dominated and preferred by *A. japonica*, characterized by downstream environments and affected by agricultural or domestic drainages, were rare on the island (Kumai, personal obs.). It is thus possible that limited habitats available for *A. japonica* due to the prior residence effect of *A. marmorata* and the scarcity of preferred habitats for *A. japonica* in the rivers may lead to the competitive exclusion of *A. japonica* in this island. If this were the case, conservation programs should protect the preferred habitats for each species, especially in sympatric rivers.

This study highlights the fact that environmental parameters are likely to play key roles in the habitat selection of the two eel species, *A. marmorata* and *A. japonica*, and might also influence the species composition in sympatric rivers, indicating that these environmental features should be considered when conserving habitats for these eels, particularly in sympatric rivers. The study, therefore, provides significant insight into protection for these two anguillids, including the determination of the most effective preservation areas or sanctuaries for each species in rivers with various environments. Notably, stagnant and muddy areas, including floodplain wetlands, irrigation channels, ponds, and backwater areas, would provide favorable habitats for *A. japonica*. Maintaining connectivity between rivers and these habitats would be important for the conservation of this species.

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336

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339

340 **Authors' contributions**

341 Y. K.: Data curation, Formal analysis, Investigation, Writing - Original Draft, Visualization.
342 M. K.: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology,
343 Writing- Original Draft, Visualization. K. M.: Investigation, Methodology, Visualization,
344 Investigation, Writing - Review and Editing.

345

346 **Competing interests:** The authors declare there are no competing interests.

347

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Figure captions

Fig. 1 Map of the study area on Yakushima Island, southern Japan. The two maps on the right are enlarged views of survey stations in Isso (ISO) (a) and Nagata (NGT) rivers (b) where species composition of anguillid eels and environmental parameters were examined. Black and white circles indicate the dominant species in each station, the Indo-Pacific eel *Anguilla marmorata* or the Japanese eel *A. japonica*, respectively M: mainstream, T: tributary, R: reservoir, D: backwater areas in mainstream affected by agricultural or domestic drainage. The map was made using data provided by Ministry of Land, Infrastructure, Transport and Tourism, Japan (2020).

Fig. 2 Photographs of each survey station. The stations were located at mainstream (ISO-M1, ISO-M2, NGT-M), tributary (ISO-T, NGT-T), agricultural reservoir (ISO-R), backwater areas of the mainstem (ISO-D1, ISO-D2), and agricultural irrigation channel (NGT-I). Words in parentheses show the dominant species in each station, with AM and AJ corresponding to the Indo-Pacific eel *Anguilla marmorata* and the Japanese eel *A. japonica*, respectively.

Fig. 3 Size frequency distribution of collected or observed eels, the Indo-Pacific eel *Anguilla marmorata* (gray bars) and the Japanese eel *A. japonica* (white bars), in each station. The numbers include specimens collected by Kumai et al. (2020).

Fig. 4 Mean water depth, substrate index, occurrence probability of riverbed covers, current velocity, water temperature, and turbidity in stations dominated by the Indo-Pacific eel *Anguilla marmorata* (AM, gray boxes) and those dominated by the Japanese eel *A. japonica* (AJ, white boxes). The top and bottom of the boxes show the upper and lower quartiles, and the line in

each box shows the median. The ends of the upper and lower whiskers show the highest and lowest datum, respectively. Asterisks show significant differences (Mann–Whitney U tests, $p < 0.05$).

Fig. 5 Principal component analysis (PCA) plots showing environmental differences between stations. Vectors show the loadings of each environmental parameter on each principal component. Open and closed circles represent stations dominated by the Indo-Pacific eel *Anguilla marmorata* and those dominated by the Japanese eel *A. japonica*, respectively. Dep, Sub, RC, CV, Temp, and Turb correspond to water depth, substrate index, occurrence probability of riverbed cover (fallen leaves and detritus), current velocity, water temperature, and turbidity, respectively.

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Fig. 3 (full size)

Fig. 4 (full size)

Fig. 5 (half size)

1 **Table 1** Survey stations of the Isso and Nagata rivers in Yakushima Island, southern Japan and their physical characters. Number in parentheses
2 shows length of the area in which environmental parameters were measured. Width corresponds to the mean river width of areas where habitat
3 measurements were conducted.

River	Station	Property	Distance from river mouth (km)	Altitude (m)	Length (m)	Width (m)	Substrate index	Riverbed cover	Depth (cm)	Current velocity (cm/s)	Temperature (°C)	Turbidity (NTU)
Isso	ISO-M1	Mainstem	1.6	4	70 (50)	9.6	3.5	0	71.3	14.9	25.8	0.29
	ISO-M2	Mainstem	1.1	0.5	70 (50)	4.8	3.7	0	73.3	9.0	26.1	0.28
	ISO-T	Tributary	1	4	170 (50)	2.7	3.7	0	27.7	13.4	26.2	0.37
	ISO-R	Reservoir	1.6	3	12 (12)	5.7	2.3	0.70	29.5	3.0	27.7	3.04
	ISO-D1	Backwater in mainstem	1.5	3	14 (14)	5.2	1.7	0.33	59.4	8.6	25.9	0.72
	ISO-D2	Backwater in mainstem	1.1	0.5	17 (17)	4.8	1.2	0.48	15.0	1.7	23.7	0.79
Nagata	NGT-M	Mainstem	2.6	40	120 (50)	6.6	4.5	0	47.0	22.0	23.7	0.46
	NGT-T	Tributary	0.3	2	120 (50)	10.5	3.7	0	18.7	19.7	25.4	0.42
	NGT-I	Irrigation	1	4	400 (50)	1.2	1.4	0.037	3.3	8.6	24.8	1.00

Table 2 Species composition and methods used to investigate the composition at each survey station. AM and AJ correspond to the numbers of the Indo-Pacific eel *Anguilla marmorata* and the Japanese eel *A. japonica*, respectively. The collection number includes individuals collected by Kumai et al. (2020).

River	Station	Method*	AM	AJ	Dominant species
Isso	ISO-M1	O	12	0	AM
	ISO-M2	O	11	0	AM
	ISO-T	E	26	0	AM
	ISO-R	P, H	0	30	AJ
	ISO-D1	E	1	6	AJ
	ISO-D2	E	0	17	AJ
Nagata	NGT-M	O, E	5	0	AM
	NGT-T	E	17	0	AM
	NGT-I	E	0	20	AJ

*O, E, P, H show the collection methods and corresponds to visual observation, electrofishing, eel pot, and hook and line, respectively.

Table 3 Loadings of each environmental parameter on principal component 1 and 2 in the principal component analysis (PCA).

Environmental parameters	PC1	PC2
Substrate index	0.442	0.352
Occurrence probability of riverbed covers	-0.508	0.085
Depth	0.219	0.571
Current velocity	0.491	0.029
Temperature	-0.202	0.697
Turbidity	-0.465	0.237

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