

1 Concise title: Trophic ecology of Japanese eels

2 Informative title: Trophic ecology of Japanese eels in small rivers of urban and agricultural
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Abstract

Yellow-phase Japanese eel *Anguilla japonica* utilize a variety of anthropogenically impacted habitats. A profound understanding of the trophic ecology and habitat use is necessary for the conservation of this endangered species. This study analysed the trophic ecology and feeding habitat of Japanese eels in two small rivers, Akugawa River and Takase River in Wakayama Prefecture, Japan, dominated by urban and agricultural land use, respectively. The main goals were to assess the dietary diversity and trophic niche width for eels of two size classes (small eels: ≤ 240 mm total length, large eels: > 240 mm total length) in both habitats. Furthermore, the suitability of both rivers as feeding habitat for eels was evaluated. Analysis of carbon and nitrogen stable isotopes, as well as stomach contents allowed inferring the trophic ecology of eels. Both rivers were suitable to accommodate eel to a varying degree. Dietary diversity of small eels and trophic niche width differed between rivers, indicating limiting factors such as insufficient food sources and unfavourable abiotic habitat requirements for small eels in urban rivers. Furthermore, the body condition of eels in urban Akugawa River was found to be lower than in Takase River.

Keywords: Japanese eel, River habitat, Trophic niche, Dietary diversity, Niche overlap, Stable isotope analysis

Introduction

The growth phase (yellow phase) of temperate anguillid eel species including Japanese eels *Anguilla japonica* in continental waters represents the longest in the eel life cycle and is crucial for the accumulation of energy reserves in order to undertake the long-distance migration to oceanic spawning grounds which mark the onset of maturity (silver phase) (Belpaire et al. 2009; Chow et al. 2010). While some eels solely reside in coastal areas during their growth phase (Arai et al. 2003), rivers may provide key habitats in which eels distribute from the brackish estuaries to the upstream freshwater habitats (Tsukamoto and Arai 2001; Yokouchi et al. 2012). Yet, almost 80% of effective Japanese eel habitats have been lost in East Asian rivers due to concrete river revetment, migration barriers (weirs and dams) and water pollution (Chen et al. 2014). Along with overfishing, those threats were reported as the causes of declining wild Japanese eel populations (Itakura et al. 2015a), leading the Japanese eels to be listed as endangered (EN) on the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN) (Pike et al. 2020 <https://www.iucnredlist.org/species/166184/176493270> “Accessed 12 November 2021”). A comprehensive understanding of habitat requirements and the trophic ecology of eels is necessary for the management and conservation of the species and the habitats they are associated with.

Yellow-phase Japanese eels are generalist top predators, utilizing a broad spectrum of food sources in fresh- and brackish water habitats (Kaifu et al. 2013; Itakura et al. 2015b; Kutzer et al. 2020). As such, they are more resilient to changing environmental conditions due to their ability to switch between food sources (Purvis et al. 2000). Nevertheless, Layman et al. (2007b) pointed out the importance of understanding the ecology of those more resilient species in regard to the effects of anthropogenic alterations on their trophic ecology and the food web of the ecosystem. Additionally, utilization of suboptimal food sources can lead to reduced fitness and, in the long-term, to negative trends in population dynamics (Ruiz-Olmo and Jiménez 2009; Moorhouse-Gann et al. 2020). Japanese eels showed lower body conditions as well as lower abundance and diversity of prey in river habitats with concrete bank revetment compared to river habitats with more natural shores (Itakura et al. 2015b), indicating that structural elements in the habitat influence the availability of food sources for eels and therefore also the fitness level of eels. Furthermore, due to their position as the top predators in river habitats and their suitability to perform as surrogate species for the conservation of freshwater biota (Itakura et al. 2020) it is important to assess the trophic ecology of eels in regard to changes in their habitat.

The trophic niche of a species is the summary of all interactions that link it to other species in an ecosystem (Leibold 1995). The niche width and diet of fishes have been most commonly quantified through the stomach content analysis (SCA) (Amundsen and Sánchez-Hernández 2019). While the taxonomic resolution of SCA is high, it only provides a snapshot of the diet and is prone to errors, such as overestimation of prey contribution, due to opportunistic prey choices. The analysis of carbon and nitrogen stable isotopic signatures (δ , expressed as the ratio of heavy to light isotope relative to a standard expressed in ‰) of consumer tissues provides a more time-integrated approach for the estimation of trophic niche width (Bearhop et al. 2004). Nitrogen stable isotope ratios ($\delta^{15}\text{N}$) allow the identification of diet and trophic position of animals within a food web (Minagawa and Wada 1984; Schoeninger and DeNiro 1984; Post 2002), while carbon stable isotope ratios ($\delta^{13}\text{C}$) allow the identification of primary carbon sources in the diet of consumers (Fry and Sherr 1989; Post 2002) based on a predictable enrichment of heavier isotope with the step-wise increase in trophic position (DeNiro and Epstein 1978, 1981). Stable isotope analysis allows a quantitative comparison of trophic niche widths of predators within communities, visualizing the possible overlap of trophic niches and therefore competition for resources. Furthermore, the trophic niche width along with other metric derived from stable isotope ratios allow to assess the diversity of resources in the diet of consumers (Layman et al. 2007a; Jackson et al. 2011) and can be used to compare communities of consumers between different habitats such as natural and anthropogenically altered rivers (Layman et al. 2007b).

Many small rivers in Japan underwent heavy alterations such as channelization for paddy field irrigation, fragmentation through weirs, and bank revetment for flood control (Yoshimura et al. 2005; Nakamura et al. 2006). Furthermore, these rivers can be heavily impacted by the anthropogenic input of nutrients, e.g. inflowing household sewage in urban areas without centralized sewage system (Furukawa et al. 2000) and occasional run offs from paddy fields in agricultural areas (Somura et al. 2012). Small rivers provide promising study sites for the application and assessment of conservation measures, as they can be monitored with less effort than larger streams (Kume et al. 2019, 2020; Matsushige et al. 2020).

The objectives of this study were to assess the trophic ecology of Japanese yellow-phase eels in two small rivers in urban and agricultural areas with varying degree of bank revetment and to assess the prey availability for eels in both rivers. Stomach content analysis and stable isotopes ratios were used to compare dietary patterns and to quantify intraspecific variation in the trophic niche of eels of 1) both size classes within a river and 2) the same size class between

121 rivers. Furthermore, body condition of eels was compared between rivers to evaluate the fitness
122 of eel size classes in context of their feeding habitat.

124 **Materials and methods**

125 **Study site**

126 This study was conducted at two freshwater sampling stations in Akugawa River (AKU,
127 approximately 4.3 km in length) and three freshwater sampling stations in Takase River (TAK,
128 approximately 8.1 km in length) in Wakayama Prefecture, Japan (Fig.1). All stations had cobble
129 and/or boulder riverbeds that created potentially available shelter for yellow phase eels (Kume
130 et al. 2019, 2020; Matsushige et al. 2020). Additionally, sand and gravel were present at TAK1
131 (Table 1). The percentage of artificial revetments (e.g., concrete-sealed banks and stone
132 masonry revetment) along riverbanks was determined using field observations and Google
133 Earth Pro (version 7.3.2.5776 [Map data: Google, Digital Globe]). It was based on a total of 3.0
134 and 3.3 km of riverbank from the river mouth to the uppermost sampling station for Akugawa
135 and Takase River, respectively.

136 Akugawa River was running through a mainly urbanized area with a few paddy field
137 patches (Online Resource S1). The bank of Akugawa River was completely reinforced by
138 concrete revetment with muddy river bottom and without bank vegetation on the first 2.5 km
139 from the river mouth. The percentage of artificial revetments from river mouth to AKU2 (3.0
140 km from the mouth) was estimated to be c.a. 90%. However, most of the riverbanks between
141 AKU1 and AKU2 were covered by vegetation and small-scale masonry revetments were
142 present at AKU1 (Table 1). Upstream of AKU2 is a concrete culvert that passes under JR
143 Shirahama Station. Several household sewage outlets were located at Akugawa River. One weir
144 (ca. 2.75 km from the river mouth, approximately 1.65 m height from the river bottom, 90°
145 angle, no fish way present) was present at the downstream of AKU1. The weir was covered in
146 a biofilm of algae, and yellow phase eels were found in AKU1 and AKU2 (Kume et al. 2021).
147 It was confirmed that elver and small-sized yellow eels climbed this weir with installed video
148 cameras (Kume et al. 2022).

149 Takase River, which meets the Tonda River mouth (approximately 41 km in length), is
150 running through an area dominated by paddy fields from the river mouth up to TAK3
151 (approximately 3.3 km from the river mouth) (Online Resource S1). The river water was
152 utilized for paddy field irrigation. Further upstream the area was dominated by mixed
153 woodlands. The banks of Takase River from the river mouth to TAK3 are alternately revetted
154 with stone masonry or concrete on one side of the river, while the opposite side has soil deposits

and vegetation growing on it. The ratio of artificial bank without vegetation was estimated to be about 55%. In addition, most of the downstream shoreline is a masonry revetment, and the gaps between the stones were used as habitat by eels. Two weirs are present between TAK1 and TAK 2 (approximately 0.2 and 0.5 m from river bottom to the top, 90° angle, no fish way present). There are also two weirs between TAK2 and TAK3, but they form gentle slopes because of the accumulation of sediment. No eel stocking has been reported for neither one of the rivers.

Sample collection and habitat assessment

Habitat assessment and sampling at all five stations were conducted in June and October 2018. The number of repetitions varied depending on the assessment. The location of the first downstream sampling station in each river was decided based on salinity levels and the earliest possible application of electric shocker for eel and prey source sampling. This resulted in AKU1 being located upstream a weir, whereas no weir was present between the river mouth and TAK1.

Eel habitat assessment included the recording of the following variables in sets of three repetitions per station and month: Dissolved oxygen (DO; mg/l), pH, water temperature (°C), electric conductivity (EC in $\mu\text{S}/\text{cm}$, AAQ-RINKO, AAQ170, JFE Advantech, Kobe, Japan) and flow velocity (cm/s, VE20/VET-200-10PII, KENEK Co., Ltd., Tokyo, Japan). Water samples were taken as single sample per station and month for the analysis of dissolved inorganic nitrogen in nitrate and nitrite (DIN) and dissolved inorganic phosphorous in phosphate (DIP) concentrations (mg/l) and later measured with an Auto-Analyzer (QuAatro2-HR, BLTEC). For the quantitative comparison of primary production, water samples were taken in triplicate per station and month to calculate chlorophyll *a* (chl *a* in $\mu\text{g}/\text{l}$) concentrations of particulate organic matter (POM) of river water. Three stones were collected from the riverbed per station and month and scraped with a knife to collect samples for determining the chl *a* concentration ($\mu\text{g}/\text{cm}^2$) of benthic microalgae (BMA). Samples of chl *a* were processed according to Kasai et al. (2010) and later analysed by a calibrated fluorometer (Turner Designs, Trilogy). Mean values were calculated for each triplicate per month and used for further analyses. Univariate differences in abiotic factors and chl *a* (pooled data for stations and sampling months within a river) between rivers were tested with Mann-Whitney U-test in the R software (R Core Team 2020, <http://www.r-project.org/> “Accessed January 10, 2020”). The above-described parameters and samples for habitat assessment were monitored over a length of approximately 100 m per station.

Riverine biota was sampled in accordance with the “Guidelines for the Use of Fishes in Research” published by the Ichthyological Society of Japan (2003 <http://www.fish-isj.jp/english/guidelines.html>. “Accessed January 18, 2018”). We collected eels at all stations by electric shocker (LR-20B, SmithRoot, Vancouver, WA, USA) in the early morning hours (5 am to 9 am). During the sampling, we moved upstream zigzagging across the river to guarantee complete coverage within each station. A total of 49 eels were collected in the sampling stations of Akugawa River and 45 eels in the sampling stations of Takase River. The numbers of eel collected varied greatly between stations and between sampling periods (Online Resource S2). Therefore, data for eels was pooled for the different stations and sample months within a river in the following analyses. Since the two rivers surveyed were small and the number of eels inhabiting them was thought to be limited, we decided to limit the number of eels collected in both rivers to a maximum of 50 individuals, because we considered that we should not collect many endangered species even for a survey. The development stage of eels was visually assessed using the silvering index (Okamura et al. 2007) and all individuals used for this study were categorized as yellow-phase eels. Additionally, benthic macroinvertebrate sampling was conducted using a Surber sampler (0.5 mm mesh size, sampled area: 625 cm²). Surber samplings were performed in triplicate per sampling and month. Collected fishes were anaesthetized with clove oil (Walsh and Pease 2002), put on dry ice for transportation together with other samples, and later stored at -30°C for later analysis.

The composition and biomass of electric shocker catches from June and October was analysed by sorting organisms into seven groups: crabs, shrimp, crayfish, gammarids, gobiid fishes, other fishes and eels and weighing potential prey items of eels. Organisms collected by Surber sampler were assessed under a binocular, grouped into seven groups: Diptera (fly larvae), Ephemeroptera (mayfly larvae), Trichoptera (caddisfly larvae), other aquatic insects, gammarids, Asellidae and Gastropoda (snails) and weighed. Mean values were calculated for samples collected by Surber sampler. Differences in overall biomass of potential prey items of eels were tested with Mann-Whitney U-test in R. To visualize the biomass compositions of community structures of aquatic organisms collected in Akugawa and Takase Rivers using electric shocker and Surber sampler, a non-metric multidimensional scaling (NMDS) was performed with the *vegan* package (Oksanen et al. 2020 <https://cran.rproject.org/web/packages/vegan/index.html> “Accessed date month 2021”) in R, relying on a Bray-Curtis similarity matrix of abundance data. Environmental vectors were fit onto the NMDS plot using the *envfit* function (permutations = 999), to analyse the influence of environmental variables (temperature, oxygen concentration, pH, nutrient concentrations), as well as chl *a*

concentrations of BMA and POM on the compositions of a Surber sampler and electric shocker catches. One-way analysis of similarity (ANOSIM), based on the corresponding rank similarities between samples was used to test for differences in the biomass composition of electric shocker and of Surber sampler catch communities between Akugawa and Takase rivers. If ANOSIM showed significant differences, similarity percentage analysis (SIMPER) was conducted to identify the groups that accounted for most of the difference between rivers for electric shocker and Surber sampler catches, respectively.

Body condition of eels

Total length (TL in mm) and body weight (BW in g) of eels were measured. To eliminate the effects of stomach fullness and recent prey choice on the calculation of body condition, values for BW were subtracted by stomach weight. The sex of eels was determined through visual assessment of the gonadal structure according to Tesch (2003). Currently, there is no scientific literature available on dividing Japanese eels based on TL for trophic ecology studies. Under the assumption that changes in eel mobility lead to changes in accessibility of food sources, 240 mm were used as criterion (Imbert et al., 2010; Wakiya et al. 2016; The Ministry of Environment of Japan 2017; Yokouchi et al. 2022). For further analysis, eels were grouped into the size classes small eels (≤ 240 mm TL) and large eels (> 240 mm TL). The body condition of eels was tested through the length-weight-relationship (LWR) calculated for all eels in this study determined by the equation $BW = a \times TL^b$. Both parameters a and b were estimated by ordinary least squares regression through a logarithmic transformation of the data: $\log(BW) = \log(a) + b \times \log(TL)$ where $\log(a)$ is the intercept and b the slope of the regression line (Froese 2006). To test for differences in LWR of eels between size classes and rivers, an analysis of covariance (ANCOVA) was performed (Vila-Gispert et al. 2000). The BW was used as the dependent variable in the ANCOVA and TL as a covariate. Homogeneity of slopes b was tested among the eels of different size classes and rivers. If no interaction between TL and groups (size class + river) was found a second ANCOVA was used to compare the intercepts $\log(a)$. Multiple comparisons of slopes with Hochberg correction was performed for group-wise comparison of LWR. Mann-Whitney U-test was performed to test for significant differences in TL of eels between rivers. The analyses were performed with the software R.

Trophic ecology of eels assessed by stomach contents

The stomach contents of eels were stored in 70% ethanol, identified under a binocular microscope, weighed (mg) and pooled for all stations and sampling months for the following

analyses. The index of relative importance (IRI) of prey categories was calculated according to Pinkas et al. (1971): $IRI = (\%N + \%W) \times \%F$, where $\%N$ is the percentage numerical composition ($\%N = \text{number of prey items of a certain category} / \text{number of all prey items} \times 100$), $\%W$ is the percentage wet weight ($\%W = \text{wet weight of a certain prey category} / \text{wet weight of all prey items} \times 100$), and $\%F$ is the percentage frequency of occurrence ($\%F = \text{frequency of occurrence of certain prey category} / \text{number of stomach containing prey} \times 100$). IRI was standardized to %IRI by the following equation: $\%IRI = (IRI / \sum IRI) \times 100$.

Pianka's niche overlap (Pianka 1973) was calculated for stomach contents of eels belonging to different size classes within a river using the $\%W$ and $\%N$ of prey categories and was compared with a null model (RA3 algorithm, 1000 repetitions) to test for significant overlap using the *EcoSimR* package (Gotelli et al. 2015) of the R software. Values of Pianka's niche overlap index close to 0 reflect utilization of different prey categories by eels of different size classes being compared whereas values close to 1 reflect similar prey categories.

Dietary diversity of eels of different size classes and rivers was assessed using the Shannon-Weaver diversity index (H') (Shannon and Weaver 1963). The higher the H' value the more diverse is the diet of the respective group.

Trophic ecology of eels assessed by stable isotopes

Dorsal white muscle tissue was taken from the flanks of eels and other fishes. For molluscs, shrimps and crabs, muscle tissue was taken from the foot, the abdominal segment and the pereiopods, respectively, and samples were observed under a binocular in order to remove possible traces of exoskeleton. Whole organisms of aquatic insects were prepared as stable isotope samples, after removing particulate matter with Mili-Q water. A brief acid treatment was found to not significantly affect $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of muscle tissue (Yokoyama and Ishihi 2006). Therefore, muscle tissue samples of gastropods were washed in 1.2 N HCl for 1 min to remove traces of carbonate and then washed with Mili-Q water. After drying at 60°C for 24 h, samples were ground to a fine powder. Lipid-rich tissues are relatively depleted in $\delta^{13}\text{C}$ (DeNiro and Epstein 1977). A previous study found significant differences in $\delta^{13}\text{C}$ for eels but not for potential food sources such as gobiid fishes and crustaceans (Kutzer et al, 2020). Therefore, samples of eel muscle tissue were divided into two sub-samples, one was kept untreated and was used to calculate $\delta^{15}\text{N}$ values, the remaining one underwent lipid removal according to Bligh and Dyer (1959) and used to calculate $\delta^{13}\text{C}$ values. Powdered samples were placed in tin capsules (approximately 1 mg) and used for stable isotope analysis.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of samples were assessed with an isotope ratio mass spectrometer (Delta V Advantage, Thermo Scientific) coupled online via the Finnigan ConFlo IV interface with the elemental analyser. The results were expressed in the standard δ unit notation as $\delta X = ((R_{\text{samples}}/R_{\text{references}}) - 1) \times 10^3$, where X denotes ^{13}C or ^{15}N and $R = ^{13}\text{C}/^{12}\text{C}$ for carbon and $^{15}\text{N}/^{14}\text{N}$ for nitrogen. Standard reference materials Vienna Pee Dee Belemnite (VPDB) and atmospheric $\text{N}_{2(\text{air})}$ were used for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Isotopic signatures were corrected by a two-point calibration using internal standards (CERKU-02 L-Alanine: $\delta^{13}\text{C}_{\text{VPDB}} = -19.04\text{‰}$; $\delta^{15}\text{N}_{\text{air}} = 22.71\text{‰}$; CERKU-03 Glycin: $\delta^{13}\text{C}_{\text{VPDB}} = -34.92\text{‰}$, $\delta^{15}\text{N}_{\text{air}} = 2.18\text{‰}$ and CERKU-05 L-Threonine: $\delta^{13}\text{C}_{\text{VPDB}} = -9.50\text{‰}$; $\delta^{15}\text{N}_{\text{air}} = -2.88\text{‰}$) between each set of 12 – 15 samples. These internal standards were corrected to multiple international standards (Tayasu et al. 2011). The standard deviation (SD) of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements was within 0.1‰. The stable isotope analysis was performed at the Center for Ecological Research, Kyoto University.

For the following analysis, stations and sampling months were pooled. The multivariate analysis of variance (MANOVA) was used to assess the variability in isotopic composition of both size classes of eels within a river and the same size class between rivers. To illustrate the isotopic food web between rivers and the positioning of the two size classes of eels, bi-plots of stable isotope signatures of eels and possible food sources were generated using the *SIAR* package (Parnell et al. 2010) of the R software and applying the trophic discrimination factor (TDF) for eels ($0.8\text{‰} \pm 0.8\text{‰}$ for $\delta^{13}\text{C}$ and $2.1\text{‰} \pm 0.5\text{‰}$ for $\delta^{15}\text{N}$) calculated by Kaifu et al. (2013) to the isotopic signature of food sources. To compare the isotopic niche spaces of different size classes of eels within a river and of same size classes between rivers, Bayesian standard ellipse area (SEA_B) and standard ellipse areas corrected for sample size (SEA_C) were estimated using a Stable Isotope Bayesian Ellipses model of the *SIBER* package (Jackson et al. 2011) in the R software. SEA_C is insensitive to bias associated with small sample size and allows robust comparison between data sets comprised of different sample sizes (Jackson et al. 2011). Niche overlap was calculated based on SEA_C . Furthermore, the total area of isotopic niche space (TA) was calculated with *SIBER* to illustrate the total occupied isotopic niche space of a group. Layman's mean distance to centroid (CD) was calculated using *SIBER*. The CD provided the average degree of trophic diversity within a food web (Layman et al. 2007a; Kroetz et al. 2017).

Results

Feeding habitat

Concentrations of DIN, DIP, chl *a* of POM and BMA, as well as EC were significantly higher in Akugawa River compared to Takase River. The DO concentration in Akugawa River was lower compared to Takase River ($p < 0.05$, respectively) (Table 2).

Benthic macroinvertebrates sampled by Surber sampler had a significantly higher ($p < 0.01$) overall biomass in Takase River (mean $\pm$ SD = 1.11 ± 0.15 mg/cm², $n = 18$) than in Akugawa River (mean $\pm$ SD = 0.39 ± 0.16 mg/cm², $n = 12$). The macroinvertebrate community in Takase River was dominated by Ephemeroptera (mayfly) larvae representing 58.8% of the macroinvertebrate biomass while they represented only 0.8% of the macroinvertebrate biomass in Akugawa. Diptera (fly) larvae contributed only 4.3% to the total biomass of Surber sampler catches in the Takase River, but were the second highest contributor in Akugawa River. Gastropoda were the highest contributor to the macroinvertebrate biomass in Akugawa River (46.2%) and the second highest in Takase River (33.8%). Trichoptera (caddisfly) larvae made up 6.4% in Akugawa River and 6.7% in Takase River of the total biomass of Surber sampler catches. Gammaridae and Asellidae contributed 4.5 and 7.7% in Akugawa River, respectively, but contributed only little in Takase River or were absent (Fig. 2a). The compositions of the benthic macroinvertebrate community collected by Surber sampler in Akugawa and Takase Rivers were significantly different (ANOSIM, global $R = 0.97$, $p < 0.01$) and allowed a clear separation by rivers along the first axis of the NMDS ordination plot (Fig. 3a). DO, chl *a* concentration of POM and BMA were significantly correlated with the biomass composition of the community of Surber sampler catches ($p < 0.05$; Fig. 3a). The comparison of average dissimilarity in abundance of prey categories collected by Surber sampler in Akugawa and Takase Rivers showed that Ephemeroptera larvae and Gastropoda contributed to 57.7% and 23.5% of the significant differences, respectively (SIMPER).

The overall biomass of potential prey organisms sampled by electric shocker was significantly higher in Akugawa River (37.0 ± 37.7 g/m², $n = 4$) than that of prey organisms in Takase River (6.0 ± 1.6 g/m², $n = 6$) ($p < 0.01$). Eels contributed 43.6 (16.1 g/m²) and 17.2% (1.0 g/m²) to the biomass collected by electric shocker at Akugawa and Takase Rivers, respectively (Fig. 2b). Amongst the crustaceans in Akugawa River crabs contributed 23.0%, shrimps 16.2% and crayfish 3.4% to the biomass of electric shocker catches, while in Takase River shrimp contributed 19.3% and crabs 13.0%. Crayfish were absent in the shocker catches at Takase River. Gobiid fishes were the dominant contributor to the biomass of electric shocker catches in Takase River (46.0%) but were less dominant in Akugawa River (13.2%). The compositions of the prey community's biomass collected by electric shocker at Akugawa River was significantly different than that collected in Takase River (ANOSIM, global $R = 0.66$, $p <$

0.01). The NMDS ordination plot showed a clear separation between rivers along the axis 1 (Fig. 3b). Significant correlations of the biomass composition of the community with DO and chl *a* concentration of POM were found ($p < 0.01$ for both, Fig. 3b). The densities of shrimp and gobiid fishes contributed to 43.0% and 42.5% of the significant differences, respectively (SIMPER).

Body condition of eels

The density of eels of the entire sampling area was 0.29 eel/m² in Akugawa River and 0.04 eel/m² in Takase River. The size of collected eels ranged from 83 to 641 mm in TL (Table 3). The comparison within the two size groups and between rivers showed that small eels were significantly larger in Akugawa River (median = 181 mm) than small eels in Takase River (median = 112 mm) ($p < 0.01$), but no significant difference in TL of large eels between rivers was found (median = 377 mm for Akugawa River and 331 mm for Takase River) ($p > 0.05$).

The ANCOVA for the log-transformed length-weight relationship (LWR) showed that the interaction between covariate (TL) and factor (size class/river) was not significant ($p > 0.05$, Online Resource S3, Model 1). A second model (without interaction) showed significant differences between the log-transformed LWR intercepts ($p < 0.001$, Online Resource S3, Model 2). According to multiple comparisons with Hochberg correction the intercept was significantly ($p < 0.001$) higher for small eels in Takase River ($a = 3.11 \times 10^{-4}$) compared to large eels in Takase ($a = 2.23 \times 10^{-4}$) and that of large eels in Takase is significantly higher than those of both size classes in Akugawa River ($p < 0.05$, $a = 2.04 \times 10^{-4}$ for small eels and $a = 2.01 \times 10^{-4}$ for large eels, respectively, Fig. 4). This shows that eels of both size classes in Takase River had a higher weight for a given size than those in Akugawa River, while small eels in Takase River had the highest relative weight of all four eel groups.

Trophic ecology of eels assessed by stomach contents

In Akugawa River, seven out of 11 small eels (58.3%) and 35 out of 38 large eels (92.1%) had identifiable stomach contents. In Takase River, 19 out of 30 small eels (66.3%) and eight out of 15 large eels (53.3%) had identifiable stomach contents (Table 4). The prey categories used to classify stomach contents of eels included various kinds of aquatic prey such as the crab species *Eriocheir japonica* (De Haan 1835) and *Chiromantes dehaani* (H. Milne-Edwards, 1853), shrimps *Caridina* spp., *Macrobrachium formosense* (Spence Bate, 1868) and *Palaemon paucidens* (De Haan, 1844), crayfish *Procambarus clarkii* (Girard, 1852), other Crustacean (Gammaridae, Asellidae), gobiid fishes (*Tridentiger* sp. *Rhinogobius* spp., not further

identified), Diptera (Chironomidae, Trabanidae, Tipulidae), Ephemeroptera (Ephemerellidae, Heptagenidae), Trichoptera (Stenopsychidae, not further identified), other aquatic insects (Coenagrionidae), Gastropoda species *Semisulcospira reiniana* (Brot, 1876) and *Clithon retropictum* (von Martens, 1879), Oligocheata (Annelidae, Criodrilidae) as well as Polycheta (Nereididae).

Shrimps had the highest %IRI contribution for the stomach contents of large eels in Akugawa River (%IRI = 64.6) but were scanty in the stomach contents of eels of both sizes classes in Takase River (Table 4). Crabs were the most important food source of eels of both size classes in Takase River (%IRI = 70.0 for small eels and 71.6 for large eels). Aquatic insects (Diptera and Trichoptera larvae), were identified as most important, most preyed upon and most abundant food source of small eels in Akugawa River (%IRI = 95.0, %F = 100, %N = 92.3) while aquatic insects (dominantly Diptera and Ephemeroptera larvae) were most abundant in the stomach contents of small eels in Takase River (%N = 62.9). Fishes the second highest contributor (%IRI = 21.5) to the diet of large eels in Takase River presented a low %IRI (1.0) in Akugawa River and were absent in the diet of small eels in both rivers.

Pianka's overlap index indicated a significant dietary niche overlap between size classes of eels in Akugawa River in respect to %W (0.44, $p < 0.01$) and %N (0.55, $p < 0.01$). The dietary niche overlap between size classes of eels in Takase River was significant in respect to %W (0.95, $p < 0.001$) and a non-significant (0.47, $p > 0.05$) in regard to %N (Online Resource S4). Dietary diversity of large eels in Akugawa River was higher ($H' = 1.67$) than that of small eels (0.69). In Takase River, large eels (1.31) had a lower dietary diversity than small eels (1.68). Moreover, the difference of dietary diversity between size classes in Takase River was smaller than that in Akugawa River.

Trophic ecology of eels assessed by stable isotopes

The isotopic signatures of both size classes of eels in Akugawa and Takase Rivers showed high variation but were within the isotopic mixing space defined by the food sources adjusted by the TDF ($\delta^{13}\text{C} = 0.8 \pm 0.8$ and $\delta^{15}\text{N} = 2.1 \pm 0.5$; Kaifu et al. 2013). Isotopic signatures of eels and food sources in Akugawa River had lower $\delta^{13}\text{C}$ values and higher $\delta^{15}\text{N}$ values compared to Takase River (Fig. 5, Online Resource S5). Significant differences were found between the isotopic signatures of small eels (Wilks = 0.29, $F = 46.7$, $p < 0.001$), as well as large eels (Wilks = 0.36, $F = 44.6$, $p < 0.001$) between Akugawa and Takase Rivers. Isotopic signatures were significantly different between small and large eels in Akugawa River (Wilks = 0.18, $F = 41.2$, $p < 0.001$) but not in Takase River (Wilks = 0.99, $F = 0.12$, $p > 0.05$).

The isotopic niche overlap based on the SEAC point-metric for eels in Akugawa River showed an overlap of 0.81 ‰² which corresponds to 61% ellipse area of small eels and nearly 26% niche area of large eels. In Takase River, niche overlap reached 3.32 ‰², corresponding to nearly 54% for small eels and nearly 100% for large eels (Table 5, Fig. 6a). Small eels in Takase River had a larger niche area compared to eels of the same size class in Akugawa River, but also to large eels of both rivers. The SEAB of large eels was similar in both rivers (Fig. 6b). Total amount of occupied niche space (TA) was largest for small eels in Takase River as well as average isotopic variation (CD), whereas both values were lowest for small eels in Akugawa River. Values of CD and TA for large eels were similar in both rivers (Table 5).

Discussion

This study presents the first attempt to assess the trophic niche space occupied by Japanese eels and to compare the occupied niche space between different size classes of eels in two different habitats. A comparative analysis of ecological characteristics of Japanese eels between rivers was attempted by a comparative characterisation of river habitats and water quality. The analysis of stomach contents and stable isotopes showed trophic and isotopic niche overlap, to varying extent, between the two size classes of eels within each of the two study rivers. Stable isotopes indicated a broader trophic niche space and dietary diversity for small eels in Takase River than for those in Akugawa River. Furthermore, eels in Takase River showed a higher body condition than eels in Akugawa River.

Feeding habitat and body condition of eels

The body condition of eels in their growth habitats is crucial for their reproduction and only individuals with high lipid contents seem to be able to contribute to the spawning population (Belpaire et al. 2009). Abiotic and biotic habitat parameters are known to influence the body condition, a measure commonly used to assess the fitness of eels (Lasne et al. 2008; Itakura et al. 2015b).

The habitat assessment allowed a clear distinction between the abiotic and biotic characteristics of the two study rivers. The occurrence of the invasive macrophyte *Elodea nuttallii* and filamentous algae mats indicated eutrophication processes in Akugawa River (Hilton et al. 2006). The high nutrient contents, higher chl *a* concentrations of POM and BMA, the lower oxygen concentrations compared to Takase River, supported the idea of eutrophication in Akugawa River. Eels are known to tolerate various kinds of water pollution and the nutrient concentrations found in this study were considered as unproblematic (Kamstra

1996). Small eels were reported to have a higher oxygen demand in combination with higher temperatures (Degani 1989) but the oxygen concentrations found in this study did not fall below those required by anguillid fishes (Tesch 2003).

While natural shorelines were present at all sampling stations to a varying degree, concrete revetment was more dominant in Akugawa River, completely covering approximately the first 2.5 km from the river mouth. Furthermore, the concrete riverbed in this part of Akugawa River was covered almost entirely by silty substrate which provided unfavourable habitat conditions for small eels (Kume et al. 2019, 2020) entering the river. In addition, small grain sizes which eels smaller than 200 mm in TL preferred (Kume et al. 2019, 2020) was present at TAK1 but absent in all stations of Akugawa River. The absence of suitable substrate could have contributed to the low catch number of small eels in Akugawa River. Although small eels were observed to climb the weir downstream of AKU1 (Kume et al. 2022), it can be expected that the presence of the weir leads to a decrease of small eel density in upstream reaches of Akugawa River.

The higher nutrient concentrations in Akugawa River could have resulted in the higher biomass of potential prey organisms of eels collected by electric shocker, as crustaceans and fishes are the main food source for larger eels (Kaifu et al. 2013; Itakura et al. 2015b). A recent study found that high DIN concentrations support higher food source availability for eels and therefore promote survival and growth rate (Kasai et al. 2021). The lower body condition found for eels of both size classes in Akugawa River is contradicting to this finding. Acou et al. (2011) found that eel habitats showed a density-based mortality of eels which may have been the result of competition for space and food. Therefore, possible explanations for the lower body condition are intraspecific competition for food sources due to a higher biomass of large eels in Akugawa River, combined with limited competition avoidance possibilities due to limited suitable habitat for eels in a small river flowing through residential areas.

Dietary diversity

Japanese eels in rivers were commonly described as opportunistic feeders with focus on bottom-dwelling/ benthic prey organisms (Kaifu et al. 2013; Itakura et al. 2015b; Kutzer et al. 2020). Amongst the potential prey organisms sampled by electric shocker crustaceans accounted for the majority of the biomass in Akugawa River and represented the main food source of large eels in this river as well. Gobiid fishes were the main contributor to the biomass of shocker catches in Takase River and had the second highest %IRI, while crustaceans had the highest %IRI and contribution. According to relative abundance and relative frequency of

occurrence in stomach contents, small eels focused on aquatic insects as prey source as described by previous studies (Tesch 2003; Wasserman et al, 2012; Kutzer et al. 2020). The %IRI of small eels in Akugawa River supported these findings, while the diet of small eels in Takase River was dominated by small crabs rather than aquatic insects.

Generalist feeding strategies are defined by a high diversity of prey with low abundances of prey categories (Bridcut and Giller 1995). Diversity indices (H') of large eels diet in both rivers and small eels diet in Takase River reflected a relatively more diverse diet, while the index of small eels diet in Akugawa River represents a more specialized diet (Kroetz et al. 2017). These findings indicated that small eels in habitats with wider potential prey availability such as Takase River tended to utilize a wider spectrum of food sources, while scarcity of potential food sources for small eels in Akugawa River imposed a less diverse prey choice. The disparity in sample sizes of stomach contents from small and large eels in Akugawa and Takase Rivers could have influenced these findings as a larger sample size would be expected to lead to a higher dietary diversity, but the values for TA and CD (Layman et al. 2007a) supported the results from stomach contents. TA and CD differed remarkably between small eels of Takase and Akugawa rivers, indicating that small eels in Akugawa River had a more specialized/less diverse diet, while those in Takase River had a generalized/more diverse diet.

The range of $\delta^{13}\text{C}$ found for eels in Takase River indicted that they use a wide range of prey sources and rely on several primary producers, as $\delta^{13}\text{C}$ values increase only little through the trophic chain (Post 2002). Contributing to such a wide of $\delta^{13}\text{C}$, can be the utilization of marine or brackish water and terrestrial food sources. The latter were not specifically identified in eel stomachs, but could be underrepresented due to the snapshot characteristics of stomach content analysis and were found by Itakura et al. (2021) to be utilized by eels after rainfall. Glass eels arriving in inland waters present marine isotopic signatures, and while gathering in estuaries, they partially utilize those habitats as feeding grounds on their way to upstream habitats (Bardonnet and Riera 2005). Smaller eels collected in this study could have contributed to a wider range of isotopic signatures, as it was the case in Takase River. Feeding on prey species that migrated from marine or brackish water to freshwater habitats could also have contributed to a wider range of $\delta^{13}\text{C}$. On the contrary, the weir just downstream of AKU1 could have lessened the degree of small eel and prey migration from downstream habitats into the sampling stations. However, eels and migratory crustaceans (*Chiromantes dehaani*, *Eriocheir japonica*) can overcome the weir, based on Kume et al. (2022) and observations during sampling, respectively. The $\delta^{13}\text{C}$ values of eels in Akugawa River showed a smaller range that indicated reliance on fewer primary producers. The high $\delta^{15}\text{N}$ values of eels and possible food

sources in Akugawa River, especially those of Gastropoda were an indicator of inorganic nitrogen input, as described by previous studies (Hartland et al. 2011; Negishi et al. 2019).

Dietary overlap

The analysis of stomach contents and stable isotopes showed trophic and isotopic niche overlap between small and large eels in both rivers to a varying degree. Differences in the ecological niche, including food sources, foraging habitat and foraging time are the basis for the coexistence of similar species, which also applies for different development stages of the same species (Schoener 1974; Martin and Martin 2001). The isotopic niche overlap in Takase River is mainly caused by the broad niche area of small eels, which are restricted to small-sized food sources. Although the isotopic niche space of large eels in Takase River almost fully overlapped with that of small eels, large eels can feed on larger-sized prey organisms. The niche overlap in Akugawa River may impact small eels more as 60% of their isotopic niche space overlapped with that of large eels and due to the restriction to small prey organisms and the low biomass of aquatic insects. This could have contributed to the lower body condition of small eels in Akugawa River compared to small eels in Takase River.

Small rivers of urban and agricultural areas as targets for eel conservation and habitat restoration

Due to their ubiquitous presence in rivers, Japanese eels are a suitable surrogate species for riverine ecosystems (Itakura et al. 2020). Given their cultural and economic importance, they represent a suitable flagship species (Simberloff 1998) and their life-cycle and in-river movements make them a good indicator species for habitat connectivity, representing a broader spectrum of riverine fishes and the river habitat itself as umbrella species (Simberloff 1998; Itakura et al. 2020). Therefore, Japanese eel could play a versatile role in future river restoration and conservation projects. The rivers in this study were characterized by anthropogenic alterations, short lengths and a close or direct proximity to urban and agricultural areas. Both rivers were found to be suitable habitats to accommodate eels of various sizes and provide interesting study sites to apply and assess future conservation and restoration measures. Furthermore, the proximity to urban settlement would provide a social aspect, additionally to the ecological, as residents and recreationist would benefit from restoration efforts (Zingraff-Hamed et al. 2018).

The stable isotope assessment of eel tissue in this study provided a linkage between the trophic ecology of eels and the food/ energy sources utilized by eels in regard to the diversity

of prey sources consumed. Tracking the trophic ecology of wild eel populations with such methods would allow assessing the *status quo* of the population and tracking changes over time, in connection to environmental changes/ habitat degradation (Layman et al, 2007b; Quevedo et al. 2009), or river restoration efforts (Palmer et al. 2005), allowing the evaluation of the success of the measure and to allow a process-orientated, stepwise-adaptive management (Geist and Hawkins 2016).

Supplementary Information

The online version contains supplementary material available at -.

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Declarations

Conflict of interest: The authors declare that they have no conflicts of interest.

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Table 1 Characteristics of sampling stations in Akugawa (AKU) and Takase (TAK) Rivers

Station	River width (m)	Water depth (cm)	FV (m/s)	Substrate	BV (%)
AKU1	5	21	14.9	boulder	75
AKU2	3	25	10.9	cobble/boulder	100
TAK1	10	28	28.5	sand/gravel/boulder	75
TAK2	6	34	26.6	cobble/boulder	25
TAK3	6	29	31.8	cobble/boulder	75

FV = flow velocity, BV = bank vegetation

Table 2 Results of the habitat assessment (mean $\pm$ SD) pooled for all samplings in Akugawa and Takase Rivers

	Akugawa River	Takase River
Temperature ($^{\circ}$ C)	24.22 $\pm$ 2.11	19.8 $\pm$ 1.35
pH	7.01 $\pm$ 0.52	7.23 $\pm$ 0.68
EC (μ S/cm)	234 $\pm$ 7.61	144 $\pm$ 1.04*
DO (mg/l)	7.3 $\pm$ 0.19	9.62 $\pm$ 0.32*
DIN (mg/l)	0.20 $\pm$ 0.08	0.12 $\pm$ 0.06*
DIP (mg/l)	0.04 $\pm$ 0.01	0.01 $\pm$ 0.01*
chl <i>a</i> POM (μ g/l)	1.90 $\pm$ 0.66	0.34 $\pm$ 0.21*
chl <i>a</i> BMA (μ g/cm ²)	9.51 $\pm$ 4.03	6.78 $\pm$ 3.18*

* $p < 0.05$ in Mann-Whitney U-test. EC = electric conductivity, DO = dissolved oxygen concentration, DIN = dissolved inorganic nitrogen, DIP = dissolved inorganic phosphorous, chl *a* POM = chlorophyll *a* concentration of particulate organic matter, chl *a* BMA = chlorophyll *a* concentration of benthic microalgae

Table 3 Median values (minimum – maximum) of total length (TL), body weight (BW) and the sex ratio of small and large eels in Akugawa and Takase Rivers

River	Sizeclass	<i>n</i>	TL (mm)	BW (g)	Sex ratio (F:M:I)
Akugawa	all	49	354 (152 – 641)	56 (2.8 – 464)	16:19:15
	small	11	181 (152 – 240)	5 (2.8 – 18)	00:00:12
	large	38	377 (279 – 641)	76 (28 – 464)	15:19:03
Takase	all	45	149 (83 – 602)	4 (0.5 – 360)	07:04:38
	small	30	112 (83 – 231)	1 (0.5 – 19)	00:00:34
	large	15	331 (271 – 602)	47 (23 – 360)	07:04:04

n = sample size, F = female, M = male, I = undifferentiated

778 **Table 4** Stomach contents of small and large eels collected in Akugawa and Takase Rivers

U.category	Prey category	%N	%W	%F	%IRI	%N	%W	%F	%IRI
Akugawa River									
		Small eels (<i>n</i> = 7)				Large eels (<i>n</i> = 35)			
Crustacea		7.69	29.81	14.29	5.02	65.85	94.46	88.57	88.22
	Brachyura					21.95	27.61	25.71	20.48
	Atyidae	7.69	29.81	14.29	5.02	36.59	51.37	45.71	64.60
	Astacidae					3.66	14.57	8.57	2.51
	Others					3.66	0.91	8.57	0.63
Pisces						3.70	1.17	13.02	1.01
	Gobiid fishes					3.66	1.16	13.02	1.01
Insecta		92.31	70.19	100.00	94.98	25.61	2.23	25.71	9.81
	Diptera	76.92	51.48	71.43	85.86	24.39	2.15	22.86	9.75
	Trichoptera	15.38	18.71	28.57	9.12				
	Others					1.22	0.08	2.86	0.06
Others						4.94	2.14	8.82	1.00
	Oligocheata					4.88	2.14	8.57	0.97
Takase River									
		Small eels (<i>n</i> = 19)				Large eels (<i>n</i> = 8)			
Crustacea		31.43	80.20	57.89	70.47	40.00	78.35	50.00	71.64
	Brachyura	25.71	79.42	47.37	69.99	40.00	78.35	50.00	71.64
	Atyidae	2.86	0.12	5.26	0.22				
	Others	2.86	0.65	5.26	0.26				
Pisces						26.67	20.73	37.50	21.52
	Gobiid fishes					26.67	20.73	37.50	21.52
Insecta		62.86	19.29	63.16	29.33	20.00	0.14	10.53	2.57
	Diptera	40.00	15.95	31.58	24.83				
	Ephemeroptera	11.43	0.54	10.53	1.77	20.00	0.14	10.53	2.57
	Trichoptera	8.57	2.80	15.79	2.52				
	Others	2.86	<0.01	5.26	0.21				
Others		5.71	0.51	10.53	0.22	13.33	0.78	25.00	4.27
	Oligocheata	2.86	0.11	5.26					
	Polychaeta	2.86	0.40	5.26	0.22				
	Gastropoda					13.33	0.78	25.00	4.27

779 U.category = upper category, %N = percentage numerical composition, %W = percentage of wet weight
780 composition, %F = percentage frequency of occurrences in eel stomachs, %IRI = index of relative importance, *n*
781 = number of stomachs with content
782

783 **Table 5** Food web metrics for eels derived from stable isotope data including mean $\delta^{15}\text{N}$ (‰) and $\delta^{13}\text{C}$ (‰) $\pm$ SD

River	Size class	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	TA	CD	SEA _B	SEA _C	SEA _C overlap
Akugawa	Small	11.76 $\pm$ 0.64	-23.54 $\pm$ 0.63	2.86	0.70	1.19	1.32	0.81
Akugawa	Large	12.69 $\pm$ 1.25	-23.20 $\pm$ 0.87	10.51	1.40	3.06	3.15	
Takase	Small	10.55 $\pm$ 1.16	-19.29 $\pm$ 2.02	24.01	1.88	5.90	6.11	3.32
Takase	Large	10.56 $\pm$ 1.06	-19.41 $\pm$ 0.96	8.13	1.29	3.21	3.45	

784 Total area of convex hull = TA, mean distance to centroid = CD, standard ellipse area = SEA_B, standard ellipse corrected for sample size = SEA_C, SEA_C overlap = SEA_C overlap
785 between size classes within a river

FIGURE CAPTIONS

Fig. 1 Study area in Wakayama Prefecture, Japan: Akugawa (AKU) and Takase (TAK) River with two and three sampling stations (black circles), respectively and weirs (black bars)

Fig. 2 Biomass (%) of prey categories sampled by surber sampler (a) and electric shocker (b) in Akugawa ($n = 4$ and 4 , respectively) and Takase rivers ($n = 6$ and 6 , respectively)

Fig. 3 Non-metric multidimensional scaling (NMDS) ordination plots of potential prey category densities of surber sampler (a) and electric shocker (b) catches in Akugawa (open squares, $n = 4$ and 4 , respectively) and Takase Rivers (solid squares, $n = 6$ and 6 , respectively) with fitting environmental variables as predictors (arrows). Only environmental variables with a significant effect ($p < 0.05$) are shown. Abbreviations are shown in Table 2

Fig. 4 Log-transformed length-weight relationship of small and large eels collected in Akugawa and Takase Rivers.

Fig. 5 The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope bi-plot for individual eels of both size classes in Akugawa River (a) and Takase River (b). Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ($\pm$ SD) of potential prey are included and are adjusted by the trophic discrimination factor ($\delta^{13}\text{C} = 0.8\text{‰} \pm 0.8\text{‰}$ and $\delta^{15}\text{N} = 2.1\text{‰} \pm 0.5\text{‰}$; Kaifu et al., 2013)

Fig. 6 Sample size-corrected standard ellipse area (SEA_C) calculated from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for eels in Akugawa River (small eels: blue, large eels: purple) and Takase River (small eels: yellow, large eels: green) (a). Bayesian estimates of the standard ellipse area (SEA_B). Ellipse area plots represent 50, 75 and 95 % credible intervals with SEA_B mode indicated by a black circle and SEA_C mode by a red cross (b)

1 Fisheries Science

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3 Article title: Trophic ecology of Japanese eels in small rivers of urban and agricultural areas

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Online resources

S1: Sampling station AKU2 in Akugawa River and sampling station TAK2 in Takase River (from left to right)



S2: Number of eel catches by station and sampling month in 2018 in Akugawa and Takase River. Numbers in parentheses indicate the ratio of small eels (≤ 240 mm total length) and large eels (> 240 mm total length).

River	Station	<i>n</i>	June	October
Akugawa	1	22 (2:20)	16 (1:15)	6 (1:5)
	2	27 (9:18)	8 (3:5)	19 (6:13)
	1	36 (27:9)	7 (5:2)	29 (22:7)
Takase	2	5 (1:4)	4 (1:3)	1 (0:1)
	3	4 (2:2)	2 (2:0)	2 (0:2)

n = sample size

S3: Results of the analysis of co-variance for the log-transformed length-weight relationship of eels. The first model (M1) tested the homogeneity of slopes amongst size classes of eels in Akugawa and Takase River. Model (M2) compared the γ -intercept of the mass-length relation amongst size classes of eels in both rivers

Model	<i>F</i>	d.f.	<i>p</i> -value
M1 (test for interaction)			
ln(TL)	48023	1	<0.001
group (size class + river)	52.7	3	<0.001
ln(TL) $\times$ group	2.77	3	0.06
M2 (no interaction)			
ln(TL)	45607	1	<0.001
Group	50.05	3	<0.001

TL = total length

S4: Results of Pianka's niche overlap for eels of both size classes in Akugawa (AKU) and Takase (TAK) River

Eels		Niche overlap	<i>p</i> -value
AKU_small vs AKU_large	%W	0.44	0.007
	%N	0.55	0.007
TAK_small vs TAK_large	%W	0.95	< 0.001
	%N	0.47	0.056

%W = percentage of wet weight composition, %N = percentage numerical composition

S5: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of potential food sources of eels (median [minimum – maximum]) collected in Akugawa and Takase River

River	Food source	<i>n</i>	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Akugawa	Gobiid fishes	3	-22.94 (-25.20 to -22.33)	13.92 (13.60 – 14.96)
	Largemouth bass	6	-25.62 (-26.23 to -24.24)	9.73 (9.36 – 10.04)
	Other fishes	5	-24.32 (-24.69 to -22.87)	12.07 (9.30 – 12.64)
	Crabs	13	-23.74 (-26.24 to -21.86)	9.58 (6.19 – 12.15)
	Shrimp	21	-24.74 (-26.58 to -21.43)	10.38 (6.92 – 12.80)
	Gastropoda	15	-23.68 (-25.00 to -20.54)	12.87 (11.34 – 14.93)
	Aquatic insects	11	-24.46 (-25.73 to -23.38)	7.45 (5.98 – 8.59)
Takase	Gobiid fishes	20	-18.72 (-22.49 to -15.35)	7.78 (5.84 – 11.51)
	Ayu	12	-16.90 (-19.04 to -14.92)	6.81 (5.33 – 8.02)
	Other fishes	12	-20.17 (-23.80 to -14.92)	8.13 (6.03 – 9.33)
	Crabs	9	-20.17 (-21.55 to -18.39)	6.78 (5.85 – 9.23)
	Shrimp	22	-19.96 (-25.23 to -17.08)	7.19 (4.82 – 9.32)
	Gastropoda	10	-17.95 (-19.14 to -15.96)	6.61 (5.00 – 7.84)
	Aquatic insects	16	-21.08 (-22.95 to -16.58)	2.21 (1.32 – 4.42)

n = sample size











