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Aquatic subsidies transport anthropogenic nitrogen to riparian spiders

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Smaller spiders assimilate anthropogenic nitrogen through the predation on aquatic subsides.

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ABSTRACT

Stable nitrogen isotopic composition (δ^{15}N) of aquatic biota increases with anthropogenic N inputs such as sewage and livestock waste downstream. Increase in δ^{15}N of riparian spiders downstream may reflect the anthropogenic pollution exposure through predation on aquatic insects. A two-source mixing model based on stable carbon isotopic composition showed the greatest dependence on aquatic insects (84%) by horizontal web-building spiders, followed by intermediate (48%) and low (31%) dependence by cursorial and vertical web-building spiders, respectively. The spider body size was negatively correlated with the dietary proportion of aquatic insects and spider δ^{15}N. The aquatic subsidies transported anthropogenic N to smaller riparian spiders downstream. This transport of anthropogenic N was regulated by spider's guild designation and body size.

1. Introduction

Riparian spiders use prey organisms originating from both terrestrial and aquatic ecosystems (Williams et al., 1995; Polis et al., 1997; Henschel et al., 2001; Collier et al., 2002); thus, spiders may indirectly uptake anthropogenic pollutants such as anthropogenic nitrogen (N), heavy metals, and chemical synthesis in the watershed through the use of aquatic prey subsidies. Stable N isotopic composition (δ^{15}N) can be useful tool for tracing N sources in aquatic biota from watersheds (e.g. Vander Zanden et al., 2005). Sewage and livestock waste N are enriched in δ^{15}N (10–20‰), while synthetic fertilizers and atmospheric N typically have low δ^{15}N (synthetic fertilizer: −3 to 3‰; atmospheric, 2–8‰) (Kreitler and Browning, 1983; Macko and Ostrom, 1994). Many previous studies have demonstrated that the δ^{15}N of primary producers in rivers and along shorelines (e.g., phytoplankton, macroalgae, vascular plants, and periphyton) identifies their use of N sources derived from the watershed (McClelland and Valiela, 1998; Toda et al., 2002; Akamatsu et al., 2008). Subsequently, aquatic consumers ingest N pollutants through their primary-producer food sources (McClelland et al., 1997; Wayland and Hobson, 2001; Howard et al., 2005). Patterns of δ^{15}N in aquatic biota can be explained by the loading of ^{15}N-enriched N from sewage and livestock waste within the watershed (Cabana and Rasmussen, 1996; Karube et al., 2010), where aquatic primary producers show temporal variability in their δ^{15}N (Chikaraishi et al., 2009; Hannides et al., 2009) because of their assimilation of various N sources (NO_3^-, NH_4^+, and N_2) and the short life span of the organisms (Dore et al., 2002). By contrast, long-lived primary consumers integrate temporal variation in δ^{15}N of the primary producers (Cabana and Rasmussen, 1996; Post, 2002). Thus, the downstream changes in δ^{15}N of riparian spiders may reflect not only the trophic enrichment of spiders (ca. 3‰, Oelbermann and Scheu, 2002), but also anthropogenic N inputs into terrestrial food webs through predation on the aquatic prey subsidies. If riparian spiders include ^{15}N derived from anthropogenic N in watersheds downstream, the spider δ^{15}N has a possibility to be one of the useful indicators of anthropogenic pollutants from the watersheds as a tracer to characterize spider exposure.

The dependence of riparian spiders on aquatic subsidies is related to prey abundance (Kato et al., 2003; Marczak and Richardson, 2007), guild designation (web type; Akamatsu et al., 2004; Kato et al., 2004), and the stability of habitat related to flooding regimes (Greenwood and McIntosh, 2008; Lambeets et al., 2008). In addition, the consumption of aquatic insects by riparian spiders is related to spider body size; specifically, stable C isotope analyses have indicated that aquatic insects may be particularly important food sources for small spiders (Akamatsu et al., 2007). Stable C isotopic compositions (δ^{13}C) have been used to identify the energy resources of consumers in riparian ecosystems (Sanzone

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et al., 2003), as $\delta^{13}C$ can detect subtle changes in energy flow within naturally occurring ranges in productivity (Post, 2002). Variation in the values of $\delta^{13}C$ of consumers is derived from the relative contributions of different sources (aquatic or terrestrial production) as well as variation of $\delta^{13}C$ within each source (Paetzold et al., 2005). For example, periphyton often have higher $\delta^{13}C$ values compared to leaves of terrestrial C_3 plants (Akamatsu et al., 2004). The $\delta^{13}C$ values of consumers relative to those of their resources have also been used to estimate the relative contributions of various resources to consumers (McCutchan and Lewis, 2002). Thus, $\delta^{13}C$ is a powerful tool for evaluating trophic structure and energy flow in ecosystems (Peterson and Fry, 1987).

To better understand the material flows from human activities in watersheds to riparian predators, we investigated the trophic transfer and longitudinal patterns of $\delta^{13}C$ and $\delta^{15}N$ in riparian spiders that varied in body size across guilds along a gravel-bed river. In riparian food webs, spiders are prey for larger predators such as frogs, lizards, and birds (Power and Dietrich, 2002; Sabo and Power, 2002); thus, spiders can function as vectors for the spread of materials derived from aquatic ecosystems, such as heavy metals and polychlorinated biphenyls, to terrestrial ecosystems (Cristol et al., 2008; Walters et al., 2008). Therefore, examining aquatic prey use by spiders and the extent of anthropogenic N pollution in relation to relative body size between predator and prey within different guilds will provide useful information for elucidating the degree of spread of anthropogenic materials throughout food webs.

2. Material and methods

2.1. Study sites

Five study sites were selected along the main channel of the Chikuma River, (Fig. 1) which is the upper portion of the longest river (the Shinano River) in Japan (see Akamatsu et al., 2004). Site 1 was located the farthest upstream (Igura, 35°55′N, 138°42′E; 357 km from the river mouth; 1978 m above sea level [a.s.l.]; bed slope of 1/5), and the channel was covered by a forest canopy (*Betula platyphylla*, *Larix kaempferi*, *Picea jezoensis* var. *hondoensis*, and *Tsuga diversifolia*). Site 2 (Hisawa, 35°58′N, 138°42′E; 329 km from the river mouth; 1119 m a.s.l., bed slope of 1/87) was surrounded by agricultural fields and mountains, and the channel was exposed to direct sunlight. The riparian area was relatively flat (<1 m above river surface) and extended for 40 m. Only herbaceous plants such as reed (*Phragmites japonica*) and mugwort (*Artemisia capillaries*) covered the riparian area, and no riparian forests developed there. Site 3 (Usuda, 36°11′N, 138°31′E; 298 km from the river mouth; 707 m a.s.l., bed slope of 1/104) was located at the boundary between the upstream mountainous area and the downstream plateau. The riparian area was relatively steep and narrows (a maximum height of 2.7 m above river surface and width of 50 m). Reed (*P. japonica*) and knotweed (*Polygonum nodosum*) dominated the shore side zone, and a riparian forest of willows (*Salix gilgiana*) and N-fixing black locust (*Robinia pseudoacacia*) developed from 25 m away from the river edge. Sites 4 (Ikuta, 36°22′N, 138°28′E; 258 km from the river mouth; 449 m a.s.l., bed slope of 1/192) and 5 (Nezumi, 36°25′N, 138°11′E; 250.5 km from the river mouth; 407 m a.s.l., bed slope of 1/200) were located in the downstream plateau and close to urban areas. In sites 4 and 5, reed (*P. japonica*), bulrush (*Typha latifolia*), and young willows (*S. gilgiana*) propagated in the shore zone (width of 30 m) and black locust (*R. pseudoacacia*) made up riparian forests from 30 m away from the river edge (width of 200 m). The entire channel meandered, with a pool–riffle sequence at the two downstream sites (4 and 5). The widths of the main channel and the riparian area were larger downstream, especially in the downstream plateau. The streambed was composed of cobbles at all sites. Several sewage treatment system outlets were located between sites 3 and 5. The contributions by sewage and livestock waste to concentrations of total dissolved N in the river increase from 0% to 40% downstream estimated by a pollutant load method using administrative data and pollutant load factors (cf. Toda et al., 2002).

2.2. Sample collection and treatment

Field surveys were conducted at the five sites under base-flow conditions in late August 2002. At each site, spiders (web-building and cursorial on grass) and terrestrial insects found within 2 m of the ground (<2 m) were collected from 10 randomly selected quadrats (1 m^2) along the river's edge using a hand net. Two collectors spent 2 h at each site. Emerged aquatic insects were collected using a black-light trap (360 nm, 6 W) for 24 h at each site. Periphyton (mainly diatom, but

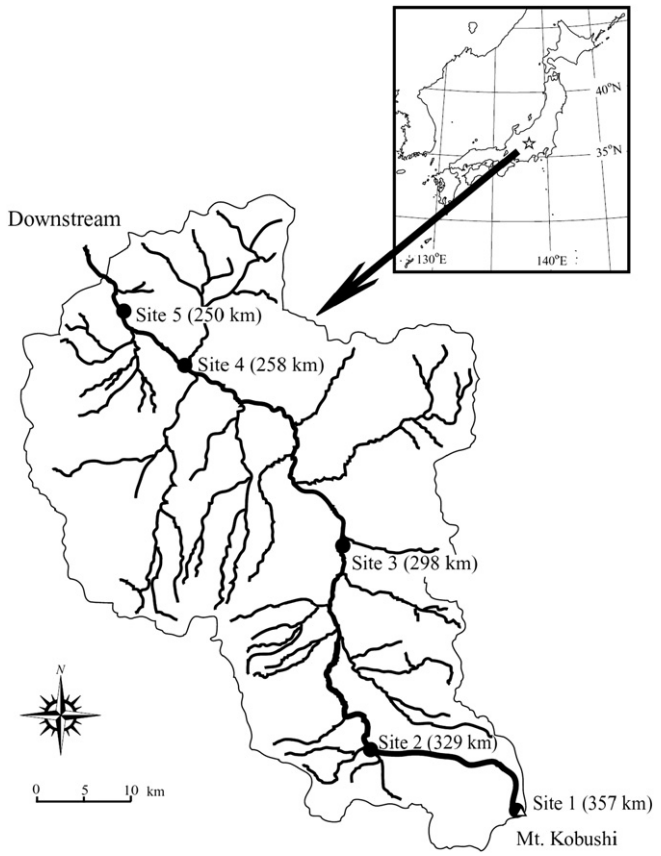

Fig. 1. Five sampling sites along the Chikuma River. The numbers in parentheses represent distances from the river mouth.

species were not identified in this study) were collected by brushing five cobbles collected from a riffle (~0.3 m water depth) in the river channel at each site. Invertebrates and debris were removed from the algal samples in the laboratory. Leaves of plant species were collected manually along a transect at each site. The collected materials were dried at 60 °C for 24 h, weighed, and ground to a powder.

River water was collected using 2-l tanks and the samples were kept cool with ice during transportation to the laboratory. River water samples were filtered through a glass-fiber filter (GF/C; Whatman, Maidstone, UK). The filtered samples (nitrate) were concentrated in an evaporator to a final concentration of 1000 mg N l^{-1} and 0.4 ml of each concentrated sample was freeze-dried in a tin cup.

2.3. Stable isotope analysis

Stable isotopic compositions were measured for each individual sample when possible, but composite samples were used for individuals <0.3 mg in dry weight. Carbon and nitrogen stable isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$, respectively) of the ground and dried samples were determined using an isotope ratio mass spectrometer (DELTA plus; Thermo Electron, Bremen, Germany) equipped with an elemental analyzer (Flash EA1112; Thermo Electron). Approximately 1.0-mg aliquot of each sample was put into a tin capsule and placed into an autosampler. Obtained CO_2 gas was separated using a Porapak QS (3 m long) at 35 °C. Results are reported in δ notation:

$$\delta X\ (‰) = \left(R_{sample}/R_{standard} - 1\right) \times 1000,$$

where $X = {}^{13}C$ or ^{15}N, and $R = {}^{13}C/{}^{12}C$ or $^{15}N/{}^{14}N$, and values are expressed relative to standard Pee Dee Belemnite for C and atmospheric N_2 for N. A working standard of known δ value (glycine and alanine) was analyzed every 5–8 runs to confirm the reproducibility and accuracy of the isotope measurements. The analytical precision (1σ) was 0.1‰ for C and 0.2‰ for N.

We used a two-end-member mixing model to estimate the contribution of aquatic insects to the diet of riparian spiders, as reported in Phillips and Gregg (2001), using $\delta^{13}C$ of aquatic and terrestrial insects collected at each site, respectively. This model includes the isotopic variances of the prey sources and the predators, resulting in more reliable estimates of food sources and their variances (Phillips and Gregg, 2001). We used a value of +1.0‰ as the isotope fractionation in C during the feeding process, similar to previous studies (DeNiro and Epstein, 1978; Oelbermann and Scheu, 2002).