Title: No such thing as a free meal: organotin transfer across the freshwater-terrestrial interface

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Summary

1. Emergent aquatic insects can represent an important subsidy to terrestrial ecosystems but may also transport accumulated contaminants across ecosystem boundaries when larvae develop in contaminated sediments.

2. We sampled tetragnathid spiders (terrestrial predators), larval chironomids (spider prey of aquatic origin) and terrestrial insects (terrestrial prey) from two contaminated and two control sites in the Norfolk Broads (UK) to determine whether the organotin compound tributyltin (TBT) is transferred by emergent aquatic insects. TBT, a biocide in anti-foulant paints, was prohibited in the UK in 1987 and globally since 2008 but persists in sediments for decades. Combining stable-isotope analyses commonly used in ecology with ecotoxicological methods enabled us to test whether aquatic subsidies could transport organotin to terrestrial predators.

3. Stable isotope mixing models ($\delta^{15}N$ and $\delta^{13}C$) indicated that chironomids contributed 31-98% to spider biomass. Subsequent organotin analyses revealed consistent, low-level butyltin (dibutyltin; DBT) contamination of chironomids from the most contaminated site but not from the other three sites. Spiders from the most contaminated site had DBT concentrations similar to those of their chironomid prey.

4. To assess bioaccumulation, we used $\delta^{15}N$ values as a proxy for trophic position of sediments, chironomids and spiders, and correlated these values with the respective DBT concentrations. Notwithstanding indications of ^{15}N-enrichment along this short food chain, chironomid and spider DBT concentrations were statistically indistinct. Biota Sediment Accumulation Factors (sediments to chironomids) and Biomagnification Factors (chironomids to spiders) were below the thresholds defining the occurrence of bioaccumulation and biomagnification.

5. Although biomagnification was not detected, it is of concern that butyltins are still present in freshwater food webs c.25 years since last known TBT use in the UK, and continue to be transferred to terrestrial consumers.

Introduction

Aquatic ecologists have long recognised the importance of terrestrially derived allochthonous subsidies, and much research has focussed on the flow of material such as senescent terrestrial plant matter to both lotic and lentic ecosystems (Wallace *et al*., 1997; Jansson *et al*., 2007). A large proportion of productivity in the recipient ecosystem may be supported via detritus-driven food chains (e.g. Grey, Jones & Sleep, 2001; Tanentzap *et al*., 2014). With a broader view of aquatic–terrestrial linkages, ecologists have also identified the reciprocal transfer of aquatic subsidies to terrestrial systems and begun to examine the complex interactions which they often support (Nakano & Murakami, 2001; Baxter, Fausch & Carl, 2005; Scharnweber *et al*., 2014).

Emergent aquatic insects (imagoes) perhaps represent the most conspicuous of freshwater subsidies as they are typically abundant and the flight period is tightly synchronised (e.g.10,000-20,000 ind m^{-2} y^{-1}; Jackson & Fisher, 1986). Productive shallow lakes support considerable littoral-benthic insect secondary production with chironomids often forming 50-80% of both production and emergence (Lindegaard & Jónasson 1979; Jónasson *et al*., 1990), and are important to higher consumers (e.g. Harrod & Grey, 2006). The synchronicity of such emergences may result in large transfers of aquatic-derived production to adjacent terrestrial habitats (e.g. 10 Kg N ha^{-1} $yr^{-1}$and 1 Kg P ha^{-1} yr^{-1}; Dryer *et al*, 2015) and, hence, consumers including adult odonates (Sukhacheva, 1996), spiders (Collier, Bury & Gibbs, 2002; Kato *et al*., 2003), bats (Power & Rainey, 2000), and birds (Murakami & Nakano, 2002). The impact of the subsidy on receiving food webs is not fully understood. However, an increasing body of literature (biased toward stream ecosystems, but see Gratton, Donaldson & Vander Zanden, 2008; Gratton & Vander Zanden 2009 and Scharnweber *et al*., 2014) documents the

influence of imagoes on various aspects of consumer ecology revealed primarily by the application of stable isotope tracers (e.g. Collier, Bury & Gibbs, 2002; Kato *et al*., 2003; Paetzold, Schubert & Tockner, 2005).

Aquatic subsidies may affect terrestrial food webs across multiple trophic levels. For example, Pacific salmon directly subsidise terrestrial predators (Gende *et al*., 2002) but they also subsidise terrestrial primary producers and consumers via indirect pathways such as nutrient recycling as carcasses decompose (Helfield & Naiman, 2001). Aquatic subsidies can also alter the dynamics of trophic interactions, sometimes with 'cascading' effects (Henschel *et al*., 2001). However, subsidies may also contain insidious, harmful components such as anthropogenic contaminants (Paetzold *et al*., 2011; Morrissey *et al*., 2013). Sediment, a rich organic matter source supporting so much secondary production, is often a sink for persistent anthropogenic contaminants (Burton, 1991). Consequently, any organisms living and feeding in or on this basal resource potentially experience increased exposure to, and accumulation of, those contaminants (Menzie, 1980; Runck, 2007). During emergence, imagoes can transport their accumulated contaminants across ecosystem boundaries, where subsequent predation transmits the contaminant to the terrestrial food web. Studies of cross-ecosystem, trophic transfer of contaminants are relatively rare (but see Echols, 2004; Walters, Fritz & Otter, 2008; Walters *et al.*, 2010; and Paetzold *et al*., 2011) but essential if we are to correctly identify contaminant fate and initiate effective management.

One such contaminant, the organotin tributyltin (TBT), was described as one of the most toxic substances ever deliberately introduced to the aquatic environment (Goldberg, 1986). Its widespread use as an antifoulant on boat hulls in the 1970-80s led to well documented examples of immune system toxicity and endocrine disruption in non-target organisms (e.g.

93 Bryan *et al*., 1986; O'Halloran, Ahokas & Wright, 1998; Omura *et al*., 2001). Restrictions
94 were eventually imposed on the use of TBT antifoulants in the UK, banning their application
95 on craft <25m in length in 1987. By 1989 similar legislation was passed in most of Europe,
96 North America, Australia, New Zealand and Hong Kong (Antizar – Ladislao, 2008), and
97 globally, the application of TBT-based antifoulants has been totally prohibited on all vessels
98 since 2008. Studies of TBT have typically been conducted in marine systems, with few from
99 fresh waters (see Kannan, 1997; Tessier *et al*., 2007). There has also been a tendency to
100 simply detail its occurrence or estimate trophic transfer between aquatic consumers only
101 (Stab *et al*., 1996; Traas *et al*., 1996). Since organotins have been detected in chironomid
102 imagoes from brackish waters of the Baltic (Lilley *et al*., 2012), we hypothesised that
103 chironomids might be an important vector of organotins across ecosystem boundaries from
104 freshwater to terrestrial ecosystems.

105 To test this hypothesis, we studied four sites in the Norfolk Broads: two control sites with no
106 boating access and thus no history of organotin contamination; and two sites which have
107 previously yielded high sediment-bound concentrations of TBT (Sayer *et al*., 2006). We used
108 stable isotope analysis to estimate the contribution of emerging aquatic insects (chironomids)
109 to terrestrial predators (tetragnathid spiders), coupled with organotin analyses. We also
110 examined the potential of the contaminant to biomagnify from its source in sediments
111 through chironomids to terrestrial spiders as predators. We envisaged the aquatic subsidy
112 could present both an important food resource and a source of toxic contaminants to the
113 spider.

114 **Methods**

115 *Sampling locations and key organisms*

Cromes Broad, situated on the River Ant and connected only by small dykes, has been non-navigable throughout its history. Cockshoot Broad is isolated from the River Bure and has been non-navigable for >20 years. These two lakes represent control sites as earlier sampling of sediments yielded no quantifiable butyltin contamination (Hoare, 2007). Malthouse and Ranworth Broad are situated adjacent to one another and are connected via a short channel; both broads are connected to the tidal River Bure via Ranworth Cut but Ranworth Broad is a designated nature reserve with Site of Special Scientific Interest status and hence boating is prohibited. Sediments from both these sites had previously yielded quantifiable butyltin contamination (Hoare, 2007). Key characteristics of the waterbodies are summarised in Table 1 and their location is shown in Fig. 1. Surficial sediments (0-1cm and 1-5cm depth) for organotin analyses were collected during March 2008 using a 7-cm diameter Glew gravity corer (Glew, 1991). Larvae of *Chironomus plumosus* were collected by Ekman grab from profundal sediments approximately every 3 months for 1 year (Cromes and Cockshoot: March 2007- March 2008; Malthouse and Ranworth: June 2007 – June 2008) to determine seasonal variability in isotope values. Seston was collected over the same period by plankton net (<100 µm mesh). Spiders of the Tetragnathidae (the most common family to be found on the surrounding *Phragmites australis* in the littoral-riparian fringe < 2m landward from lake margins) were collected manually at roughly 2-week intervals from April to early August, then monthly until early October. Putative terrestrial insect prey was collected with beating trays during the same sampling events and from similar habitats as spiders. Following capture, all organisms were immediately placed into hexane-rinsed glassware to avoid contamination.

Organotin analyses

Analyses of the organotin compounds tributyltin (TBT) and triphenyltin (TPhT), and the di-, and mono-substituted daughter compounds (DBT and MBT, and DPhT and MPhT, respectively), were carried out according to Waldock *et al*. (1989). The presence of the daughter compounds in water, sediments and biota is a result of the stepwise degradation of the parent compound which occurs principally as a result of aerobic biological activity (Dowson *et al*., 1993). Briefly, samples were homogenised, then organotin compounds were extracted from the sample matrix by addition of sodium borohydride and methanol, converted to hydrides, and partitioned into hexane. In the case of sediments and seston, a separate solid determination was performed in order to express concentrations on a dry mass basis. Derivatives were then analysed by gas chromatography with flame photometric detection (GC-FPD). Quality control in each sample batch included an analytical blank, a certified EU reference material (CRM 477 for biota and CRM 646 for sediments and seston) spiked with target compounds, and a Response Factor sample (containing known concentrations of all organotin compounds) analysed prior to every three environmental samples. Relative Percent Difference (RPD) for CRM 477 and CRM 646 were <15% for butyltin compounds (TBT, DBT and MBT) in all sample runs (see Appendix S1 in Supporting Information for mean values and standard deviations). Each sample contained tripropyltin (TPT) as an internal standard. All quantification was calculated through comparison of the known concentration of the standard. No contamination was detected in any analytical blank. Limits of detection for the method were c.2ng g^{-1} for butyltins and 14ng g^{-1} for phenyltins. Coefficients of Variation (CVs) of the Response Factor sample heights were < 15 % for butyltins for all sample runs. Organotin concentrations are expressed in terms of the cation (TBT^{+}, DBT^{+} and MBT^{+}) as ng g^{-1} dry mass for sediments and seston and ng g^{-1} wet mass for biota. One pooled sample of chironomids (c. 80-120 individuals) was analysed from each of four

sampling events (each site: *n*=4). A single pooled sample of terrestrial insects was analysed from each site (each site: *n*=1). Single pooled samples of spiders (c. 60-80 individuals) were analysed from a total of eight sampling events at Malthouse and Cromes and five sampling events at Ranworth and Cockshoot. Three replicate samples of surficial sediments (0-1cm depth) from a single sampling event were used for sediment analyses (*n=3* at each site). For seston analyses, a single bulked sample was used from each of four sampling events (*n*=4 for each site). For organisms and seston, replication within each sampling event was not possible due to the large amount of biological material required for organotin analysis (c.3g wet mass).

Stable isotope analyses

Chironomid larvae and terrestrial insect prey were held for 24 h to ensure gut clearance and reduce potential sources of error to isotopic values *sensu* Feuchtmayr & Grey (2003). Since tetragnathid spiders are suctorial, gut clearance was unnecessary, and so spiders were frozen immediately on return from the field. All tissues were dried at 65°C for 48 h before homogenisation using an agate pestle and mortar. Samples were weighed (0.6 ± 0.05 mg) into tin capsules and analysed by continuous-flow isotope-ratio mass spectrometry (Thermo-Finnigan, Delta Matt Plus, Waltham, USA). Results are given using the standard δ notation: $\delta = [(X_{sample} / X_{reference}) - 1] \times 1000$, expressed in per mille (‰), where X = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Reference materials were international standards of ammonium for nitrogen, and sucrose for carbon. A secondary internal standard of a known relation to international standards was run every 10 samples to provide a measure of instrumental precision (± 0.15‰ for $\delta^{13}C$ and $\delta^{15}N$).

Isotope mixing models and data analyses

The relative contributions of potential prey sources to spider biomass were estimated using SIAR Bayesian mixing models (Parnell *et al*., 2010). Annual mean chironomid $\delta^{15}N$ and $\delta^{13}C$ values (see Table S2 in Supporting Information) were used as one source in the model as it was not logistically possible to sample chironomids and spiders concomitantly. We assumed no significant change in $\delta^{13}C$ or $\delta^{15}N$ due to metamorphosis between 4^{th} instar *C. plumosus* larvae and adults based upon our own unpublished data for this species and results reported by Doi *et al.* (2007). We also assumed that chironomid imagoes dominated aquatic insect emergence based upon abundance data derived from profundal surveys using an Ekman grab (*C. plumosus* larvae were typically 5-6x more abundant, 800 to >1000 individuals m^{-2}, than any other insect). This was borne out by visual inspection of spider webs. Mean isotope values of terrestrial insects (four most abundant families; n=14 derived from all sites) was used as the second source in the model, with spiders assumed to be a mixture of the two. We used trophic enrichment factors (TEF) of -0.4 ± 0.6‰ for $\delta^{15}N$ and 0.2 ± 0.4‰ for $\delta^{13}C$ in our models based on values for fluid feeders from a meta-analysis by McCutchan *et al*. (2003). Normality and equality of variances were ascertained for all data by performing Anderson-Darling and Levene's tests, respectively. As the isotope data of terrestrial insect were non-normal and variances were unequal between terrestrial and chironomid isotope values, non-parametric Kruskal–Wallis tests were used in place of ANOVA. Regression analysis was used to examine the relationship between chironomid contribution to spider biomass (using mode values; the most probable solution in the Bayesian context) and the total butyltin concentration in spiders (e.g. the sum of all quantified butyltin compounds in a sample); where butyltins were not detected, 50% of the limit of detection for the relevant sample was assumed for statistical purposes (Murai *et al*., 2008).

To assess biomagnification, concentrations of organotins in spiders, chironomids and sediments were plotted against the corresponding $\delta^{15}N$ values as a proxy for trophic position. The regression analysis approach often used to relate trophic position and contaminant biomagnification (e.g. Kidd *et al*., 1995) was not appropriate in this instance (see Jardine *et al*., 2006), hence Welch's t-test, which is robust to small sample sizes and unequal variances, was used to test for differences between sediment, chironomid and spider butyltin concentrations. Statistical analyses were conducted using Minitab 15 (Minitab inc. 2007 State College PA, USA), with a significance level of ≤ 0.05.

Biota Sediment Accumulation Factors (BSAFs) and Biomagnification Factors (BMFs) were used to test for accumulation of butylins between sediments and chironomids, and biomagnification between chironomids and spiders, respectively. By definition, bioaccumulation from sediments occurs when the BSAF value is > 1. Similarly, compounds are biomagnified between predator and prey when the BMF value is >1 (Strand & Jacobsen, 2005). BSAFs and BMFs were calculated using the following equations:

$$BSAF = C_b/C_s \quad (1),$$

$$BMF = C_b\,(predator)/\,C_b\,(prey) \quad (2),$$

where C_b is the concentration in the whole body of the organism (ng DBT g^{-1} dry mass) and C_s the concentration in sediment (ng DBT g^{-1} dry mass). Dry mass was estimated for chironomids and spiders using literature values for moisture content (spiders; Pulz, 1987; chironomids; Cole & Underhill, 1965; Frouz & Matena, 2015).

Results

Stable isotope data

Chironomid $\delta^{13}C$ was similar across sites although variability at uncontaminated sites was c.5‰ lower than for conspecifics at contaminated sites (Fig. 2a). Larval chironomid tissue $\delta^{15}N$ was also similar across sites, although variability was slightly higher at Cockshoot Broad (Fig. 2b). Terrestrial insect $\delta^{13}C$ values were isotopically distinct from chironomids (Fig. 2a; Kruskal-Wallis test, $\chi^2=21.70$, df = 1, $p < 0.05$). Variability of terrestrial insect $\delta^{15}N$ values was greater than for $\delta^{13}C$ values (Fig. 2b) but remained isotopically distinct from chironomids (Kruskal-Wallis test, $\chi^2=28.75$, df = 1, $p < 0.05$). Spider $\delta^{13}C$ values spanned 6‰ among sites, tending to increase throughout the study period while the range of spider $\delta^{15}N$ values spanned 7‰ among sites but <3‰ within sites in any month (Table S3 in Supporting Information). At Malthouse, Cromes and Cockshoot, the contribution of the aquatic insects to spider biomass was considerable (>50%) and greatest from April to early July (Fig. 3); thereafter, the contribution generally decreased, particularly at Ranworth and Cockshoot.

Organotin analyses

No detectable phenyltins were found in any sample. No butyltins were quantified in seston samples or terrestrial insects from any site, nor in chironomid larvae from Cromes or Cockshoot. Chironomids from Ranworth exhibited detectable butyltins in one sample only (3ng g^{-1} DBT). All chironomid samples from Malthouse yielded detectable of DBT; the mean (±SD being 7.8 ± 1.3ng g^{-1} wet mass. Butyltins were not detected in spiders from the control sites, Cromes and Cockshoot. Spiders around Ranworth yielded detectable MBT (15 ng g^{-1} wet mass) only in late August. In contrast, both MBT and DBT were detectable in spiders from Malthouse but not TBT was not. Thus, the total butyltin concentration (∑MBT+DBT) in spiders from Malthouse in mid-April was 14ng g^{-1} wet mass (MBT= 8ng g^{-1}; DBT = 6ng g^{-}

^{1}), 23 ng g^{-1} wet mass in late April (MBT= 16ng g^{-1}, DBT = 7ng g^{-1}), and 19 ng g^{-1} wet mass (MBT= 11ng g^{-1}, DBT = 8ng g^{-1}) in May. No butyltins were detected in June, July or October. Spiders yielded low concentrations of MBT only during August (7ng g^{-1} wet mass) and September (4ng g^{-1} wet mass). The estimated carbon contribution to spider biomass derived from chironomids and the total butyltin concentration of spiders from Malthouse Broad were positively correlated ($P< 0.05$; Fig. 4).

Bioaccumulation and Biomagnification

DBT was used throughout to calculate BSAF and BMF values as it was the only compound consistently detected in sediments, chironomids and spiders from Malthouse Broad. Sediments from Malthouse exhibited the lowest $\delta^{15}N$ value but the highest DBT concentrations (Fig. 5). Spider $\delta^{15}N$ values from those samples also yielding quantifiable concentrations of DBT were consistently enriched by at least 1‰ relative to chironomids (Fig. 5). Concentrations of DBT were significantly greater in chironomids compared to spiders (Welch's t-test, t = 3,85 $P = < 0.05$); sediment DBT concentrations were significantly greater than those measured in spiders and chironomids (t= 19.41 $P = < 0.05$). Both BSAF and BMF values were <1, suggesting no bioaccumulation of DBT from sediments to chironomids or biomagnification of DBT from chironomids to spiders, respectively (Table S4 in Supporting Information).

Discussion

By combining stable isotope and ecotoxicological analyses, we found that a long-banned substance, butyltin, is still detectable in lake sediments, and that emergent aquatic insects transfer the contaminant from aquatic to terrestrial ecosystems. Isotopic signatures of aquatic and terrestrial prey available to a group of terrestrial predators, the tetragnathid spiders, were

277 sufficiently distinct for stable isotopes to be used as tracers. The remarkably invariable $\delta^{13}C$
278 values of terrestrial prey (-26.3 ±0.7‰) reflect consumption of C3 plant material, typically
279 around -28 to -26‰ (Peterson & Fry, 1987). In contrast, the greater temporal variability of
280 chironomid $\delta^{13}C$ values at both contaminated and control sites (~10‰ and ~5‰,
281 respectively) is fairly typical of chironomids, even in relatively shallow lakes that do not
282 stratify seasonally (Grey *et al*., 2004). However, larval chironomids were clearly more ^{13}C-
283 depleted than terrestrial insects throughout the study period, which enabled us to distinguish
284 between aquatic and terrestrial prey in the spider diet. This was further facilitated by more
285 consistent $\delta^{15}N$ values of chironomids over the season, which were also isotopically distinct
286 from those of terrestrial insects.

287 Based on the estimates from isotopic mixing models, emergent chironomids were clearly an
288 important resource to terrestrial spiders. Patterns of chironomid contribution to spider
289 biomass were similar from June to September at all sites, indicating a temporal decline in the
290 importance of the subsidy to spiders. It therefore seems likely that earlier in the season,
291 spiders from Ranworth and Cockshoot consumed chironomids to a similar degree as
292 conspecifics from Malthouse and Cromes. Our data (31-98% contribution to spider biomass)
293 correspond to several other studies that span a range of geographical locations and habitats.
294 In New Zealand, Collier, Bury & Gibbs (2002) reported that an average of 58% of spider
295 carbon was obtained from in-stream sources. In an extreme case, spiders from the riparian
296 zone of a desert stream in Arizona (USA) were supported by up to 100% by aquatic insects
297 (Sanzone *et al.*, 2003). Similarly high subsidies to terrestrial habitats have been reported
298 from lakes. For example, Walters *et al.* (2009) estimated emergent invertebrate contribution
299 to spider biomass up to 92% in a lake of the southeastern USA, and Gratton, Donaldson &

300 Vander Zanden (2008) reported greater use of carbon derived from aquatic compared to
301 terrestrial prey by spiders inhabiting the riparian margins of subarctic Icelandic lakes.

302 The peak of spider dependence upon chironomid biomass at Cromes occurred earlier than at
303 Malthouse. Given that Cromes is shallower and smaller than Malthouse and temperature is a
304 key environmental variable affecting chironomid emergence (Pinder *et al*., 1991), a plausible
305 explanation for this difference could be that the critical 'trigger' temperature was reached
306 earlier in the season at Cromes. The increases in chironomid contribution to spider biomass
307 during late summer and early autumn at Malthouse, Ranworth and Cockshoot suggest a
308 smaller, secondary peak in chironomid emergence, as has been observed from similar shallow
309 lakes in northern Germany (Jones & Grey, 2010). This bimodal pattern is not surprising as
310 many chironomid species are bi-voltine in temperate climates. Indeed, Mason (1977) inferred
311 a similar pattern from the relative abundance of different *C. plumosus* instars at Alderfen
312 Broad near our study sites, where an initial peak in abundance of 4th instar larvae recorded
313 during the summer was followed by a secondary peak during the autumn. Such a secondary
314 pulse of aquatic subsidy was absent at Cromes. Sampling from this site consistently revealed
315 not only high densities of homogenously distributed *C. plumosus* but numerous other
316 chironomid larvae associated with dense macrophyte stands, largely free-floating
317 *Ceratophyllum demersum* in the pelagic habitat. Macrophytes support high abundances and
318 diversity of chironomids by providing surface area for epiphyton growth (Balci & Kennedy,
319 2003) and refugia from predators such as fish (Hershey, 1985). Such factors may increase the
320 abundance and diversity of aquatic invertebrates, accounting for the consistently higher
321 dependence of spiders on aquatic prey at this site. In contrast, macrophytes were much less
322 abundant at Cockshoot and absent from Malthouse and Ranworth, and sampling of profundal

sediments yielded a predominance of *C. plumosus* larvae, suggesting that this particular chironomid species was the major aquatic contributor to spider biomass.

We had hypothesised that chironomids emerging from organotin contaminated sediments would carry a contaminant load across the aquatic-terrestrial interface where the trophic transfer would be reflected in spiders. Results from Malthouse support this hypothesis. The lack of butyltins in spiders from Ranworth, the other originally contaminated site but now long disconnected to boating, is perhaps unsurprising, given that butyltins were detected only once in *C. plumosus* from this lake and at a concentration close to the limit of detection. Measured $\delta^{15}N$ values from Malthouse were lowest in sediments, intermediate in chironomids and highest in spiders. Despite this, concentrations of DBT were significantly higher in chironomids than their spider predators and sediment DBT concentrations significantly exceeded those measured in the invertebrates. Thus, the data provide no evidence of biomagnification along the short food chain from sediment organic matter to chironomids and spiders at Malthouse. The BSAFs and BMFs we calculated indicated little potential for chironomids to bioaccumulate DBT from sediments, or for DBT to biomagnify following transfer from chironomids to spiders. This lack of biomagnification or bioaccumulation may be related to chironomid feeding mode and physiology, and the prevailing physical, chemical and biotic conditions of water and sediment that control the distribution of butyltins in the aquatic environment; none of these are mutually exclusive.

The lack of detectable butyltins in seston suggests that seston can be ruled out as a source of butyltin to chironomid larvae. Therefore, one possible explanation of the low concentrations of butyltins in chironomids is a higher dietary dependence on uncontaminated seston (e.g. filter feeding) and/or freshly deposited FPOM on the sediment surface (e.g. deposit feeding)

346 than contaminated sediments. Moreover, sediments from the Norfolk Broads contain up to
347 40% total organic carbon, (TOC; unpubl. data) and butyltin compounds have a log organic
348 carbon partition coefficient (log K_{oc}) of ~4.2-5.0 (Meador *et al*., 2002) indicating a high
349 affinity for organic carbon. Thus, strong binding to the organic carbon in sediments probably
350 reduces butyltin bioavailability to consumers such as chironomids, limiting the amount of
351 butyltin assimilated even if it is ingested along with contaminated sediment (Meador *et al*.,
352 1997; 2002). In line with this interpretation, various authors have demonstrated a negative
353 relationship between butyltin concentration in benthic organisms and sediment organic matter
354 (e.g. chironomids - Looser *et al*., 1998; marine polychaetes - Meador *et al*., 1997).

355 Despite high concentrations of TBT in Malthouse sediments at 0-1 cm depth (1368±117.8 ng
356 g^{-1} dry mass), chironomids contained no quantifiable concentrations of the parent compound.
357 However, TBT often undergoes a stepwise biological conversion to DBT and MBT in
358 organisms. This includes members of the genus *Chironomus* (e.g. *C. riparius*) with an
359 efficient metabolic capacity for TBT degradation (Looser *et al*., 1998; Looser *et al*., 2000).
360 Sediment concentrations at Ranworth were an order of magnitude lower (285.5±31.5) than
361 those measured at Malthouse. Therefore, one possible explanation for the absence of butyltins
362 in most chironomids from Ranworth is that the rate of metabolic elimination (e.g. de-
363 butylation) of the contaminant exceeded that at which it was assimilated (see Looser *et al*.,
364 2000) at the lower exposure concentration of TBT.

365 Another potential route of butyltin contamination is passive uptake which is virtually sure to
366 occur in soft-bodied and tubiculous chironomids that are in contact with contaminated
367 sediment pore water. Attempts to measure butyltins in pore water and quantify the
368 importance of this uptake route in chironomids yielded no detectable organotins (unpubl.

data); concentrations were invariably below our LOD of 5ng L^{-1}. This suggests that the amount of freely dissolved butyltins in porewater (e.g. the fraction that is readily bioavailable) is very low and other mechanisms also control butyltin uptake. For example, Arnold *et al*. (1998) demonstrated that up to 70% of TBT was sorbed to dissolved organic matter (in the form of humic acids) under similar anion and pH conditions to those found in the Norfolk Broads (unpubl. data). Similarly, Looser *et al.* (2000) demonstrated that the presence of DOM (humic acids) significantly reduced the bioavailability of butyltins to chironomids. Given the organic character of sediments in our study lakes, the binding of butyltins to DOM in pore water could be an important mechanism in addition to butyltin sorption to particulate organic matter (POM) in sediments. Thus, the low concentrations of butyltins in chironomids we observed even at contaminated sites are likely due to a combination of sorption of butyltins to sediment POM and DOM in porewater, which limits bioavailability, an avoidance of butyltin ingestion by selective feeding, and a metabolic capacity to de-butylate any assimilated TBT.

The positive relationship we observed between spider dietary contribution derived from chironomids and butyltin concentrations was weak, probably reflecting the mechanisms above causing variable butyltin concentrations in their prey, as well as some capability for spiders to de-butylate. To gain a clearer picture of this relationship would require analysis of both isotopes and butyltins from the same samples, and ideally from individual spiders, but the biomass required to determine butyltins was prohibitive in this respect, and we had to resort to samples pooled from many individuals.

Emergent chironomids from contaminated sediments in reed beds in the Baltic Sea contained organotins (Lilley *et al*., 2012). Chironomid imagos had up to 1487 ng TBT g^{-1} body dry

392 mass, compared to the highest recorded sediment concentrations of 527 ng g^{-1} dry mass. The
393 TBT concentrations reported in chironomids are around two orders of magnitude greater than
394 the 96h LC_{50} reported for *C. plumosus* (~ 50 ng L^{-1}; Fargasova, 1997). In contrast, only
395 DBT was detected in our study and estimated dry mass concentrations in larval chironomids
396 and their spider predators were an order of magnitude lower than those recorded in
397 sediments. Concentrations of some lipophilic compounds increase during metamorphosis
398 when insects lose mass but retain lipids (Bartrons *et al.* 2007). Accordingly, a meta-analysis
399 revealed increasing contaminant concentrations between larvae and adults for organic
400 compounds with a log octanol water partition coefficient (log K_{ow}) > 5.0, but decreasing
401 concentrations for compounds with log K_{ow} values < 5.0 (Kraus *et al*., 2014).. TBT is
402 moderately lipophilic (log K_{ow} of 3.7 – 4.4; Arnold et al., 1997) and has a higher affinity with
403 proteins (Strand & Jacobsen, 2005). Therefore, a more likely explanation is that
404 bioavailability of TBT is higher in the Baltic than in the Norfolk Broads. Fresh inputs of TBT
405 into the water column in the Baltic could result in the presence of the compound in its freely
406 dissolved form where it is readily available for biological uptake (Hoch, 2001); as a result of
407 high sediment concentrations (up to 8550 ng g^{-1} dry mass), and/or dredging, and stripping and
408 painting of large cargo and passenger ships (Lilley et al., 2012). The use of TBT based
409 antifoulants on vessels of this size has only been prohibited since 2008. In the Norfolk
410 Broads, the last legal application of TBT based antifoulants on all vessels was in 1987, and
411 hence, the presence of freely dissolved TBT in the water column is less likely.

412 Some organic contaminants biomagnify in food webs. For example, Kidd *et al*. (2001) and
413 Power *et al*. (2002) found increasing DDT and mercury concentrations with increasing
414 trophic position (measured as $\delta^{15}N$) of freshwater invertebrates and fish. Similarly,
415 chlorinated bi-phenol (PCB) accumulated in terrestrial spiders relying on aquatic prey at

416 contaminated sites (Walters, Fritz & Otter, 2008; Walters *et al*., 2009). However, DDT and
417 PCBs are highly persistent and have a strong affinity for lipids, whereas butyltins are only
418 moderately lipophilic, relatively unstable and highly persistent only in anoxic conditions,
419 sediments rich in organic matter (Sun *et al*., 1999). This, coupled with varying capacities of
420 butyltin degradation among species (see discussion above), indicates that biomagnification of
421 butyltins is less predictable than that of DDT and PCBs (see Takahashi *et al.*, 1999).

422 Over twenty-five years have elapsed since TBT-based antifoulants were last legally applied
423 to vessels < 25m in length in most of Europe, North America, Australia, New Zealand and
424 Hong Kong. In our study system the legacy is still quantifiable not only in sediments, but
425 also in aquatic invertebrates and terrestrial predators of the surrounding riparian area.
426 Therefore, redistribution of organotins from aquatic to terrestrial ecosystems by chironomid
427 (or indeed other) imagoes to could be globally widespread and requires further investigation.
428 . When combined with a lack of biomagnification (*sensu* Hu *et al*., 2006; Murai *et al*., 2008),
429 the concentrations we measured in chironomids are probably too low to pose a hazard to
430 terrestrial predatory invertebrates. Many birds such as swallows (Hirundinidae) prey upon
431 chironomid imagoes and spiders and target their breeding to coincide with insect mass
432 emergence. Chicks and juvenile birds are more vulnerable to contaminant exposure due to
433 their small body size and high ingestion rate (Walters *et al*., 2009). This risk has been clearly
434 documented in tree swallows (*Tachycineta bicolour*), where PCB concentrations were up to
435 three-fold higher in young than adults, principally as a result of extensive feeding on
436 emergent Diptera (Echols *et al*., 2004). It would be prudent to consider this risk potential also
437 in the case of butyltin contamination legacies.

438

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References

Antizar – Ladislao, B. (2008). Environmental levels, toxicity and human exposure top tributyltin (TBT) contaminated marine environments. A review. *Environment International* **31,** 292-308.

Arnold C.G. Weidenhaupt A., David M.M., Muller S.R., Haderlein S.B, & Schwarzenbach R.P. (1997). Aqueous speciation and 1-octanol-water partitioning of tributyl- and triphenyltin: Effect of pH and ion composition. *Environmental Science and Technology* **31**, 2596-2602.

Arnold, C.G. Ciani A., Muller S.R., Amirbahman A. & Schwarzenbach R.P. (1998) Association of triorganotin compounds with dissolved humic acids.*Environmental Science and Technology* **32**, 2796- 2983.

Balci P. & Kennedy J.H. (2003) Comparison of chironomids and other macroinvertebrates associated with *Myriophyllum spicatum* and *Heteranthera dubia*. *Journal of Freshwater Ecology* **18**, 235–247.

461 Bartrons M., Grimalt JO., &Catalan J. (2007) Concentration changes of organochlorine
462 compounds and polybromodiphenyl ethers during metamorphosis of aquatic insects.
463 *Environmental Science & Technology* **41**, 6137-6141

464 Baxter C.V., Fausch, K. D. & Carl S. (2005) Tangled webs: reciprocal flows of invertebrate
465 prey link streams and riparian zones. *Freshwater Biology* **50**, 201-226.
466 Burton G.A. (1991) Assessing the toxicity of freshwater sediments. *Environmental*
467 *Toxicology and Chemistry* **10**, 1585-1627.
468 Bryan G.W., Gibbs P.E., Hummerstone L.G. & Burt G.R. (1986) The decline of the
469 gastropod *Nucella lapillus* around south-west England: evidence for the effect of tributyltin
470 from antifouling paints. *Journal of the Marine Biological Association of the U.K.* **66**, 611-
471 640.

472 Cole A.C. & Underhill, J.C. (1965). The summer standing crop of sublittoral and profundal
473 benthos in Lake Itasca, Minnesota. *Limnology and Oceanography* **10**, 499-620.

474 Collier K.J., Bury S. & Gibbs M. (2002) A stable isotope study of linkages between stream
475 and terrestrial food webs through spider predation. *Freshwater Biology* **47**, 1651-1659.
476 Doi H., Kikuchi E, Takugi S. & Shikano S. (2007) Changes in carbon and nitrogen stable
477 isotopes of chironomid larvae during growth, starvation and metamorphosis. *Rapid*
478 *Communications in Mass Spectrometry* **21**, 997-1002.
479 Dowson P.H., Bubb J.M. & Lester J.N. (1993) A study of the partitioning and sorptive
480 behaviour of butyltins in the aquatic environment. *Applied Organometallic Chemistry* **7**, 623-
481 633.
482 Dryer J., Townsend P.A., Hook J.C., Hoekman D., Vander Zanden J.M. & Gratton, C (2015)
483 Quantifying aquatic insect deposition from lake to land. *Ecology* **96,** 499-509.

484 Echols K. R., Tillit D.E., Nichols J.W., Secord A.L. & McCarthy J.P. (2004) Accumulation
485 of PCB congeners in nestling tree swallows (*Tachycineta bicolor*) on the Hudson River, New
486 York. *Environmental Science and Technology* **38**, 6240–6246.

487 Eisler R. (1989) Tin hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish
488 and Wildlife Services Biological Report 85 (1.15) 83.pp.
489 Fargasova A. (1998) Comparison of effects of tributyl,- triphenyl, - and tribenzyltin on
490 freshwater benthos and alga *Scendesemus quadiricauda. Bulletin of Environmental*
491 *Contamination and Toxicology* **60,** 9-15.
492 Feuchtmayr H. & Grey J. (2003) Effect of preparation and preservation procedures on carbon
493 and nitrogen stable isotope determinations from zooplankton. *Rapid Communications in*
494 *Mass Spectrometry* **17**, 2605- 2610.
495 Frouz J. & Matena, J. (2015) Desiccation resistance of chironomid larvae. *European Journal*
496 *of Environmental Science* **5**, 31-34.
497 Gende S.M., Edwards R.T., Willson M.F. & Wipfli M. (2002) Pacific salmon in aquatic and
498 terrestrial ecosystems. *BioScience* **52**, 917-928.
499 Glew J.R. (1991) Miniature gravity corer for recovering short sediment cores. *Journal of*
500 *Paleolimnology* **5**, 285-287.
501 Goldberg E.D. (1986) TBT: an environmental dilemma. *Environment* **28**, 42-44.
502 Gratton C., Donaldson J. & Vander Zanden M.J. (2008) Ecosystem linkages between lakes
503 and the surrounding terrestrial landscape in Northeast Iceland. *Ecosystems* **11**, 764-774.
504 Gratton C., & Vander Zanden M.J. (2009) Flux of aquatic insect productivity to land:
505 comparison of lentic and lotic ecosystems. *Ecology* **10**, 2689 -2699.

506 Grey J., Jones R.I. & Sleep (2001) Seasonal changes in the importance of the source of
507 organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope
508 analysis. *Limnology and Oceanography* **46**, 505-513.
509 Grey J., Kelly A., Ward S., Sommerwerk N. & Jones R.I. (2004) Seasonal changes in the
510 stable isotope values of lake-dwelling chironomid larvae in relation to feeding and life cycle
511 variability. *Freshwater Biology* **49**, 681-689.
512 Harrod, C and Grey, J. (2006) Isotopic variation complicates analysis of trophic relations
513 within the fish community of Plußsee: a small, deep, stratifying lake. *Archiv für*
514 *Hydrobiologie* **167**, 281-299

515 Helfield J.M. & Naiman R.J. (2001) Effects of salmon-derived nitrogen on riparian forest
516 growth and implications for stream productivity. *Ecology*, **82**, 2403-2409.
517 Henschel J.R., Mahsberg D. & Stumpf H. (2001) Allochthonous aquatic insects increase
518 predation and decrease herbivory in river shore food webs. *Oikos* **93**, 429-438.
519 Hershey A.E. (1985) Effects of predatory sculpin on the chironomid community of an Arctic
520 lake. *Ecology* **66**, 1131-1138.
521 Hoch M. (2001) Organotin compounds in the environment - an overview. *Applied*
522 *Geochemistry* **16**, 719-743.
523 Hu J., Zhen H., Wan Y., Gao J., An W., An L., *et al.* (2006) Trophic magnification of
524 triphenyltin in a marine food web of Bohai Bay, North China: comparison to tributyltin.
525 *Environmental Science and Technology* **40**, 3142- 3147.
526 Jardine T.D., Kidd K. & Fisk A. (2006) Applications, considerations, and sources of
527 uncertainty when using stable isotope analysis in ecotoxicology. *Environmental Science and*
528 *Technology* **40**, 7501- 7511.

529 Jónasson P.M. Lindegaard C. Dall C.P., Hamburger K. & Adalsteinsson H. (1990) Ecosystem
530 studies on temperate Lake Esrom and the subarctic Lakes Myvatn and Thingvallavatn.
531 *Limnologica* **20**, 259–266.
532 Jones R.I., & Grey J. (2011) Biogenic methane in freshwater food webs. *Freshwater Biology*
533 **56**, 213-229.
534 Kannan K., Senthilkumar K., & Sinha R.K. (1997) Sources and accumulation of butyltin
535 compounds in Ganges River dolphin, *Platanista gangetica. Applied Organometallic*
536 *Chemistry* **11**, 223-230.
537 Kato C., Iwata T, Nakano S. & Kishi D. (2003) Dynamics of aquatic insect flux affects
538 distribution of riparian web-building spiders. *Oikos* **103**, 113-120.
539 Kidd K.A., Schindler D.W., Hesslein R.H. & Mui, D.C.G. (1995) Correlation between stable
540 nitrogen isotope ratios and concentrations of organochlorines in biota from a freshwater food
541 web. *Science of the Total Environment* **160**/**161**, 381-390.
542 Lilley T.M., Meierjohann A., Ruokolainen L., Peltonen J., Vesterinen E., Kronberg L. *et al.*
543 (2012) Reed beds may facilitate transfer of tributylin from aquatic to terrestrial ecosystems
544 through insect vectors in the Archipelago Sea, SW Finland. *Environmental Toxicology and*
545 *Chemistry* **8**, 1781 -1781.
546 Lindegaard C. & Jónasson P.M. (1979) Abundance, population dynamics and production of
547 zoobenthos in Lake Myvatn, Iceland. *Oikos* **32**, 202-227.
548 Looser P.W., Bertschi S. & Fent K. (1998) Bioconcentration and bioavailability of organotin
549 compounds: influence of pH and humic substances. *Applied Organometallic Chemistry***12**,
550 601-611.
551 Looser P.W., Fent K., Berg M., Goudsmit G.H. & Schwarzenbach R.P. (2000) Uptake and
552 elimination of triorganotin compounds by larval midge *Chironomus riparius* in the absence

553 and presence of Aldrich Humic Acid. *Environmental Science and Technology* **34**, 5165-
554 5671.
555 Mason, C.F. (1977) Population and production of benthic animals in two contrasting shallow
556 lakes in Norfolk. *Journal of Animal Ecology* **46**, 147-172.
557 McCutchan J.H., Lewis W.M., Kendall C. & McGrath C.C. (2003) Variation in trophic shift
558 for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* **102**, 378-390.
559 Meador J.P., Krone C.A., Dyer W.D. & Varanasi V. (1997) Toxicity of sediment-associated
560 tributyltin to infaunal invertebrates; species comparison and the role of organic carbon.
561 *Marine Environmental Research* **43**, 219-241.
562 Meador J.P., Collier T.K. & Stein J.E. (2002) Determination of a tissue and sediment
563 threshold for tributyltin to protected prey species of juvenile salmonids listed under the USA
564 endangered species act. *Aquatic Conservation: Marine and Freshwater Ecosystems* **39**, 2401-
565 2408.
566 Menzie C.A. (1980) Potential significance of insects in the removal of contaminants from
567 aquatic systems. *Water, Air and Soil Pollution* **13**, 473-479
568 Morrissey C. A., Stanton D.W.G., Pereira M.G., Newton J., Durance I., Tyler C.R. *et al*.
569 (2013) Eurasian dipper eggs indicate elevated organohalogenated contaminants persist in
570 urban rivers. *Environmental Science and Technology* **47**, 8931-8939.
571 Murai R., Sugimoto A., Tanabe S. & Takeuchi I. (2008) Biomagnification profiles of
572 tributyltin (TBT) and triphenyltin (TPT) in Japanese coastal food webs elucidated by stable
573 isotopes. *Chemosphere* **73**, 1749- 1756.
574 Murakami M. & Nakano S. 2002. Indirect effect of aquatic insect emergence on a terrestrial
575 insect population through bird predation. *Ecology Letters* **5**, 333-337.

O'Halloran K., Ahokas J.T. & Wright P.F.A. (1998) Response of fish immune cells to in vitro organotin exposures. *Aquatic Toxicology* **40**, 141-56.

Omura M., Ogata R., Kubo K., Shimasaki Y., Auo S., Oshima Y., *et al.* (2001) Two generation reproductivity study of tributyltin chloride in male rats. *Toxicological Science* **64**, 224-232.

Paetzold A., Schubert., C.J. & Tockner, K. (2005). Consumer-specific responses to riverine subsidy pulses in a riparian arthropod assemblage. *Freshwater Biology* **51**, 1103-1115

Paetzold A., Smith M., Warren., P.H., & Maltby., L. (2011). Environmental impact propagated by cross-system subsidy: chronic stream pollution controls riparian spider populations. *Ecology* **92**, 1711-1716.

Parnell, A.C., Inger R., Bearhop, & Jackson A.L. (2010). Source partitioning using stable isotopes: coping with too much variation. *PloS ONE* **5**, (3): e9672. doi: 10.1371/journal.pone.0009672

Peterson B.J. & Fry B. (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* **18**, 293-320.

Phillips D.L. & Gregg J.W. (2001) Uncertainty in source partitioning using stable isotopes. *Oecologia* **127**, 171-179.

Pinder A.M., Trayler K.M., Mercer J.W. & Arena, J. (1991) Diel periodicities of adult emergence of some chironomids (Diptera: Chironomidae) and a mayfly (Ephemeroptera: Caenidae) at a western Australian wetland. *Journal of the Australian Entomological Society* **32**, 129-135.

Power, M.E. & Rainey, W.E. (2000). Foodwebs and resource sheds: towards spatially delimiting trophic interactions. – in: *Ecological consequences of habitat heterogeneity*. M.J. Hutchings, E.A. John & A.J.A. Stewart (Eds). Blackwell, pp.291-341.

Power M., Klein G.M., Guiger K. & Kwan M. 2002. Mercury accumulation in the fish community of a sub-Arctic lake in relation to trophic position and carbon sources. *Journal of Applied Ecology* **39**, 819-830.

Pulz R. (1987). Thermal and water relations. – in: *Ecophysiology of spiders.* Nentwig, W (Ed). Springer – Verlag, pp.26-55.

Runck C. (2007) Macroinvertebrate production and food web energetics in an industrially contaminated stream. *Ecological Applications***17**, 740–753.

Sanzone D.M., Meyer J.L., Marti E., Gardinier E.P., Tank J.L. & Grimm, N.B. (2003) Carbon and nitrogen transfer from a desert stream to riparian predators. *Oecologia* **134**, 238-250.

Sayer C.D. Hoare D.J., Simpson G.L., Henderson A.C.G., Liptrot E.R., Jackson M.J., *et al*. (2006) TBT causes regime shift in shallow lakes. *Environmental Science and Technology* **40**, 5269-5275.

Sayer C.D. Davidson, T.A. & Jones, J.I. (2009) Seasonal dynamics of macrophyte and phytoplankton biomass in 39 shallow lakes: a eutrophication-driven pathway from plants to plankton? *Freshwater Biology* **55**, 500-515.

Scharnweber K., Vanni M.J., Hilt S., Syväranta J. & Mehner T. (2014) Boomerang ecosystem fluxes: organic carbon inputs from land to lakes are returned to terrestrial food webs via aquatic insects. *Oikos* **123**, 1439-1448.

Stab J. A., Traas G., Stroomberg G., van Kesteren J., Leonards, P., van Hattum, B., *et al*. (1996) Determination of organotin compounds in the food web of a shallow freshwater lake in the Netherlands. *Archives of Environmental Contamination and Toxicology* **31**, 319-328.

Strand J & Jacobsen J.A. (2005) Accumulation and trophic transfer of organotins in a marine food web from the Danish coastal waters. *Science of the Total Environment* **350**, 72-85.

624 Sukhacheva G.A. (1996) Study of the natural diet of adult dragon flies using an
625 immunological method. *Odonatologica* **25**, 397-403.
626 Sun H.W., Dai S-G. & Huang G-L. (1999) Bioaccumulation of butyltins through an estuarine
627 food chain. *Soil, Water and Air Pollution* **125**, 55-68.
628 Tanentzap A.J., Szkokan-Emilson E.J., Kielstra B.W., Arts M.T., Yan N.D. & Gunn J.M.
629 (2014) Forests fuel fish growth in freshwater deltas. *Nature Communications* **5**, 4077.
630 Takahashi S., Tanabe S., Takeuchi I. & Miyazaki, N. (1999) Distribution and specific
631 bioaccumulation of butyltin contamination in a marine ecosystem. *Archives of Environmental*
632 *Contamination and Toxicology* **37**, 50-61.
633 Tessier E., Amouroux D., Morin, A., Chrstian L. Thybaud E., Vindimian E., *et al.* (2007)
634 (Tri) butyltin degradation rates and pathways in different compartments of a model
635 freshwater ecosystem. *Science of the Total Environment* **388**, 214-233.
636 Traas T. P., Stab J.A., Roel P., Kramer G., Cofino W.P. & Aldenberg, T. (1996) Modelling
637 risk assessment of tributyltin accumulation in the food web of a shallow freshwater lake.
638 *Environmental Science and Technology***30**, 1227-1237.
639 US EPA (2003) Ambient aquatic life water quality criteria for tributyltin (TBT) US
640 Environmental Protection Agency. Accessed online on 01/07/2014
641 http://www.epa.gov./waterscience/criteria/tributyltin.
642 Waldock M., Waite M. E., Miller D., Smith D. J. & Law R. J. (1989) The determination of
643 total tin and organotin compounds in environmental samples. MAFF, Lowestoft. Aquatic
644 Environment Protection: 4. Analytical Methods.
645 Wallace J.B., Eggert S.L., Meyer J.L., & Webster J.R. (1997) Multiple trophic levels of a
646 forest stream linked to terrestrial litter inputs. Science **277,** 102-104.

647 Walters D.M., Fritz K. & Otter R. (2008) The dark side of aquatic subsidies: adult stream
648 insects export organic contaminants to riparian consumers. *Ecological Applications* **18**, 1835-
649 1841.
650 Walters D.M., Mills M.A., Fritz K. & Raikow, D.F. (2009) Spider-mediated flux of PCBs
651 from contaminated sediments to terrestrial ecosystems and potential risks to arachnivorous
652 birds. *Environmental Science and Technology* **44**, 2849-2856.
653

Table 1. Limnological characteristics of the study sites: surface area (SA) and mean annual values (± SE,) for mean depth (Z_{mean}), Secchi depth, plant richness and concentrations of total phosphorus (TP), total nitrate, sediment TBT and sediment DBT (*n*=22 at each site except for TBT and DBT where *n* = 3); ND - not detected; NA -, data not available. Plant species richness refers to number of species of submerged and floating-leaved macrophytes in the lake according to Sayer *et al*. (2009)

Site	SA (ha)	Z_{mean} (m)	Secchi depth (m)	Plant richness	TP (µg L^{-1})	NO_3^- (mg N L^{-1})	TBT (ng g^{-1} dry mass)	DBT (ng g^{-1} dry mass)
Cromes Broad	2.3	0.6 ± 0.3	0.6 ± 0.1	5	59.8 ± 7.7	0.2 ± 0.1	ND	ND
Cockshoot Broad	5.1	1.0 ± 0.5	0.9 ± 0.1	4	48.0 ± 5.8	0.1 ± 0.4	ND	
								ND
	10.0	1.2 ± 0.8	NA	1	NA	NA	1368 ± 6	
Malthouse Broad								266 ± 11
Ranworth Broad	40.7	1.3 ± 0.7	0.8 ± 0.2	1	72.1 ± 8.1	0.7 ± 0.3	234 ± 3	
								78 ± 4

660 **Figure legends**

661 Fig. 1. Map of the Norfolk Broads, showing main broads and the four study sites. Cockshoot
662 Broad (River Bure), and Cromes Broad (River Ant), were control sites. Ranworth and
663 Malthouse Broads (adjacent on River Bure) were contaminated study sites.

664 Fig. 2. Box and whisker plots (a) $\delta^{13}C$ values and (b) $\delta^{15}N$ values for larval *C. plumosus*
665 collected during 2007-2008 from four Norfolk broads. Boxes represent inter-quartile ranges,
666 the solid line represents the median, the whiskers minimum and maximum values, and
667 outliers are included as open circles. Terrestrial insects from the riparian zone are shown for
668 comparison.

669 Fig. 3. Proportional chironomid contribution to spider biomass between April and October
670 2008 modelled from $\delta^{15}N$ and $\delta^{13}C$ values. Each plot shows 25%, 75% and 95% high density
671 ranges of the posterior estimates that chironomids contribute to spider biomass during each
672 sampling event. Malthouse and Ranworth (top panels) are butyltin-contaminated sites;
673 Cromes and Cockshoot (bottom panels) are control sites.

674 Fig. 4. Relationship between total butyltin concentration (MBT + DBT) in spider tissues and
675 chironomid contribution to spider biomass from Malthouse Broad. The regression line (±
676 95% CI) was fitted by the equation $y = -12.9 + 31.8x$ ($R^2 = 0.60$; $P < 0.05$).

677 Fig. 5. Scatter plot showing $\delta^{15}N$ and DBT in sediments (filled circles), chironomids (open
678 circles) and spiders (filled triangles) from Malthouse Broad. Data are shown only for
679 chironomid and spider samples that yielded quantifiable concentrations of DBT.

680