THE DARK SIDE OF SUBSIDIES: ADULT STREAM INSECTS EXPORT ORGANIC CONTAMINANTS TO RIPARIAN PREDATORS

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Abstract. Aquatic insects provide a critical energy subsidy to riparian food webs, yet their role as vectors of contaminants to terrestrial ecosystems is poorly understood. We investigated relationships between aquatic resource utilization and contaminant exposure for a riparian invertivore assemblage (spiders and herptiles) along a stream contaminated with polychlorinated biphenyls (PCBs). Stable carbon ($^{13}$C) and nitrogen ($^{15}$N) isotopes indicated that aquatic insect utilization varied among predators, with progressive enrichment of $^{13}$C and depletion of $^{15}$N as predators shifted from aquatic to terrestrial prey. PCB concentrations significantly increased along these isotopic gradients; $^{13}$C and $^{15}$N explained 65% and 15% of the variance in predator PCBs, respectively. PCBs in predators were high, exceeding 2000 ng/g wet mass (the human-health advisory prohibiting any consumption of fish tissue) in three species. Greater consideration should be given to streams as lateral exporters rather than simply as longitudinal conduits for contaminants. Persistent contaminants are underutilized for addressing landscape-level questions in subsidy research, but our results demonstrate they are an ideal in situ tracer of stream-derived energy because they label stream organic matter and invertebrates over large distances. Likewise, riparian predators such as tetragnathid spiders have great potential as biological monitors of stream condition and as an assessment tool for risk management of contaminated aquatic sediments.

Key words: biomagnification; PCBs; Sanguino-Weston; stable isotopes; Superfund.

INTRODUCTION

Subsidies, or the transfer of materials between habitats, have been a primary focus of food web and landscape ecology for the last decade (Polis et al. 1997, Ballinger and Lake 2006). Stream ecologists have long recognized that terrestrial systems fuel streams via inputs of nutrients and organic matter, but recent studies have highlighted the importance of aquatic insect subsidies to riparian predators like arthropods, birds, bats, and herptiles (Baxter et al. 2005). This subsidy affects the behavior, production, and diversity of riparian predators as well as initiating ecosystem-level responses such as trophic cascades (Baxter et al. 2005, Ballinger and Lake 2006).

In addition to providing critical nutrient subsidies, donor habitats can also supply unwanted contaminants to recipient food webs (Vander Zanden and Sanzone 2004). Few studies have addressed contaminant flux from streams to riparian zones, even though stream contamination is widespread. The U.S. Environmental Protection Agency (2002) recently assessed 700,000 of the nation’s 400,000 stream miles and reported that 38% (266,000 stream miles) were listed as “impaired” for fish consumption, primarily due to persistent bioaccumulative contaminants such as mercury and polychlorinated biphenyls (PCBs). Aquatic insects are key exporters of contaminants to terrestrial systems (Menzie 1980, Runck 2007), and their consumption is linked to exposure of terrestrial predators such as tree swallows (Echols et al. 2004). However, current knowledge of exposure extends to only a small subset of riparian consumers and contaminants, limiting our ability to predict the fate of stream-derived contaminants in complex riparian ecosystems.

Natural abundance of stable isotopes is increasingly used to quantify material transport and biomagnification in food webs (Jardine et al. 2006) and to track contaminant flux between ecosystems (Blais et al. 2005). Here we used natural abundance of stable carbon ($^{13}$C) and nitrogen ($^{15}$N) isotopes to determine putative food resources (aquatic vs. terrestrial) for riparian predators with varying foraging behaviors. We then used these isotopes to predict contaminant exposure to riparian predators from a stream with a legacy of severe PCB contamination. We expected that riparian predators would be contaminated with PCBs via consumption of aquatic insects, and that predators showing the greatest reliance on aquatic resources (as inferred from stable
isotopes) would have correspondingly high PCB concentrations.

**Methods**

The Sangamo-Weston capacitor plant (Pickens, South Carolina, USA) discharged 181.4 metric tons of PCBs into Twelvemile Creek from 1955 to 1977, and the stream food web remains highly contaminated (Walters et al. 2008). We sampled the stream and riparian food web for PCBs and stable isotopes at four sites from 0–25 km downstream of the Sangamo-Weston site as well as an upstream reference site (Appendix A: Fig. A1). Sampling was conducted for the stream food web during 2003–2004 and for riparian predators and terrestrial arthropods (at two sites) from April to October 2005. Collection methods, replication, and chemical analyses for stream organic matter and consumers are provided in Walters et al. (2008). Here we report data for stream insects (Diptera, Ephemeroptera, Megaloptera, Plecoptera, and Trichoptera) that are likely prey for riparian invertivores and for insectivorous fishes, the primary in-stream predators of aquatic insects (Appendix B: Table B1).

We sampled web-building and ground-dwelling spiders and herptiles which presumably vary in their dependence on aquatic insect prey (Table B1). Web-building spiders included tetragnathid and araneid (Araneus spp., Argiope aurantia) orb-weaving spiders. Tetragnathid spiders inhabit stream and lake margins, build weak, horizontal orb webs, and specialize in consumption of aquatic insects (Gillespie 1987, Williams et al. 1995, Ballinger and Lake 2006) (see Plate 1). Araneid spiders are distributed from riparian to upland habitats, build strong, vertical orb webs, and feed on aquatic and terrestrial insects (Kato et al. 2004). Ground-dwelling Dolomedes spiders forage at the stream margins and in forest litter and capture a mix of aquatic and terrestrial prey (Collier et al. 2002). Less is known regarding aquatic resource utilization by herptiles, even though they may dominate the biomass of stream riparian predators (Ballinger and Lake 2006).

Riparian predators were collected by hand from reaches spanning 200 m of stream bank. Web-building spiders were collected from vegetation over the stream and up to 1 m from the water edge (Williams et al. 1995). Dolomedes were collected from the debris along stream margins, and by turning rocks within 3 m of the stream (Collier et al. 2002). Herptiles were collected from within 5 m of the stream. We expanded the search area for herptiles because they are more mobile and presumably forage over a wider area than spiders. Sites were sampled on three occasions between April and October 2005 to ensure adequate replication. Whenever possible, three replicates of each taxon were collected for stable isotope and PCB analysis during each sampling event. Samples were usually composites of several similar-sized individuals. Separate replicates were obtained for stable isotope and PCB analysis for abundant spider taxa (e.g., Tetragenatha). Dolomedes were less abundant, so two legs from individuals in composite PCB samples were used for stable isotope analysis (Collier et al. 2002). Herptile whole bodies and thigh muscle tissue was used for analysis of PCBs and stable isotopes, respectively.

Samples were combusted to CO₂ and N₂ and analyzed in a Carlo Erba NA 1500 CHN analyzer (Carlo Erba Instrumentazione, Milan, Italy) connected to a Finnigan Delta isotope ratio mass spectrometer (Thermo Electron, Waltham, Massachusetts, USA) to determine δ¹³C and δ¹⁵N. Reference standards were PeeDee belemnite carbonate for δ¹³C and atmospheric N₂ for δ¹⁵N. Reproducibility was monitored using bovine liver (NIST No. 1577b), and precision was better than 0.2% (1 SD) for both analytes.

PCBs were extracted using EPA Method 1668, Revision A (See Appendix C). A fraction of extract was used to determine lipids gravimetrically. Surrogate recovery standards (PCB14 and 169) were added to samples, matrix spikes, and blanks prior to extraction. Matrix spikes, matrix spike duplicates, and matrix blanks were run with each extraction batch of 20 samples. Surrogate recoveries were 63% ± 11% (mean ± 1 SE) and 48% ± 11% for PCB 14 and 169, respectively. Concentrations were not corrected for recovery of standards. Extracts were analyzed for PCBs using an Agilent 6890-GC (Agilent Technologies, Santa Clara, California, USA) equipped with a 60-m fused silica column (Rtx-5; Restek, Bellefonte, Pennsylvania, USA; 60 m length, 0.25 mm diameter, 0.25 µm film thickness) and a 63Ni electron capture detector (ECD). This method quantifies 102 GC peaks representing 135 congeners (Appendix C: Table C1). ΣPCB concentration was calculated as the sum of individual congeners. Additional information on methods and quality control are provided in Appendix C.

Utilization of aquatic insect prey was evaluated using stable isotope data. First, we used plots of δ¹³C and δ¹⁵N to identify predators whose isotopic signature was most similar to aquatic insects and other components of the stream food web. Mean δ¹³C and δ¹⁵N were calculated for organic matter and consumers by pooling samples among sites to illustrate both aquatic and terrestrial components of the Twelvemile Creek food web. Among riparian predators, only tetragnathid spiders were collected at all sites and all sampling dates. We observed no consistent temporal trends in tetragnathid δ¹³C or δ¹⁵N, so samples were also pooled over time for all taxa. Next, we compared among-site differences in δ¹³C and δ¹⁵N of aquatic insects, insectivorous fishes, and riparian predators using ANOVA and Tukey’s post hoc tests. Correspondence in spatial variation of δ¹³C and δ¹⁵N between predators and aquatic insects indicates greater use of aquatic prey, whereas lack of correspondence indicates greater reliance on terrestrial prey. Samples were again pooled across time to calculate mean δ¹³C and δ¹⁵N for among-site comparisons. No
herptile was collected at all sites, so all species were pooled within sites for analysis.

We used linear regression to determine relationships between predator PCBs, the percentage of body mass that was lipids, and stable isotopes. ΣPCB concentrations were log10-transformed and the percentage of lipids was arcsine-square-root transformed prior to analysis. PCB concentrations and lipid content were uncorrelated ($r = 0.23, P = 0.43$), so analyses used ΣPCB ng/g wet mass (Hebert and Keenleyside 1995). ANCOVA was used to test the effect of site and stable isotopes on ΣPCB concentrations for each predator. Finally, we estimated the annual mass of PCBs exported from the stream. We assumed an average annual export of 5.3 g/m² insect dry mass using the mean of 15 studies summarized in Jackson and Fisher (1986). Area of the stream was calculated by multiplying the mean stream width from six reaches surveyed by Walters et al. (2007) by the total stream length (25 km) among sites. PCB export was then calculated using the mean PCB concentration measured in larval insects (Appendix B: Table B1).

RESULTS

Isotopic signatures of riparian predators were highly variable, with mean values ranging from 3% to 10% δ15N and −27% to −23% δ13C (Appendix B: Table B1). *Tetragnatha* was most similar to aquatic insects and plotted within the area described by the stream food web (Fig. 1). *Dolomedes* was also similar to aquatic insects and plotted just outside of the stream food web. The remaining riparian predators generally plotted along an isotopic gradient showing progressive enrichment of δ13C and depletion of δ15N. This pattern suggests greater dependence on terrestrial resources, as predators plotted increasingly distant from the stream food web.

Aquatic insect δ15N and δ13C varied significantly among sites (Fig. 2). The largest differences were enrichment of δ15N at site 4 and depletion of δ13C at site 3. Insectivorous fishes and *Tetragnatha* tracked these among-site differences in aquatic insect signatures. *Dolomedes* was also significantly enriched in δ15N at site 4. *Dolomedes* was depleted in δ13C at site 3, but the trend was not significant. Neither *Argiope* nor herptiles tracked spatial variation in aquatic insect isotopic signatures.

ΣPCB concentrations in riparian consumers ranged from 180-2740 ng/g, with higher values approaching those of insectivorous fishes (2870 ng/g; Appendix B: Table B1). ΣPCBs at the reference site were an order of magnitude lower for *Dolomedes* (4.1 ng/g) and *Tetragnatha* compared with contaminated sites. ANCOVA of δ13C or δ15N, ΣPCBs, and site showed no effect of site and interaction terms were not significant. Since site had no effect, we compared ΣPCB with δ15N using linear regression (Fig. 3). ΣPCB significantly decreased with predator δ13C enrichment (Fig. 3, $r^2 = 0.27, P = 0.003$; $r^2 = 0.65, P < 0.0001$ excluding outliers). ΣPCB was also weakly correlated with δ15N ($r^2 = 0.15, P = 0.03$).
biotransport in streams have linked anadromous salmon to exposure of freshwater food webs and riparian predators such as bears (Krummel et al. 2003, Sarica et al. 2004). By way of comparison, our estimate of aquatic insect export of 6.1 g/yr of PCBs is equivalent to the PCB mass delivered by 50,000 returning Chinook salmon (Compton et al. 2006). Considering that hundreds of thousands of miles of streams are impaired by persistent contaminants (U.S. EPA 2002), our results suggest that aquatic insects are likely key movers of contaminants from lotic to terrestrial systems at the continental scale.

Enrichment of predator δ13C and depletion of δ15N values indicated a shift from aquatic to terrestrial prey use, as reported for other stream riparian arthropod predators (Sanzone et al. 2003, Kato et al. 2004, Paetzold et al. 2005) and herring gulls (Hebert et al. 1999). This pattern was confirmed by correspondence between these isotopic signatures and PCB concentrations as well as spatial analysis showing that spiders like...
**Plate 1.** *Tetragnatha* sp. at Twelvemile Creek, Clemson, South Carolina, USA. Tetragnathid spiders are nocturnal predators that seek refuge during the day under limbs or foliage. At dusk, they emerge to build their webs on riparian vegetation, bridges, and docks. During prolific hatches (e.g., *Hexagenia* mayflies), they also ambush prey from their daytime refuges. Photo credit: Ryan Otter.

*Tetragnatha* and *Dolomedes* that were isotopically similar to aquatic insects also tracked spatial variation in stream insect δ¹³C and δ¹⁵N. Many predators, particularly herptiles, were much more enriched in δ¹³C than the terrestrial arthropods we sampled. This suggests the existence of other δ¹³C-enriched terrestrial prey (e.g., soil arthropods) that were not sampled in this study. Some of the δ¹³C enrichment observed in herptiles may also be attributed to difference in trophic fractionation rates for predators analyzed as whole-body (i.e., spiders) vs. muscle tissue (i.e., herptiles). McCutchan et al. (2003) noted that the trophic shift for δ¹³C was 0.3‰ for whole-body samples compared with 1.3‰ for muscle tissue.

δ¹³C is commonly used to identify organic matter pathways, whereas δ¹⁵N is typically used to estimate trophic position (Jardine et al. 2006). δ¹³C was the better predictor of predator ΣPCBs in this study, even though both δ¹³C and δ¹⁵N appeared to vary with consumption of aquatic prey. The weaker relationship between δ¹⁵N and ΣPCBs may be related to herptiles feeding at higher trophic levels in the terrestrial food web. The lateral extent of stream insect subsidies is typically confined to the first few meters of riparian habitat (Baxter et al. 2005, Ballinger and Lake 2006), so herptiles feeding on predacious arthropods just a short distance from the stream margin could demonstrate higher δ¹⁵N values without a concurrent increase in ΣPCBs. Four herptile species with the highest δ¹⁵N values in this study had PCB concentrations much lower than predicted (Fig. 3b), suggesting that δ¹⁵N in these taxa was related to trophic position rather than aquatic resource utilization.

**Conclusions**

Recent reviews in stream subsidy research emphasize the need to increase the scale of investigations from reaches to entire riverscapes (Baxter et al. 2005, Ballinger and Lake 2006). A key step toward advancing this goal is to identify and validate ecological tracers of stream subsidy that are applicable across a range of stream conditions (Hebert et al. 2006). For example, a key unresolved question is how the lateral extent of aquatic insect flux varies with longitudinal changes in stream size. The extent in upland streams is limited to a narrow swath of riparian habitat (Baxter et al. 2005, Ballinger and Lake 2006), but lateral flux is hypothesized to increase in large, lowland rivers which produce more emergent insects and where wind advection is likely to increase lateral transport (Power and Rainey 2000). δ¹⁵N tracer additions have been used to quantify...
the lateral extent of aquatic insect utilization in upland riparian food webs, but logistical constraints limit these experiments to reach-scale (tens or hundreds of meters) studies in small, low-order streams (e.g., Sanzone et al. 2003, Briers et al. 2005). Persistent contaminants may be a useful tool in resolving this question because they are proven ecological tracers of energy flow within food webs and between habitats (Fisk et al. 2002, Blais et al. 2005, Hebert et al. 2006). Here, we show that persistent contaminants (1) label large streams over tens of kilometers and (2) serve as indicators of predator reliance on aquatic resources. Thus, these contaminants are ideal in situ tracers of stream-derived energy in riparian food webs and provide a valuable, but underutilized, tool for scaling subsidy research from the reach to landscape level.

Management agencies have a pressing need for biological monitors of water quality and assessment tools for risk management of contaminated aquatic sediments. Birds such as tree swallows have been used to assess contaminant levels and to evaluate the bioavailability of aquatic contaminants in riparian predators (Echols et al. 2004), but they have some limitations as sentinels of pollution. These include high mobility (although this is somewhat mitigated by the positioning of nesting boxes near contaminated sites), reliance on both aquatic and terrestrial insect prey, difficulty in obtaining large sample sizes, and ethical concerns over sacrificing charismatic vertebrates (particularly juveniles) for contaminant analysis. Tree swallows typically frequent larger lotic systems and have limited applicability to wadeable and headwater streams. Other riparian predators such as tetragnathid spiders deserve greater consideration as sentinels of aquatic contamination. Tetragnathids are relatively sedentary, specialize in the consumption of aquatic insects, typically occur in high densities ideal for sample replication, and as we found in this study, are highly labeled by persistent contaminants exported from aquatic systems. Since tetragnathid spiders are globally distributed in the margins of stream, river, and lentic systems (Williams et al. 1995), they could also form the basis for larger-scale, regional comparisons of contaminant flux among disparate aquatic–terrestrial ecosystems.

Our findings underscore the need to view streams as lateral exporters of contaminants with linear "hot strips" (sensu Vander Zanden and Sanzone 2004) of contamination along their margins. This departs from the traditional viewpoint of streams as longitudinal conduits for contaminant flux, either through upstream biotransport by anadromous fishes (e.g., Krummel et al. 2003) or downstream export to lakes and estuaries (Hasler 1975). Riparian insectivores facilitate the transfer of aquatic contaminants to higher trophic levels (e.g., birds and mammals), thus serving as a vital link in the dispersal of aquatic contaminants to the broader terrestrial food web (Cristol et al. 2008). Given the widespread contamination of streams, the ubiquity of stream insects, and the importance of insect subsidies to riparian predators, more research is needed to quantify the magnitude and risk of exposure to riparian food webs.

Acknowledgments

Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy. We thank B. Johnson, W. Cross, and E. Rosi-Marshall for reviewing a draft of this manuscript and A. Sowers for help with sampling.

Literature Cited


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APPENDIX A
Sample sites in Twelvemile Creek (Ecological Archives A018-063-A1).

APPENDIX B
Summary PCB, $\delta^{15}$N, and $\delta^{13}$C values for stream and riparian food webs (Ecological Archives A018-063-A2).

APPENDIX C
Targeted PCB analyses and methods for extraction and analysis of samples by GC-ECD (Ecological Archives A018-063-A3).