

# Large contribution of pulsed subsidies to a predatory fish inhabiting large stream channels

Hikaru Itakura, Yoichi Miyake, Takashi Kitagawa, Takuya Sato, and Shingo Kimura

**Abstract:** Resource subsidies exert critical influences on recipient habitats with relatively higher perimeter-to-area ratios, such as headwaters in watersheds. However, little is known about how those subsidies contribute to the energy sources in recipient habitats where the perimeter-to-area ratio is low, such as large stream channels. Here, we show that the diet of small Japanese eels (*Anguilla japonica*) <500 mm in total length inhabiting natural shoreline areas in large stream channels consists largely of terrestrial earthworms (*Metaphire* spp.). Stable isotopic analyses showed that the earthworms were the prey animal that contributed most to the eels' diet (45%–47%). Earthworms constituted the largest portion of the eels' stomach contents (7%–93%). Eels ingested earthworms within 2 days after rainfall during spring, summer, and autumn, and their consumption increased as the precipitation increased. These findings indicate that the pulsed earthworm subsidy that is driven by rainfall could temporarily bias the eels' diet toward this allochthonous resource, which may explain the large contribution of the subsidy for consumers inhabiting large stream channels. Furthermore, diverse earthworm species could drive multiple pulsed subsidies across seasons and provide the predators with a prolonged subsidy, enhancing the long-term contribution of the subsidy to the predators' diet.

**Résumé :** Les apports de ressources exercent des influences clés dans les habitats récepteurs caractérisés par des rapports périmètre:superficie relativement importants, comme les cours supérieurs de bassins versants. Les connaissances sur la contribution de ces apports aux sources d'énergie dans les habitats récepteurs où le rapport périmètre:superficie est faible, comme les chenaux de grands cours d'eau, sont toutefois limitées. Nous démontrons que le régime alimentaire de petites anguilles du Japon (*Anguilla japonica*) de moins de 500 mm de longueur totale vivant le long de rives naturelles de chenaux de grands cours d'eau est majoritairement constitué de petits lombrics terrestres (*Metaphire* spp.). Des analyses d'isotopes stables montrent que les lombrics constituent les proies animales les plus importantes dans le régime alimentaire des anguilles (45 % – 47 %) et représentent la plus grande proportion de leur contenu stomacal (7 % – 93 %). Les anguilles ingèrent des lombrics dans les deux jours suivant une pluie au printemps, à l'été et à l'automne, et la consommation de lombrics augmente parallèlement aux précipitations. Ces constatations indiquent que les apports épisodiques de lombrics modulés par la pluie pourraient biaiser provisoirement le régime alimentaire des anguilles vers cette ressource allochtone, ce qui pourrait expliquer l'importante contribution de cet apport pour les consommateurs vivant dans les chenaux de grands cours d'eau. En outre, différentes espèces de lombrics pourraient produire de multiples épisodes d'apport au fil des saisons et fournir aux prédateurs un apport soutenu, rehaussant la contribution à long terme de l'apport au régime alimentaire des prédateurs. [Traduit par la Rédaction]

## Introduction

Local habitats are linked tightly by reciprocal energy subsidies from contiguous habitats, and across-habitat transfers of both materials and organisms frequently have key effects on local consumers and their communities (Polis et al. 1997; Nakano and Murakami 2001). In particular, the effects of subsidies are thought to be strongest where recipient habitats have high perimeter-to-area ratios (hereinafter referred to as PAR), such as headwater streams surrounded by riparian forests and small islands encompassed by ocean (Polis and Hurd 1996; Polis et al. 1997; Marczak et al. 2007). However, the effects of subsidies are related not only

to the PAR of habitats, but also to the ratio of subsidy resources to equivalent resources in the recipient habitat (Marczak et al. 2007). Pulsed resource subsidies infrequently occur with short duration (Holt 2008; Yang et al. 2008), but can temporally increase the ratio of the subsidy resources to the equivalent resources in the recipient habitat, which may explain the effective input of resource subsidies to habitats with low PAR. Despite there being ubiquitous evidence for the positive effects of resource subsidies on consumers in habitats with high PAR (Polis et al. 1997), little is known about either how the subsidies contribute to the energy source in recipient habitats with low PAR or how they are supplied to these habitats.

Received 2 January 2020. Accepted 15 September 2020.

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Within a river system, riverine PAR decrease continuously from the headwaters to the river mouth; thus, riverine food webs often shift from a reliance on allochthonous energy in the headwaters to a reliance on autochthonous production in the lower reaches (Vannote et al. 1980; Rosi-Marshall and Wallace 2002). For example, in a headwater where the PAR is relatively high, the input of allochthonous plant detritus from riparian forests represents an important energy source of stream production (Naiman and Décamps 1997), and inputs of terrestrial invertebrates, in particular, are known to be an important energy subsidy for stream consumers, including fish (Kawaguchi and Nakano 2001; Utz and Hartman 2007; Sato et al. 2011; Syrjänen et al. 2011). Contrary to those examples, an autochthonous energy source appears to be a major component in large stream channels such as the lower reaches of rivers, where the PAR are low (Vannote et al. 1980; Rosi-Marshall and Wallace 2002). Although consumers in the large stream channels can also receive various types of subsidies from the upper reaches or riparian environments (Polis et al. 1997), the contribution of terrestrial resources to consumers in the large stream channels is poorly understood.

Terrestrial earthworms are highly abundant in soils throughout basins (Tsukamoto 1986; Stewart 2004). Earthworms emerge seasonally from the soil to the surface of the ground both during and after rainfalls (Ohno 2007), and there can be large quantities of earthworms in rivers (Kobayashi et al. 2015). Furthermore, earthworms are found from stomachs of fishes such as salmonids and anguillid eels during flood seasons (e.g., Warlow and Oldham 1982; Ryan 1986; Jellyman 1989; Kawaguchi et al. 2007; Itakura et al. 2015). These facts indicate that earthworm inputs can be regarded as a pulsed resource subsidy that is driven strongly by rainfall. When the pulsed earthworm subsidy is available, fishes may shift their dietary intake temporarily in favor of the earthworms, which may represent an important energy subsidy for predatory fishes inhabiting large stream channels.

It has been reported that anguillid eels feed heavily on earthworms in various habitats, not only in the upper reach of rivers (Denoncourt and Stauffer 1993), but also in the lower reaches of rivers, in lakes, and in lagoons (Jellyman 1989; Bouchereau et al. 2009; Van Liefferinge et al. 2012; Itakura et al. 2015). Jellyman (1989) found that earthworms constituted more than 50% by weight of the food of the *Anguilla australis* and *Anguilla dieffenbachii* in a lake during the flood season. Earthworms are also a common food item for Japanese eels (*Anguilla japonica*) in temperate rivers (Itakura et al. 2015; Wakiya and Mochioka 2020). However, since the results of these previous studies were based solely on stomach content analysis of eels obtained from samplings over one to few months, the seasonality (i.e., frequency) and the importance of earthworm inputs as an energy subsidy on eels are still unclear. Given that earthworms can be provided as a pulsed subsidy for riverine environments, evaluating snapshots of eels feeding on earthworms based solely on stomach content analysis can lead to underestimating the contribution of earthworms to the food utilization of eels. Thus, we also used carbon and nitrogen stable isotopic ratios, which are very effective in enabling discrimination between allochthonous and autochthonous production in riverine food webs (Finlay 2001). A combination of stomach content and stable isotope analyses hence can help in evaluating the food utilization of eels over longer periods (Vander Zanden et al. 2015).

In this study, by examining the food utilization of *A. japonica* via a combination of stomach content and stable isotope analyses throughout the seasons in multiple years, we first show that the diet of small eels (<500 mm in total length) inhabiting natural shoreline areas (i.e., lacking in revetments) in the large stream channels depends largely on terrestrial earthworms. Our aim was to estimate the relative contributions from both aquatic and terrestrial origins, including the specific contribution of terrestrial earthworms (*Metaphire* spp.) to the body tissue of *A. japonica*

inhabiting the lower reaches of a large river. We also tested whether (i) eels feed on terrestrial earthworms either during or after rainfall (i.e., timing), (ii) increase of precipitation promotes changes in eels' consumption of earthworms (i.e., magnitude), and (iii) the eels feed on earthworms throughout the seasons, based on the detailed analysis of the eels' stomach contents. By combining the stomach analysis data with isotope analysis, we explore and discuss the possibility that the multiple resource pulses driven by the diversity of earthworms prolongs the contribution of terrestrial subsidies for eels inhabiting environments in large stream channels with low PAR.

## Materials and methods

### Study species

*Anguilla japonica* spawn in the waters west of the Mariana Islands located in the western North Pacific Ocean (Tsukamoto et al. 2011), and their larvae drift westwards to growth habitats in East Asia. After metamorphosing into glass eels (early juvenile phase), they migrate to brackish and freshwater habitats, in which they remain as growth-phase yellow eels. They then grow in a wide range of habitats within rivers, from brackish estuaries to upland headwaters, lakes, and inner bays (Yokouchi et al. 2008; Kaifu et al. 2010; Itakura et al. 2019), preying on a wide range of aquatic animals, especially benthic species and small fishes (Kaifu et al. 2013; Itakura et al. 2015). Yellow eels are generally nocturnal (Itakura et al. 2018), tending, during daytime, to either hide in refuges such as holes and crevices or burrow into mud (Aoyama et al. 2005). After ~10 years' growth, they metamorphose into reproductive-stage silver eels (Yokouchi et al. 2009), following which they migrate from the rivers and estuaries to their spawning areas. In East Asia, *A. japonica* are a commercially and ecologically important species, but they have been classified as Endangered on the IUCN Red List of Threatened Species because of a marked decline in their abundance (Jacoby and Gollock 2014).

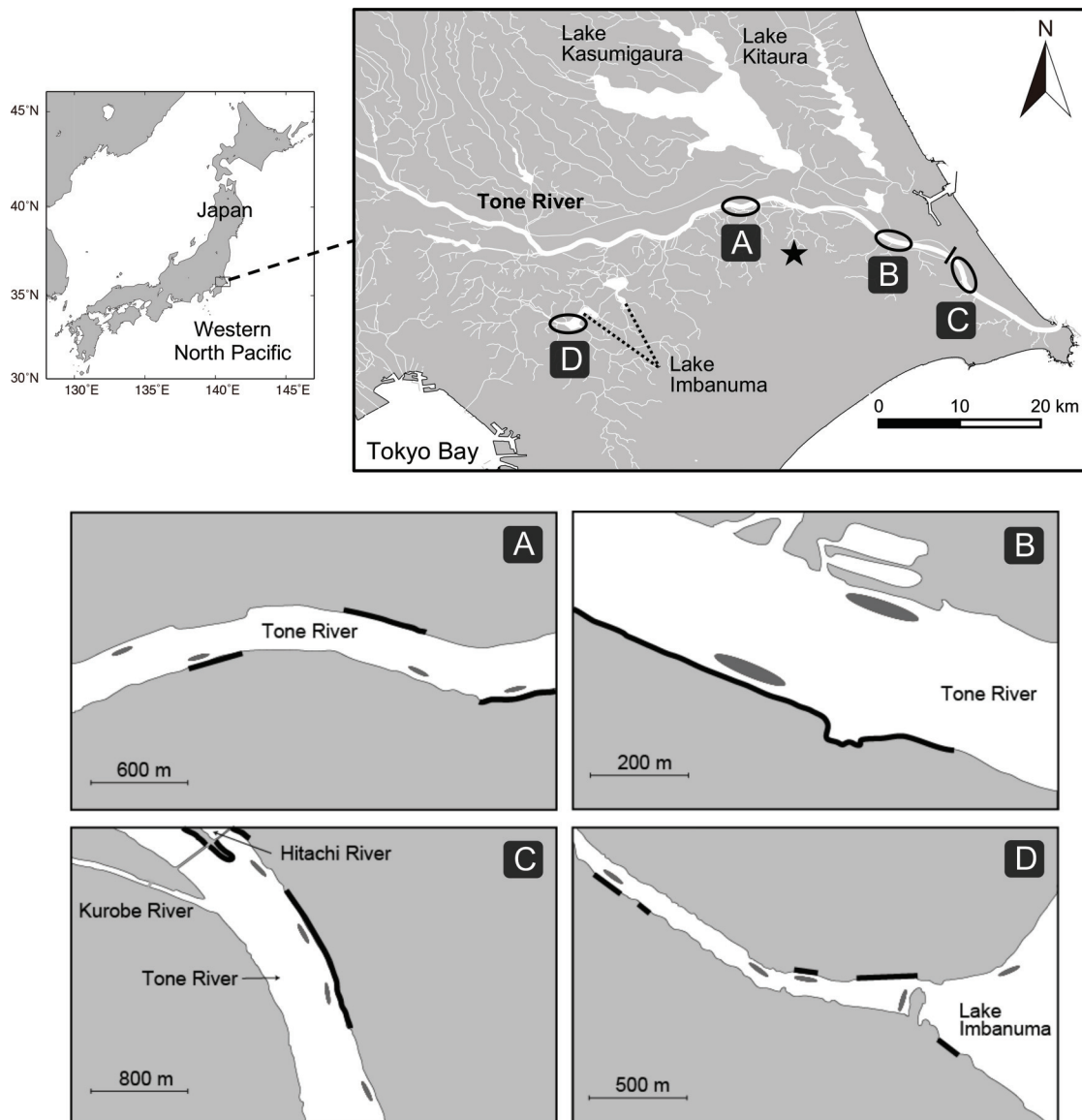
### Study area

This study was conducted in the lower reaches of the watershed of Japan's Tone River, which empties into the Pacific Ocean (Fig. 1). The Tone River has the second-longest stream length (322 km) and the largest basin area (16 840 km<sup>2</sup>) in Japan. Fresh water and brackish water are separated by the Tone River Estuary barrage, which is located 18.5 km upstream from the river mouth and is equipped with fish ladders.

Four sampling sites (A to D) were set in the lower reaches of the watershed (Fig. 1; see Itakura et al. 2015 for details about environmental conditions). Sites A, B, and C were located in the main stream, whereas site D was located in Lake Imbanuma. Sites A, B, and D were located in the freshwater nontidal habitats, and site C was located in the area of brackish water below the tidal barrage. Depth ranges of study sites A, B, C, and D were 1.3–5.1, 1.9–3.6, 1.3–2.0, and 0.8–1.0 m, respectively (Itakura et al. 2015). The PAR for a given river reach was calculated for each sampling site by dividing the perimeter (i.e., length of both right and left banks in metres) by the water surface area of the reach (m<sup>2</sup>). As a result, the PAR values of study sites A, B, C, and D were 0.0056, 0.0037, 0.0032, and 0.0099, respectively, all of which were several orders of magnitude lower than those of previous studies that included PARs (1–32; Polis and Hurd 1996; Witman et al. 2004).

At each sampling site, the riverbank consists of both revetment and natural shore areas; in this study, eels were sampled from seven revetment areas and eight natural shore areas. We defined a revetment as an artificial shore that is covered with materials such as concrete and poling board, and we defined other bank types as natural shore. Riverbanks of natural shore areas in sites C and D consist of vegetation, and those of sites A and B also include exposed soil. The bottom sediments consist of fine soil and fine-grained materials in sites A and D, fine-

**Fig. 1.** Maps of the study areas and of each sampling site in the lower reaches of the Tone River watershed in Japan where yellow-phase Japanese eels (*Anguilla japonica*) were collected. The open ovals denote sampling sites (A–D). The star denotes the location of the observatory for determining the amount of rainfall (Katori station). The black line crossing rivers denotes the Tone River Estuary barrage. The bold lines denote revetment shorelines, and the remaining shorelines are natural shore banks. The gray ovals in front of shorelines denote the approximate locations of the sampling areas where traps were set for catching eels. Maps were created using Generic Mapping Tools and QGIS, and the river, lake, and coastline data were acquired from the Geospatial Information Authority of Japan, Ministry of Land, Infrastructure, Transport and Tourism of Japan (<http://nlftp.mlit.go.jp/ksj/>).



grained materials in site B, and sand and fine soil in site C (Itakura et al. 2015).

### Sampling

Sampling was conducted from June 2011 to September 2013 at sites A, B, and D and from August 2012 to September 2013 at site C. Eels were collected using refuge traps consisting of two cylinders that were open at both ends. Thirty traps were attached to a rope at intervals of ~5 m, and ropes were then set longitudinally in the waters at distances of approximately <1 m (site D) to 50 m (sites A, B) from shore at the freshwater sites, and at ~80 m from

shore at the brackish water site (site C), because of the tidal range, which exposes the nearshore areas at low tide. Within each sampling site, the distance between revetment and natural shore areas was consistent. In all the sites, 450 traps were deployed at 15 sampling areas to constantly remain in the river or lake throughout the study period, and they were checked periodically for eels. At each sampling site, the deployed traps were sampled using a boat twice each month, at ~2-week intervals, during daytime, mainly in the morning. At all sites, potential prey organisms for eels were collected using set nets and refuge traps and via direct sampling for stable isotopic analysis (see online Supplementary Table S1<sup>1</sup>). The

<sup>1</sup>Supplementary data are available with the article at <https://doi.org/10.1139/cjfas-2020-0004>.



sampling was conducted both under the guidance and with the permission of the Fisheries Adjustment Rules of Ibaraki Prefecture.

Captured eels and their potential prey animals were euthanized immediately on the boat by holding them in ice water, according to the methods of Itakura et al. (2015) to satisfy both national and institutional standards, and they were stored at  $-20^{\circ}\text{C}$  until they were examined. All the eels captured were dissected for measurement and diet analysis. The total length of each eel was measured to the nearest 1 mm, and the body weight was measured to the nearest 0.1 g. The growth stages of eels (yellow eel or silver eel) were confirmed from body and pectoral fin colour, in accordance with the silvering index (Okamura et al. 2007). We excluded one silver eel from the study, because it may have already begun its early migration to the ocean to spawn. In all, 554 yellow eels were captured during the study period.

### Stomach content analysis

As eels forage mainly at night (Kaifu et al. 2013; Itakura et al. 2015), only eels captured before noon ( $n = 518$ ) were used for analysis of stomach content to avoid underestimating the easily digestible prey organisms. About half of captured eels had empty stomachs (Itakura et al. 2015), and the remaining 226 eels were used for analysis. Stomach contents were identified to the lowest possible taxonomic levels and were categorized into seven prey-type categories (Table S2<sup>1</sup>): aquatic annelid (sandworm (*Hediste atoka*) and leech (*Limnotrachelobdella okae*)), aquatic insecta (Chironomidae (*Chironomus plumosus*), Ephemeroptera (*Ephoron eophilum*), and dragonfly (*Pseudothemis zpnata*) larvae), bivalve (mostly *Limnoperna fortunei*), crustacean (shrimp (*Macrobrachium nipponense* and *Palaemon paucidens*), crayfish (*Procambarus clarkii*), and crab (*Eriocheir japonica*)), fish (species unidentified), oligochaete (earthworms (*Metaphire* spp.)), terrestrial Insecta (Lepidoptera larva (*Streltzoviella insularis*)), and unidentified material (Itakura et al. 2015). The food items found in each eel were weighed to the nearest 0.1 g.

### Stable isotope analysis

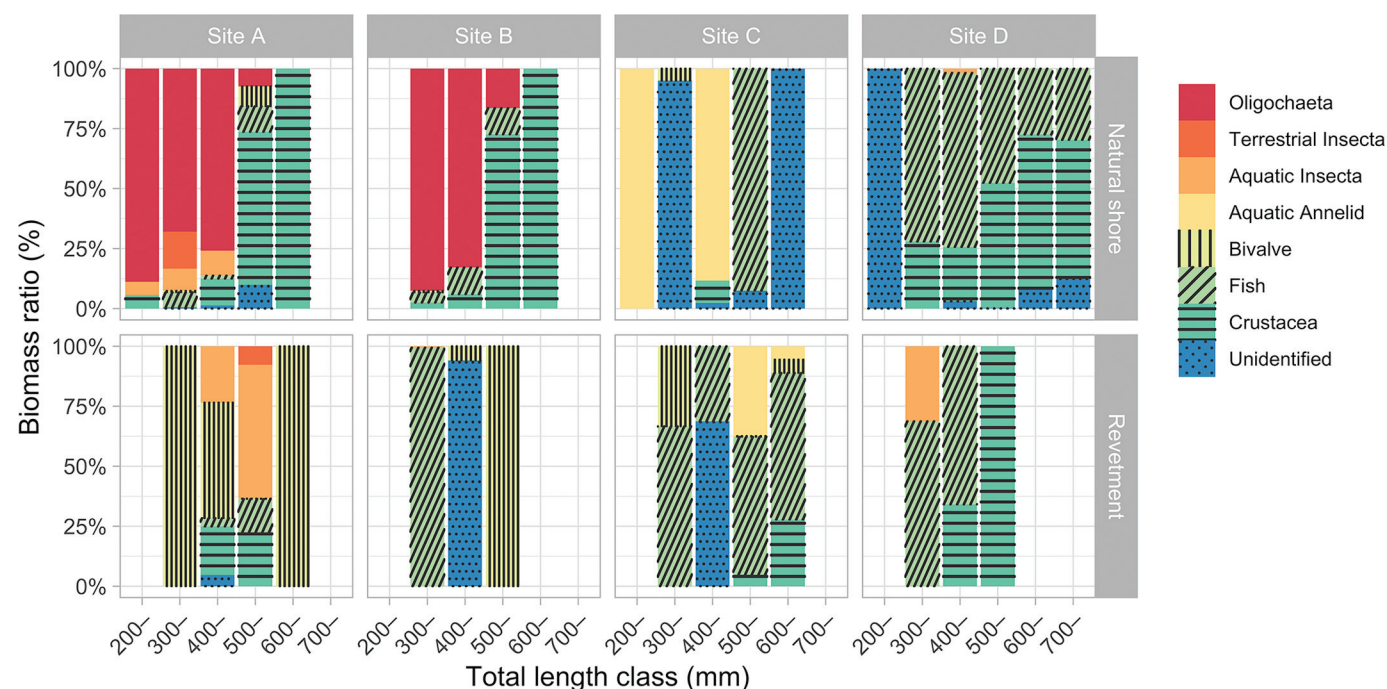
All the captured eels and their potential prey animals were used for stable isotope analysis (see Supplementary material, Table S1 and Fig. S1<sup>1</sup>). The potential prey animals used were collected either by additional sampling or from the stomach contents of the eels. The muscle tissues of these animals were used, as muscle has slow turnover rate, providing a history of food assimilation over a period of months and thereby excluding short-term variability (Guelinckx et al. 2007). All samples were dried in an oven at  $60^{\circ}\text{C}$  for 24–48 h and ground to a fine powder using a mortar. Lipids were removed from half of the ground samples using 1:1 chloroform–methanol solution (v/v) and centrifugation (Folch et al. 1957). The lipid-removed samples were then oven-dried once more, after which 0.5–1.0 mg of each sample was sealed in a tin capsule. The other half of the ground samples was also sealed into a tin capsule without lipid extraction (treatments). As the lipid-removed samples for fish had higher  $\delta^{15}\text{N}$  than did the treatments (Sotiropoulos et al. 2004; Mintenbeck et al. 2008), we used the treatments for  $\delta^{15}\text{N}$  and the lipid-removed samples for  $\delta^{13}\text{C}$ . Analysis of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  was performed using an elemental analyzer (FLASH 2000, Thermo Electron, Italy) interfaced with a mass spectrometer (Delta V advantage, Thermo Finnigan, Germany) via a ConFloIV open split interface (Thermo Finnigan, Germany). The isotope ratios were expressed as per mille (‰) deviation, according to the international standard of Vienna Pee Dee Belemnite for carbon and atmospheric  $\text{N}_2$  for nitrogen, in which  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$ , where  $R = {}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{15}\text{N}/{}^{14}\text{N}$ . Repeated analysis of the standards yielded an external reproducibility of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measurements of better than  $\pm 0.15\text{‰}$ .

### Statistical analysis

All statistical analyses were conducted using R 3.6.0. To determine how rainfall and its seasonality affected eels feeding on earthworms, we used a generalized additive model (GAM; gam in the package mgcv; Wood 2019), which included either the presence or the absence of earthworms in the stomach contents of each eel (i.e., 1 or 0, respectively) as a response variable and the number of days after rainfall, the number of days from 1 January (hereinafter referred to as seasonality), daily precipitation (mm), total length of each eel, and the interaction term between the number of days after rainfall and seasonality as predictor variables. Moreover, to determine how rainfall affected the eels' consumption of earthworms, we also used a GAM, which included the weight of earthworms in stomach contents of each eel (i.e., eels' consumption of earthworms) as a response variable; the daily precipitation, seasonality, total length of each eel, and the interaction term between the daily precipitation and seasonality as predictor variables; and the body weight of each eel as an offset term. In the model, we used body weight of each eel as an offset term to standardize eels' consumption of earthworms, whereas total length of each eel was used as a predictor variable to test ontogenetic shifts of feeding on earthworms by eels. All predictor variables in these analyses were used as a spline function. We used a binomial distribution for the response variable of the first model with a logit-link function and a gamma distribution for the second model with a log-link function. As the earthworms were found only in the stomachs of eels that were collected in three natural shore areas of the freshwater sampling sites (two areas in site A and one area in site B; Itakura et al. 2015), only the data collected from these areas were used. The daily rainfall data at the Katori station (Fig. 1), which was the closest observatory to both sites A and B, was provided by the Japan Meteorological Agency (<https://www.data.jma.go.jp/obd/stats/etrn/index.php>). The models were assessed through considering values of the Akaike's information criterion (AIC); those with  $\Delta\text{AIC} < 2$  were chosen for descriptive purposes (Burnham and Anderson 2002). We carried out the model selections using the dredge function in the package MuMIn (Bartoń 2019). Then, we evaluated whether zero was included in the 95% confidence interval of the coefficients (Wald statistics) of the explanatory variables that were selected by the lowest AIC models (i.e., the best models) using Wald tests.

To ascertain the contribution of each prey animal to the eels' diet, we used a Bayesian isotopic mixing model (MixSIAR package; Stock and Semmens 2016), which provides a valuable way of allocating the relative contributions of more than two sources to obtain potential dietary solutions as true probability distributions. We applied an a posteriori aggregation method to combine food sources sharing common attributes such as same taxa following recommendations in Phillips et al. (2005) for meeting the assumptions of the mixing model analysis. All fish species were combined into one food item as "Fish", while *M. nipponense* and *P. paucidens* were combined into one food item as "Shrimp". This aggregation would reduce statistical uncertainty of diet estimation that can arise from having too many possible sources. The results of the mixing model depend on the trophic enrichment factor (TEF) values for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between food sources and consumer tissue. We set the TEF values at  $0.8\text{‰} \pm 0.8\text{‰}$  (mean  $\pm$  SD) and  $2.1\text{‰} \pm 0.8\text{‰}$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively, per trophic level, following Kaifu et al. (2013), who estimated unique TEF values of reared yellow-phase *A. japonica*. We did not use  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for prey animals that were either not found or were found at low frequency in stomach content analyses for the mixing model. In the study watershed, yellow eels have been reported to have a narrow home range and to exhibit strong fidelity to a particular site; they tended to be distributed on one particular side of the river (right or left bank) and in one particular shore type (revetment or natural shore) and changed these preferences rarely

**Fig. 2.** Stomach contents of yellow-phase Japanese eels (*Anguilla japonica*), collected in the Tone River watershed, Japan, shown as biomass ratio (%) per each total length (mm) class. [Colour online.]



(Itakura et al. 2018). Moreover, there were considerable differences in the stomach content compositions of yellow eels from the different shore types of sampling areas in each site (Itakura et al. 2015). Consequently, we calculated the rates of contribution of each prey animal to the diet of eels per each sampling area at each site using biomass ratios of the stomach contents corresponding to each sampling area as informative priors following previous studies (Moore and Semmens 2008; Stock et al. 2018). The model was run under the “long” setting with the following parameters: 300 000 chain length, 200 000 burn-in, and 100 thinning for three parallel Markov chain Monte Carlo (MCMC) chains.

The trophic levels of eels were estimated using the Bayesian model (tRophicPosition package; Quezada-Romegialli et al. 2018). In the model, the trophic levels were calculated using the following equations (Post 2002; Quezada-Romegialli et al. 2018): trophic level =  $(\{\delta^{15}\text{N}_{\text{consumer}} - [\delta^{15}\text{N}_{\text{base1}} \times \alpha + \delta^{15}\text{N}_{\text{base2}} \times (1 - \alpha)]\} / \Delta\delta^{15}\text{N}) + 2$ , and  $\alpha = \{[\delta^{13}\text{C}_{\text{base2}} - (\delta^{13}\text{C}_{\text{consumer}} + \Delta\delta^{13}\text{C})] / (\text{trophic level} - 2)\} / (\delta^{13}\text{C}_{\text{base2}} + \delta^{13}\text{C}_{\text{base1}})$ , where  $\delta^{15}\text{N}_{\text{consumer}}$  and  $\delta^{13}\text{C}_{\text{consumer}}$  are the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of the consumer, respectively;  $\delta^{15}\text{N}_{\text{base1}}$ ,  $\delta^{13}\text{C}_{\text{base1}}$ ,  $\delta^{15}\text{N}_{\text{base2}}$ , and  $\delta^{13}\text{C}_{\text{base2}}$  are the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of baselines 1 and 2, respectively;  $\Delta\delta^{15}\text{N}$  and  $\Delta\delta^{13}\text{C}$  are the TEF for nitrogen and carbon, respectively; and the value 2 denotes the trophic level of the organism (primary consumers) used to establish the baselines. The TEF was set at  $0.8\text{‰} \pm 0.8\text{‰}$  and  $2.1\text{‰} \pm 0.8\text{‰}$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively (Kaifu et al. 2013). We considered that eels acquire sources from both aquatic and terrestrial food webs (see Results). Japanese eels inhabiting rivers primarily belong to the littoral food web (Kaifu et al. 2013). Thus, we used the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of Viviparidae (*Bellamyia* spp.) for  $\delta^{15}\text{N}_{\text{base1}}$  and  $\delta^{13}\text{C}_{\text{base1}}$  as the end-member of the aquatic (littoral) food web, while we used those of earthworm for  $\delta^{15}\text{N}_{\text{base2}}$  and  $\delta^{13}\text{C}_{\text{base2}}$  as the end-member of the terrestrial food web, because these animals are primary consumers (trophic level = 2), with protracted isotopic turnover rates integrating spatial-temporal variability (Cabana and Rasmussen 1996). We calculated the trophic levels of animals collected from sites A and B, because the diet of eels inhabiting these

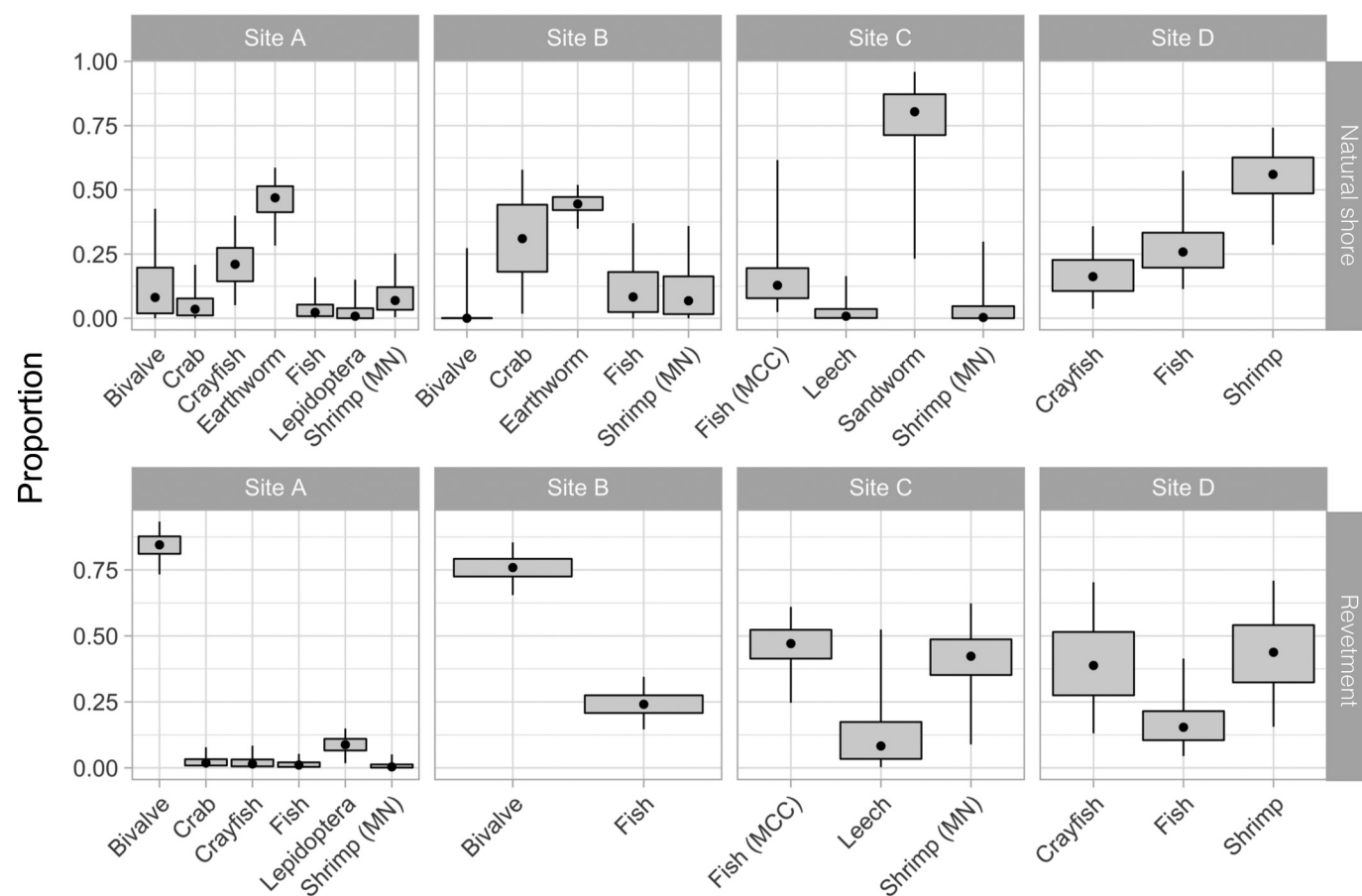
sites depend largely on terrestrial animals (see Results). The model was run under the following parameters: 20 000 adaptive samplings, 20 000 iterations, 20 000 burn-in, and 10 thinning for five parallel MCMC chains.

## Results

### Relative contribution of earthworms to eels' stomach contents

The stomach content compositions of *A. japonica* collected in the Tone River watershed differed substantially by total length or shore types of sampling areas (Fig. 2; Supplementary Table S2<sup>1</sup>). For smaller eels in the natural shore areas of the freshwater river sites A and B, the most dominant food item was terrestrial earthworms, accounting for 88.9%, 67.9%, 75.8%, and 7.4% of the food items for eels in the total length size classes 200, 300, 400, and 500 mm in site A and 92.6%, 82.8%, and 16.4% of the food items for eels in the size classes 300, 400, and 500 mm in site B, respectively. However, the ratios of earthworms accounting for eels' food items tended to be lower in larger size classes. On the other hand, no earthworms were found in the stomachs of eels in the revetment areas of these sites. In the natural shore area of the brackish water site of the river (site C), aquatic annelids, such as sandworms and leeches, were the prey found most commonly in the stomachs of smaller eels, but their ratios also tended to be lower in larger size classes. Conversely, fishes and crustaceans were found in the stomachs of eels from almost all size classes in both shore type areas of all sampling sites, and these food items accounted for a large percentage of the stomach contents of larger eels (total length > 500 mm). The ratios of these food items tended to be higher in larger size classes, with the exception of eels collected at the lake (site D). In the revetment areas, there was no clear relationship between total length of eels and their stomach content compositions, with the stomachs of eels from all size classes being dominated by bivalve, fishes, and crustaceans.

**Fig. 3.** Results of the mixing model for estimating prey contributions to the diet of yellow-phase Japanese eels (*Anguilla japonica*) collected in the Tone River watershed, Japan. The points, vertical lines, and boxes indicate posterior medians and 95% and 50% credible intervals, respectively. Fish in site A includes *Acanthogobius lactipes*, *Ictalurus punctatus*, and *Tridentiger brevispinis*; fish in site B includes *Acanthogobius lactipes* and *Tridentiger brevispinis*; MCC in site C is *Mugil cephalus cephalus*; fish in site D includes *Acanthogobius lactipes*, *Carassius langsdorfii*, *Hyporhamphus intermedius*, *Hypomesus nipponensis*, *Lepomis macrochirus*, *Opsariichthys uncirostris*, *Pseudorasbora parva*, *Tridentiger brevispinis*, and *Zacco platypus*; shrimp in site D include *Macrobrachium nipponense* (MN) and *Palaemon paucidens*.



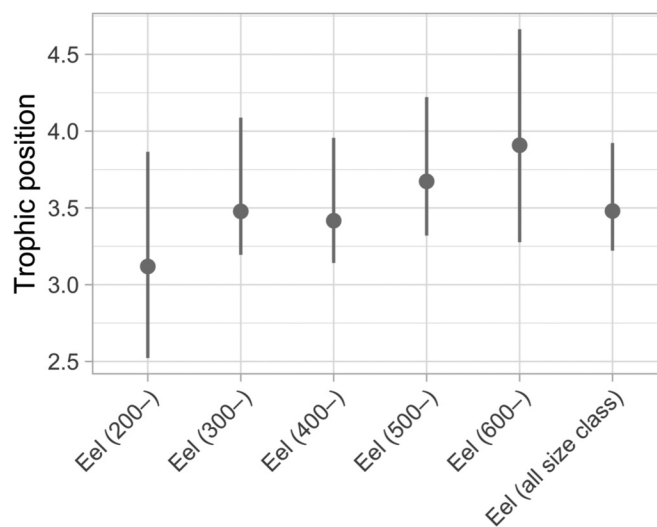
#### Relative contribution of earthworms to eel's body tissues

In the freshwater river site, the largest proportion of the prey animals found in eels caught in the natural shore area consisted of terrestrial earthworms (*Metaphire* spp.; median: 46.9% at site A; 44.5% at site B), with crayfish (*Procambarus clarkii*; 21.0%) and crab (*Eriocheir japonica*; 31.0%) appearing to be the second-largest food item in terms of quantity at sites A and B, respectively (Fig. 3). On the other hand, eels in the revetment areas of these sites, where earthworms were unavailable, largely utilized bivalves (*Limnoperna fortunei*; 84.5% at site A; 75.9% at site B), and eels in site B also consumed fish (24.1%). Eels caught in the revetment areas in the brackish water site of the river (site C) utilized mullet (*Mugil cephalus cephalus*; 47.1%) and shrimp (*Macrobrachium nipponense*; 42.3%), whereas eels caught in the natural shore areas utilized the sandworm (*Hediste atoka*; 80.4%; Fig. 3). In the lake (site D), eels caught in both shoreline type areas utilized mainly shrimp (*Macrobrachium nipponense* and *Palaemon paucidens*; 56.0% at natural shore area; 43.8% at revetment area). Eels in natural shore and revetment areas of this site also consumed fish (25.8%) and crayfish (38.8%), respectively (Fig. 3).

#### Stable isotopic ratios and trophic level

There was a slight, but both significant and positive, relationship between the  $\delta^{15}\text{N}$  of eels and total length at all the sampling sites ( $R^2 = 0.03\text{--}0.22$ ,  $p < 0.05$ ), whereas such a consistent trend was not

**Fig. 4.** Trophic levels of yellow-phase Japanese eels (*Anguilla japonica*) collected in the freshwater sites A and B of the Tone River watershed, Japan. Values in parentheses in the horizontal axis represent the total length (mm) class for eels. The points and lines indicate posterior medians and 95% credible intervals, respectively.





**Table 1.** Akaike's information criterion (AIC) ranking of the models that explain either the presence or the absence of earthworm in stomach contents of Japanese eels (*Anguilla japonica*) (A) and coefficient values and associated probability of the best model (B).

A. AIC ranking of the models							
Rank	Predictor variable			Precipitation	Total length	AIC	$\Delta$ AIC
	No. of days after rainfall	Seasonality	No. of days after rainfall $\times$ seasonality				
1			+	+	+	109.290	0.000
2	+		+	+	+	109.290	0.000
3		+	+	+	+	109.290	0.000
4	+	+	+	+	+	109.290	0.000
5			+	+		110.163	0.873
6		+	+	+		110.163	0.873
7	+		+	+		110.163	0.873
8	+	+	+	+		110.163	0.873
9	+	+		+		111.169	1.879
B. Coefficient values and associated probability of best model							
Predictor variable		Effective df	Reference df	$\chi^2$	$p$		
No. of days after rainfall $\times$ seasonality		5.911	5.991	16.985	0.010		
Precipitation		6.299	6.812	11.155	0.131		
Total length		1.601	1.997	2.883	0.248		

**Note:** A plus symbol (+) indicates significant effect of the predictor variable that was used as a spline function on the response variables; a blank cell indicates no significant effect.  $\Delta$ AIC, differences between Akaike's information criterion values of the best model (rank 1) and selected model; df, degrees of freedom. The seasonality indicates number of days from 1 January.

found between the  $\delta^{13}\text{C}$  of eels and total length among sites (Supplementary Fig. S2<sup>1</sup>). The trophic level of all eels captured was 3.5 (median) (Fig. 4). The trophic levels of the eels tended to increase slightly with increasing total length, and those of largest size class (total length > 600 mm) was 3.9 (median) (Fig. 4).

### Relationship between eels' consumption of earthworms and rainfall

The GAMs ranked with low AICs suggested that precipitation and the interaction term between the number of days after rainfall and seasonality were related consistently to the presence of earthworms in the diet of the eels (Table 1A). The best GAM (deviance explained = 56.7%) showed that this interaction term was related significantly to the presence of earthworms in the eels' diet (Table 1B); earthworms were present for only 1 or 2 days after rainfall in spring, summer, and autumn (Fig. 5a). Precipitation and total length of each eel were also included in the best model; the probability of presence of earthworms peaked at around precipitation of 26 mm and it decreased at eels with total length > 500 mm (Supplementary Fig. S3<sup>1</sup>), but they were not related significantly to the presence of earthworms in the diet of the eels (Table 1B). Moreover, the optimal GAMs for the eels' consumption of earthworms revealed that precipitation was included in all candidate models with  $\Delta$ AIC < 2 (Table 2A). The best model (deviance explained = 63.5%) revealed that the precipitation and the interaction term between precipitation and seasonality were related significantly to the eels' consumption of earthworms (Table 2B). This consumption peaked in summer and increased significantly when the precipitation exceeded 25 mm (Fig. 5b). Seasonality and total length of each eel were not included in the best model (Table 2A).

### Discussion

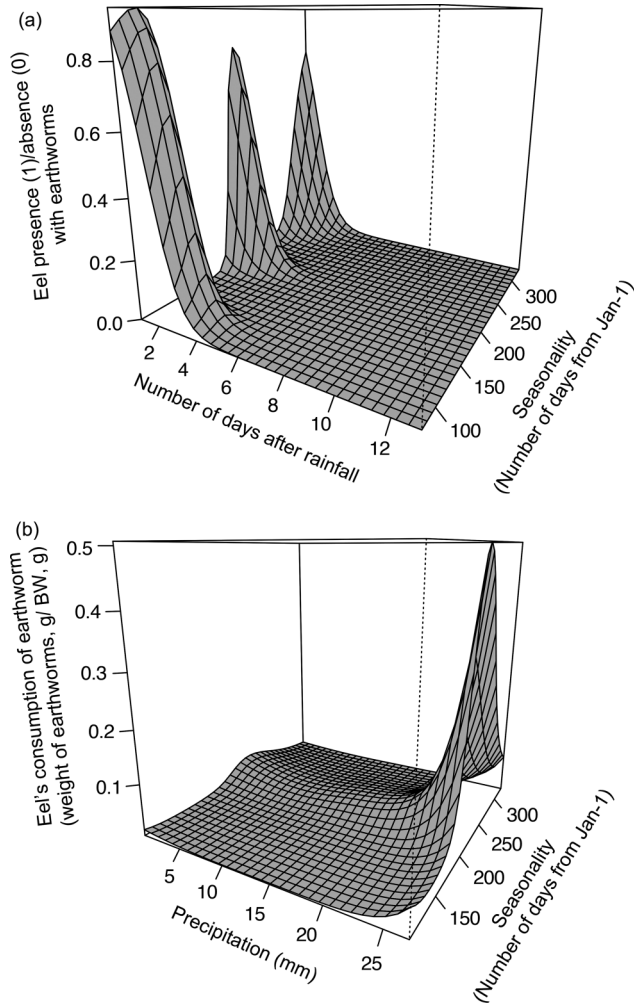
Though allochthonous resource subsidies have key effects on recipient habitats, particularly when the PAR is very high, such as in headwaters and on small islands (Polis et al. 1997; Kawaguchi and Nakano 2001; Sato et al. 2011), these effects are thought to be not important in habitats with low PAR, such as in the lower reaches of large rivers, which have large stream channels (Polis

et al. 1997). Our findings from the stomach content analysis demonstrated that the predatory *A. japonica*, particularly smaller eels (total length < 500 mm), inhabiting the lower reaches of a large river fed largely on oligochaete terrestrial earthworms. This is likely a result of earthworm pulsed subsidies related to rainfall that could temporally increase the ratio of this subsidy resource to equivalent resources in recipient habitats in the large stream channels, causing eels' diet to be biased toward earthworms. Additionally, the stable isotopic mixing models showed that the diet of eels can depend largely on allochthonous terrestrial resources that feed on terrestrial matter. Consequently, these results suggest that earthworms were resource subsidies that contributed the most to the diet of eels.

Earthworms can be supplied to rivers as a pulsed resource subsidy through mass movement, driven by rainfall, into river waters. In this study, there was significant association between the presence of earthworms in the eels' diet and the interaction term between the number of days after rainfall and seasonality, and the eels' consumption of earthworms was also significantly related to precipitation and its interaction term with seasonality. This suggests that eels fed intensively on earthworms within 2 days after rainfall during spring, summer, and autumn and that their consumption of eels peaked during summer, tending to increase with increasing precipitation. These findings support a previous study that reported mass appearances of earthworms on the ground, occurring mainly either during or after rainfall in spring, summer, and autumn, with a peak in summer (Ohno 2007). Earthworms emerge often from the soil to the surface of the ground, probably because of factors such as a rapid fall in soil temperature, rainfall, and a subsequent increase in the soil's carbon dioxide concentration (Friend 1921; Stewart 2004; Ohno 2007), and they can appear in rivers in large quantities (Kobayashi et al. 2015). The eels' intense consumption of earthworms, related to rainfall and its seasonality, as observed in this study, coupled with these previous findings, suggests that earthworm inputs can be regarded as a pulsed resource subsidy in terms of "low frequency", "large magnitude", and "short duration", as defined by Yang et al. (2008).

Moreover, the significant effect of the interaction term between the number of days after rainfall and seasonality on eels' feeding

**Fig. 5.** Graphic summaries of generalized additive models assessed relationships between rainfall and feeding on earthworms by yellow-phase Japanese eels (*Anguilla japonica*), collected in the freshwater sites of the Tone River watershed, Japan. (a) The effect of the interaction term between the number of days after rainfall and seasonality on either the presence or the absence of earthworms in the eels' stomachs. (b) The effect of the interaction term between the precipitation and seasonality on the eels' consumption of earthworms. The surfaces indicate the predictive value of the models.



indicates that the pulsed earthworm subsidies occur in multiple seasons. This is supported by a study for salmonids showing that earthworms were found in stomachs of the fishes after floodings in spring and summer (Warlow and Oldham 1982). A previous study reported that the rainfall-related mass appearances of earthworms on the ground happen throughout the seasons, but the season of appearance differed among earthworm species with different phenology (Ohno 2007). The previous study identified that a total of four earthworm species appeared on the ground. Although all four species appeared in summer, one species also appeared from the end of spring, while the appearance of another species lasted until winter. In addition to these four species, the appearance of unidentified earthworm species was confirmed in the early spring (Ohno 2007). Although the earthworms on which the eels fed were not identified to species in this study, the diversity of earthworm species could drive

**Table 2.** Akaike's information criterion (AIC) ranking of the models that explain the consumption of earthworm by Japanese eels (*Anguilla japonica*) (A) and coefficient values and associated probability of the best model (B).

A. AIC ranking of the models					
Predictor variable				AIC	$\Delta$ AIC
Rank	Seasonality	Precipitation	Precipitation $\times$ seasonality		
1		+	+	96.876	0.000
2		+	+	97.331	0.455
3	+	+		97.505	0.629
4	+	+	+	97.505	0.629
5	+	+		97.883	1.007
6	+	+	+	97.883	1.007
B. Coefficient values and associated probability of best model					
Predictor variable		Effective df	Reference df	$\chi^2$	p
Precipitation		3.390	4.006	7.451	<0.001
Precipitation $\times$ seasonality		3.187	3.622	7.332	<0.001

**Note:** A plus symbol (+) indicates significant effect of the predictor variable that was used as a spline function on the response variables; a blank cell indicates no significant effect.  $\Delta$ AIC, differences between AIC values of the best model (rank 1) and selected model; df, degrees of freedom. The seasonality indicates number of days from 1 January.

multiple earthworm-pulsed subsidies related to rainfall across seasons, providing predators in the large stream channels with prolonged subsidies.

The effects of subsidies on consumers are related to both the ratio of subsidy resources to equivalent resources in the recipient habitat and the PAR of the habitat (Marczak et al. 2007). When the rainfall-related pulsed earthworm subsidy takes place in a large stream channel with low PAR, the intensive input of earthworms can temporally increase the ratio of the allochthonous resource to equivalent resources in the recipient habitat, resulting in the eels' diet being biased toward earthworms. Moreover, this temporal increase of the ratio happens in multiple seasons, likely driven by the diversity of earthworm species with different phenology (Ohno 2007). This might lead to earthworms exerting considerable influences on the body tissues of eels over a long period, as was observed in this study by means of stable isotopic analyses. Stable isotopic analyses also revealed that the trophic level of eels was greater than three — these values are normally associated with secondary consumers. Therefore, the earthworm inputs during or after rain events could be one of dominant pathways of terrestrial subsidies to consumers inhabiting large stream channels such as the lower reaches of a large river.

The findings of stable isotopic analysis and of its mixing model showed that allochthonous terrestrial resources that feed on terrestrial matter can contribute to the diet of eels. As earthworms are only one component of many types of terrestrial resources, it is difficult to ascertain from using stable isotopic analysis what types of the terrestrial resources can contribute to the tissues of consumers in aquatic habitats; however, the analysis of the eels' stomach contents in this study revealed no terrestrial resources other than earthworms. These findings, provided from a combination of stomach content and stable isotope analyses, imply strongly that earthworms are the allochthonous terrestrial resource that contributed to the diet of the eels in this study.

The prey contributions to the diet of eels estimated by stable isotopic mixing models were consistent with the results of stomach content compositions, as shown for earthworms. In the revetment area of the freshwater sites A and B, bivalves contributed most to the eels' stomach contents, and it was estimated as the prey animal that contributed the most to the diet of eels by mixing models. Similarity, sandworm and shrimp were the prey



animals that contributed the most to the diet of eels in the natural shore areas of sites C and D, respectively, while fish (*Mugil cephalus cephalus*) and Crustacea (shrimp and crayfish) were the prey animals that contributed to the diet of eels in the revetment areas of sites C and D, respectively, all of which constituted most of the eels' stomach contents in each area of each site. These results suggest that the results from stomach content compositions observed in this study can reflect not only temporal feeding by eels, but also the food utilization of eels over longer periods.

As eels, depending on their body size, may selectively eat suitable prey animals, the contribution of earthworms to the diet of the eels changed as the eels grew. Smaller eels fed mainly on annelids, including earthworms and insects with relatively small and soft bodies, whereas larger eels never fed on earthworms and consumed mainly fish and crustaceans, which have relatively large and hard bodies, indicating an ontogenetic diet shift, as reported previously for anguillid eels (Jellyman 1989; Michel and Oberdorff 1995; Tzeng et al. 1995). The GAM also showed that the probability of presence of earthworms decreased in eels with total length > 500 mm, although there was no significant relationship between them. The stable isotopic analyses supported this ontogenetic diet shift; the trophic levels of larger eels tended to be higher than those of smaller eels, suggesting that eels change their diets from prey animals with lower trophic levels to higher ones as the eels grew. Gape size correlated with body size has a strong influence on the available size of prey animals (Brönmark and Hansson 1998), and handling time for feeding decreases as the predator's total length increases (Werner 1974). As larger prey animals provide a predator fish with more energy, there is a suitable size of prey animal for each body size of the predator. Thus, earthworms seem to be suitable food items for smaller eels due to the ease of feeding, whereas fish and crustaceans appear to be suitable food items for larger eels.

Shoreline revetments may block one of the important linkages between terrestrial and freshwater ecosystems, which is supplying earthworms from land to rivers. A study by Itakura et al. (2015) reported that the earthworms were the most dominant food item for eels inhabiting the natural shore areas in the lower reaches of the river, but they were never part of the eels' diet in any of the revetment areas. Moreover, condition factors of eels inhabiting the revetment areas of the river were significantly lower than were those of eels inhabiting the natural shore areas, in part because earthworms were not available for eels in the revetment areas (Itakura et al. 2015). As earthworms are common food items for anguillid eels (Jellyman 1989; Denoncourt and Stauffer 1993; Bouchereau et al. 2009; Van Liefferinge et al. 2012), revetments may block the supply of this important allochthonous subsidy for eels. Shoreline modifications often result in reduced abundance and diversity of freshwater animals because of loss of structural diversity along riverbanks (e.g., Taniguchi et al. 2001; Wolter 2001), and these ecological impacts have given rise to increasing restoration efforts (Yoshimura et al. 2005; Nakamura et al. 2006). Our results may provide another strategy that is worth consideration for restoring the connectivity between terrestrial and freshwater ecosystems.

In summary, the present study has shown that the diet of a predatory fish in large stream channels depends largely on terrestrial earthworms using a combination of stomach content and stable isotope analyses, suggesting that multiple earthworm-pulsed subsidies related to rainfall across seasons provide a prolonged subsidy to predators inhabiting habitats in large stream channels with low PAR. Allochthonous prey inputs into rivers can alter the flow of energy both through and across ecosystems. For example, terrestrial orthopterans that are manipulated by nematomorph parasites to enter streams become a large food subsidy item for stream fishes, and this can reorganize stream communities and alter ecosystem function (Sato et al. 2011, 2012). Earthworms are ubiquitous and very abundant in soils through-

out basins (Tsukamoto 1986), and they are supplied to rivers via processes that differ from the host-parasite process. Therefore, future studies are needed to examine how this subsidy affects freshwater species, communities, and ecosystems in entire basins, and they may increase understanding of the role of the dynamics of terrestrial invertebrates in the linkages between terrestrial and freshwater ecosystems.

## Acknowledgements

We are deeply grateful to M. Machida and T. Miyajima for their help on the stable isotopic analyses. We also thank S. Miyazaki and H. Morioka for their advice on the stable isotopic analyses, K. Sakuma for his advice on identifying stomach contents, and H. Hino, T. Kaino, and A. Takeshige for help in the fieldwork. H.I. was financially supported by the Research Fellowship for Young Scientists from the Japan Society for Promotion of Science. This study was financially supported by the River Fund of the River Foundation, Japan, the Futaba Electronics Memorial Foundation, and the JSPS KAKENHI Grant (Nos.: JP21380119, JP13J06053). The authors have no conflict of interest to declare.

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