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Environmental Toxicology and Chemistry, Vol. 30, No. 9, pp. 2167–2174, 2011
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Printed in the USA
DOI: 10.1002/etc.608

AQUATIC TO TERRESTRIAL TRANSFER OF SEDIMENT ASSOCIATED PERSISTENT ORGANIC POLLUTANTS IS ENHANCED BY BIOAMPLIFICATION PROCESSES

JENNIFER M. DALEY,† LYNDA D. CORKUM,‡ and KEN G. DROUILLARD*†
†Great Lakes Institute for Environmental Research, University of Windsor, Windsor, Ontario, Canada
‡Department of Biological Sciences, University of Windsor, Windsor, Ontario, Canada

(*Submitted* 18 *March* 2011; *Returned for Revision* 3 *May* 2011; *Accepted* 14 *June* 2011)

Abstract—Ephemeral emergent insects, such as mayflies (*Hexagenia* spp.), are commonly used as biomonitors of persistent organic pollutants (POPs) and provide a vector for aquatic-terrestrial contaminant transfer. Mayflies bioaccumulate sediment-associated contaminants by bioconcentration and biomagnification during the aquatic stage and concentrate POP residues postemergence due to bioamplification, which occurs as a result of weight and lipid loss without contaminant loss. The present study quantified polychlorinated biphenyl (PCB) bioamplification in male and female emergent mayflies at three sites. Male mayflies used 36 to 68% of their lipids during emergence, with the exception of caged males that were prevented from flight. Females did not lose lipid content between pre-emergent nymph and emerged life stages. Mass balance indicated no PCB elimination between life stages. The mean PCB bioamplification factor, expressed as the ratio of lipid-equivalent PCB concentrations across life stages, was 2.05 ± 0.38 for male imagos/nymphs and 1.91 ± 0.18 for male imago/subimago life stages. For females, bioamplification factors were close to unity. Wildlife consumers of imago stages of emergent mayflies can potentially increase their total daily intake of PCBs by 36% depending on the sex-ratio composition of their diet relative to animals that feed predominantly on nymph or subimago stages during mass emergence events. Environ. Toxicol. Chem. 2011;30:2167–2174. © 2011 SETAC

Keywords—Bioaccumulation Biota-sediment accumulation factor Persistent organic pollutants Trophic transfer *Hexagenia*

INTRODUCTION

Aquatic insect larvae readily bioaccumulate sediment-associated contaminants, such as metals and persistent organic pollutants [1,2]. As an important component of their respective food webs [3], aquatic insects act as vectors for contaminant transfer of in-place pollutants to upper food web components through benthic to pelagic coupling [4]. This process can be extended to terrestrial wildlife when considering aquatic-terrestrial food web coupling, which commonly occurs for emergent aquatic insects during mass mating swarms [5,6].

The bioaccumulation of persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and organochlorine pesticides, from sediments to larval stages of aquatic invertebrates has received considerable attention [2,7]. Hydrophobic organic contaminants bioaccumulate in benthic invertebrates through bioconcentration and biomagnification processes. Through bioconcentration, the benthic invertebrate equilibrates with pore water by respiratory exchange and can approach or achieve chemical potentials similar to the sediment it inhabits and/or the water that it respires [2,8]. Under biomagnification, additional chemical exposures occur by ingested diet items in the gastro-intestinal tract [9,10], causing the chemical potential of an animal's tissues to exceed that of its ingested food; for benthic invertebrates, this can translate into elevated chemical potentials in the organism compared to the surrounding sediments and overlying waters [7,11]. Biota-sediment accumulation factors greater than one are considered indicative of biomagnification and have been reported to range from one to >10 for PCBs and organochlorines in a variety of aquatic invertebrates [12,13].

A third bioaccumulation process that can mediate chemical potential in an organism is bioamplification. Here, the term *bioamplification* is defined as a physiological process whereby an organism loses body weight and chemical partitioning capacity at a faster rate than it can eliminate contaminants [14]. This causes an elevation of both the chemical concentration and chemical potential in the animal's tissues independent of contaminant uptake mechanisms. Bioamplification generally occurs under specific life history stages when an organism experiences major bioenergetic bottlenecks. This process has been recognized and verified in a variety of species, including bird and fish embryos during egg development [14,15], spawning salmon and silver eels [16–18], overwintering yellow perch [19], and amphibians during tadpole-frog metamorphosis [20]. Bioamplification has the potential to occur in emergent aquatic insect adults that rely on accumulated energy reserves obtained during the larval stages to satisfy reproductive costs, such as flight activity, following emergence. Adult mayfly species within the order Ephemeroptera do not feed following emergence from the water; their gut fills with air, aiding in flight [21]. In addition, emergent insects revert to air breathing, which is a much less efficient POP elimination pathway compared to respiratory losses to water [22], so the elimination of sediment-accumulated POPs during the emerged life stages is likely to be negligible. Bioamplification in the emerged life stage is therefore expected to occur as an additive factor above bioaccumulation processes contributed by bioconcentration and biomagnification experienced during the aquatic larval stages.

Bioamplification of POPs by emerged aquatic insects can also have significant consequences, from the perspective of trophic transfer of sediment-associated contaminants, to

All Supplemental Data may be found in the online version of this article.

* To whom correspondence may be addressed (kgd@uwindsor.ca).

Published online 28 June 2011 in Wiley Online Library (wileyonlinelibrary.com).

terrestrial predators that consume them. Bioamplification raises the chemical potential of food items above that of the earlier life stage, resulting in a higher potential for gastrointestinal magnification to be experienced by the predator while consuming emerged aquatic invertebrates over sustained periods of time [9,10]. Even when the predator is in non-steady state bioaccumulation with an ephemeral food source, bioamplification occurs in conjunction with decreases in the energy density of the ingested food items. Thus, a predator consuming organisms that have undergone bioamplification will need to consume a larger number of insects and higher biomass to satisfy daily metabolic requirements [23]. This will result in higher total daily influx of contaminants and greater overall exposures to contaminated sediments than risk-based models of aquatic-terrestrial transfer typically assume.

The objective of the present study was to evaluate bioamplification in emerged forms of aquatic insects using mayflies (*Hexagenia* spp.) as a case study. *Hexagenia* in both its larval and emerged forms are commonly used as biomonitors of sediment contamination [13,24] and as a routine laboratory bioassay organism to assess contaminant bioavailability from field-collected sediments [25]. Burrowing mayflies are considered ideal biomonitors of sediment contamination for several reasons: they inhabit organic-enriched sediments of shallow lakes and large rivers that are likely to contain high POP concentrations [26]; they are relatively large in body size and achieve high biomass (especially on emergence), facilitating ease of collection [24]; and they are an important source of food to a variety of terrestrial animals, including birds, bats, amphibians, and other insectivores [5,6,27]. Mayfly larvae achieve steady state with their associated sediments for POP compounds, such as PCBs, within approximately 30 d [12] and exhibit biomagnification, as exemplified by biota-sediment accumulation factors ranging from 1.4 to 9.4 for PCB and organochlorines [12,13]. Finally, adult stages of mayflies do not feed after they emerge and deplete accumulated lipids due to bioenergetically expensive flight costs associated with mating swarms [21]. To our knowledge, bioamplification as a bioaccumulation process for POPs compounds has not been described for invertebrates, nor has the potential impact of this process on the trophic transfer of sediment associated contaminants to terrestrial insectivores been evaluated. In the present study, pre-emergent and two emerged life stages (subimago and reproductively active imago life stages) of mayflies were collected from three study locations in the Huron-Erie corridor to verify and quantify bioamplification across life stages. The present study also examined sex-based differences in bioamplification of emerged mayflies and tested whether or not bioamplification could be specifically attributed to flight costs as evaluated using a caging study.

MATERIALS AND METHODS

Life history of Hexagenia *spp.*

Great Lakes populations of burrowing mayflies are largely of two species, *Hexagenia limbata* and *Hexagenia rigidia* [24]. Both species have similar morphology and life history characteristics and co-occur during nymphal and emergence periods. In the present study, no efforts were made to distinguish between the two species. Depending on mean seasonal temperatures, the aquatic life stage of *Hexagenia* spp. lasts from one to three years [28,29], but lasts two years in Lake Erie [30]. Approximately two to three weeks prior to emergence, the wing pad of the mayfly nymph thickens and turns black, representing the final instar before emergence. Past research has shown that male mayfly larvae accumulate more lipids than females [31], which can potentially influence bioconcentration/biomagnification processes and sex-differences in the wet chemical concentration of animals at emergence. On emergence, mayflies exist as the subimago molt stage and are not yet reproductively active. The subimagos fly or are carried by wind to shore (seldom more than 5 km; [32]), where they rest for a day before molting into a sexually mature adult, known as an imago. The combined emerged stages seldom last for more than 3 d, after which the animal dies [21,24]. Mass emergence activity of mayflies occurs over extended periods of two to four weeks through June and early July at the study sites [33]. Subimagos and imagos are active and collected at different times. Male imagos establish mating swarms approximately 1 h before sunset. Individual females fly into the swarm, and males and females drop to the ground to mate. These swarms continue after dusk for several hours. Imagos, especially females, are attracted to lights approximately 30 to 60 min after sunset, while subimagos arrive later (90–120 min after sunset).

Sampling strategy

Emerged mayflies (subimagos and imagos, $n = 5$ per life stage, sex and location) were collected from three locations in Ontario, Canada: Middle Sister Island (MSI; 41°51′N 83°00′W) in western Lake Erie; Colchester Harbour (CH; 41°59′N 82°56′W), a marina on the north shore of the western basin of Lake Erie, located approximately 15 km away from Middle Sister Island; and Lakeview Marina (LVM; 42°20′N 82°55′W) on the Detroit River, where Lake St. Clair enters the river (Fig. 1). Subimagos and imagos were collected using light traps or near street lamps in late June of 2006 and 2007 during their period of greatest emergence activity [24]. Adults were separated and placed into solvent-rinsed glass jars according to their life stage (subimago or imago) and by sex (males and females). Black wing pad–staged nymphs ($n = 5$ per sex and site) were collected from two of the three sampling locations in early June of 2006 and 2007 prior to peak emergence activity. The sites included adjacent waters to Middle Sister Island and at Peche Island (42°20′N 82°56′W), located in the upper Detroit River within 1 km of Lakeview Marina (Fig. 1). Mature nymphs were identified by their black wing pads and sorted by sex. The nymphs were sampled using a petite Ponar grab sampler, washed through a 500-μm sieve, and transported to the lab for analysis in solvent rinsed jars on ice. Black wing pad nymphs were not collected from Colchester Harbour due to difficulties finding samples in the heterogeneous substrates surrounding the harbor.

To control for reproductive flight, a caging study was performed in which male and female subimagos were collected from each location and placed in cages (14.5 cm × 10.5 cm × 10 cm) to allow molting but not flight. After sampling a portion of the collected subimagos for analysis, the remaining animals were placed in cages for 48 h, consistent with the normal emergence period. At the end of the caging period, all subimagos had completed the final molt into the imago stage. The imagos were then removed from cages, sorted by sex, and stored frozen until chemical analysis ($n = 3$ per sex and site).

Chemical analysis

Sampled organisms were analyzed to determine dry weight, neutral lipid weight (dry wt basis), lean dry protein (LDP), and congener-specific PCB concentrations. Polychlorinated biphenyls and lipids were analyzed using a micro-extraction

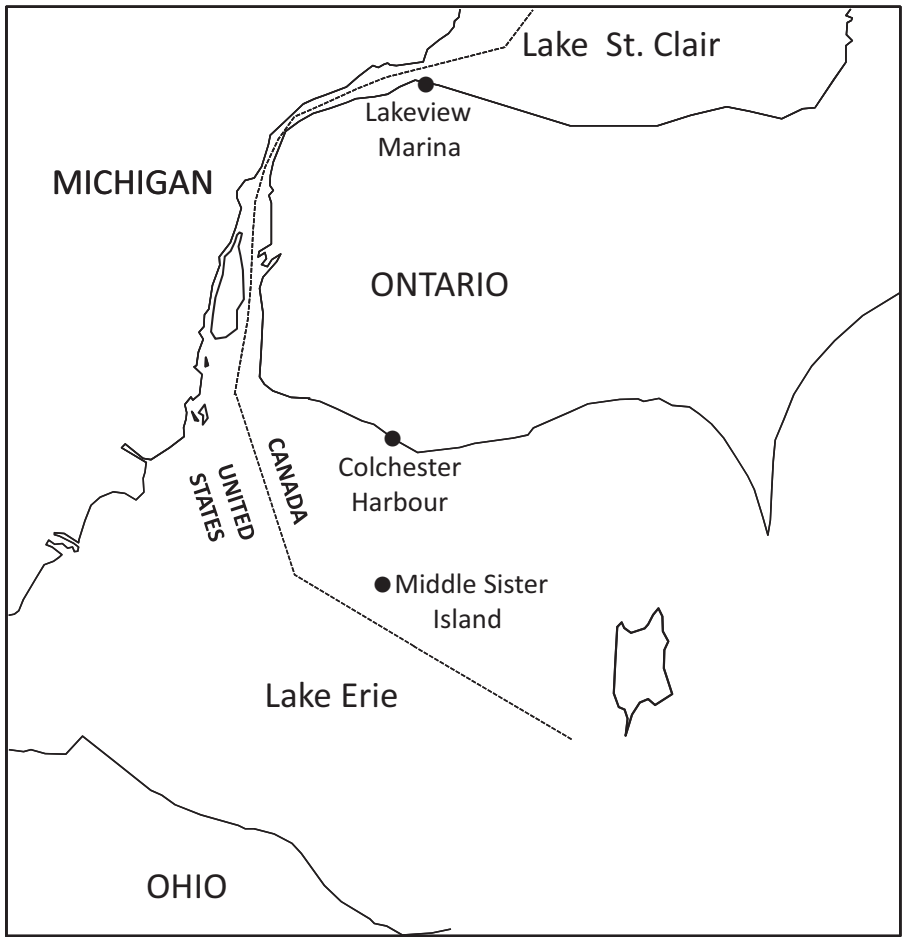

Fig. 1. Collection locations for *Hexagenia* spp. The study sites included Lakeview Marina (42°20′N 82°55′W) on the Detroit River, Colchester Harbour (41°59′N 82°56′W) on the western basin of Lake Erie, and Middle Sister Island (41°51′N 83°00′W) in western Lake Erie.

technique described in Daley et al. [14], with modifications described below. Mayflies were pooled and kept as close to 0.2 g (wet wt) as possible. The extraction used a 0.45-μm glass fiber syringe filter in place of a 1-μm filter between extraction columns and the vacuum manifold. The final volume of the extraction solvent (dichloromethane:hexane, 1:1 v/v; VWR International) was adjusted to 40 ml rather than 30 ml. The extracts were concentrated by rotary evaporator and diluted to 10 ml in hexane. One milliliter of sample was removed for the gravimetric determination of neutral lipids using a microbalance [34]. The remaining extract was concentrated to 2 ml and sample cleanup was performed by florisil chromatography as described by Lazar et al., [35]. After florisil chromatography, extracts were concentrated to 1 ml by rotary evaporator. Samples were analyzed for individual PCBs by gas chromatography-electron capture detection [35]. For each batch of six samples, a reference homogenate, method blank, and an external PCB standard (Quebec Ministry of Environment Congener Mix; AccuStandard) were analyzed. Although forty PCB congeners present in the external standard were examined for, only PCB congeners 49, 95, 101, 118, 138, 149, 153, 158, 170, 180, 194, and 201 (International Union of Pure and Applied Chemistry) were detected with sufficient frequency to be included in the data analysis. Sum PCBs therefore refers to the sum of the above 12 congeners measured in the different samples. Recoveries of individual PCB congeners in the in-house reference tissue extracted with each batch of samples were within two standard deviations of the mean laboratory control charts values for the Great Lakes Institute for Environmental Research accredited organic analytical laboratory. Mean recovery for the sum of 32 PCB congeners in the reference tissue compared to control charts was $96.7 \pm 7.2\%$. The mean recoveries for four individual congeners compared to control charts were as follows: PCB 52 was 108.0 ± 7.7, PCB 101 was 100.3 ± 7.3, PCB 153 was 96.1 ± 6.5, and PCB 180 was 97.4 ± 6.9.

Total nitrogen was determined for pooled mayflies from each life stage and sex as a surrogate measure of tissue protein content. Total nitrogen was determined using an ECS 4010 CHNSO Analyzer. The lean dry protein (%) was calculated by multiplying the percentage of nitrogen in the sample above by a factor of 6.25, determined by the Kjeldahl method.

Data analysis

Polychlorinated biphenyl concentrations were expressed on a lipid equivalents basis to provide an appropriate metric for changes in chemical potential of PCBs. The lipid equivalents concentration was calculated for each PCB congener according to the following equation:

$$C_{\text{org(lipid)}} = C_{\text{org(dry)}} / (X_{\text{lipid}} + 0.05 \cdot X_{\text{LDP}})$$

where $C_{\text{org(lipid)}}$ is the PCB concentration normalized for lipid equivalents (ng/g), $C_{\text{org(dry)}}$ is the dry weight PCB congener concentration (ng/g) in the pooled sample and X_{lipid} and X_{LDP} are the mass fractions (unitless) of neutral lipids and lean dry

protein (LDP), respectively, in the sample. The constant of this equation specifies the partition capacity difference of LDP relative to neutral lipids [36].

Unless otherwise stated, all data presented in the text and figures are reported as mean ± standard error (SE). Statistical differences between mayfly life stages, sex, and site for measured variables, including dry weight, percent lipids, LDP, and lipid-equivalent sum PCB concentrations, were evaluated using analysis of variance (ANOVA). Differences among groups were determined using Tukey's multiple comparison. The normality assumption was tested using the Shapiro-Wilk's test. All statistics were performed using SPSS version 18 (IL, USA). For all statistical tests, a probability value of 0.05 or less was considered a significant difference.

Bioamplification factors (BAmF) were calculated as the ratio of mean lipid-equivalent PCB concentration in imago to subimagos collected from a given site and experimental treatment. For the two locations where black wing pad nymphs were collected, BAmFs were also evaluated between the subimago/nymph and imago/nymph life stages, respectively. Bioamplification ratios in mayfly life stages were evaluated for sum PCBs, but also compared on a congener-specific basis to examine for trends in BAmF with chemical hydrophobicity.

RESULTS

Proximate composition changes across sexes, life stages

Females showed no significant change in dry mass between the subimago and imago life stages. Males showed evidence of significant weight loss ($p < 0.05$; ANOVA) between the subimago and imago life stage at all sites. For the emerged life stages, lipid equivalents differed significantly between sex and life stage ($p < 0.05$; two-way ANOVA). Male subimagos had lower total lipid equivalents compared to the larger female subimagos but generally higher lipid percentages (Fig. 2). Females did not show a major depletion in lipid equivalents between the subimagos and imago life stages across sites or any changes in caged female imagos relative to subimagos (Fig. 2). Males lost a significant ($p < 0.05$; ANOVA) amount of lipid equivalents between the two emergent life stages at all sites (31–61%). The exception was for caged males, which did not undergo significant weight loss or lipid equivalents loss between the subimago to imago stages.

PCB mass and concentration changes

For the locations in which black wing pad nymphs were collected, sum PCB concentrations in nymphs were generally similar to those measured in subimagos, with the exception of female subimagos collected at Lakeview Marina (Fig. 3). At this location, female subimagos had higher sum PCB concentrations compared with values measured in the other female life stages. Generally, lipid-equivalent sum PCB concentrations in male and female nymphs and male and female subimagos were similar (Fig. 3). This observation held for the two immature life stages at Colchester Harbour and Middle Sister Island, and for the black wing pad nymph stages at Lakeview Marina. However, differences were observed between nymphs and subimagos at Lakeview Marina because of the high lipid-equivalent sum PCB concentration noted in subimago females measured at this site.

Lipid-equivalent sum PCB concentrations in male imagos were significantly higher ($p < 0.05$; ANOVA) compared to black wing pad nymphs and subimagos at each of the sites (Fig. 3). Lipid-equivalent sum PCB concentrations between female subimago and imago were not significantly different at Middle Sister Island but did show differences at the other two sites. The PCB concentrations in caged male imagos were not significantly different from field subimagos at any site. The concentrations in caged female imagos compared to subimagos were not different at Middle Sister Island but were at the other two sites. Despite the above-noted differences in lipid-

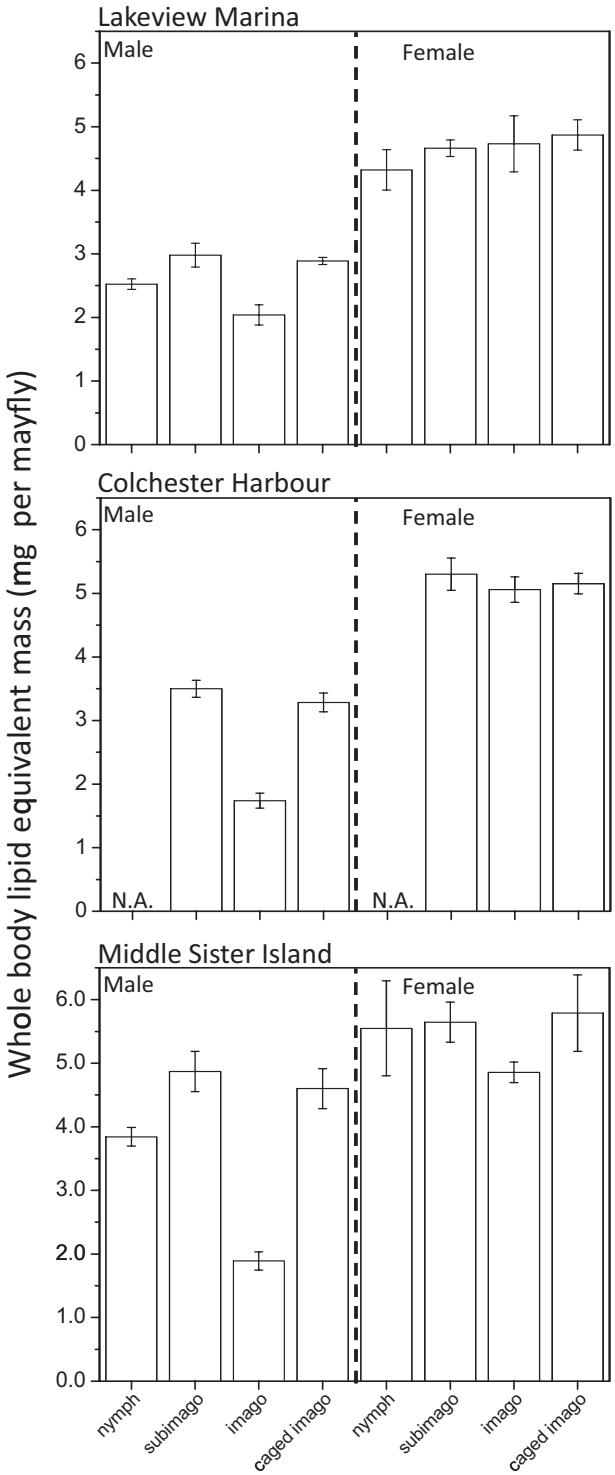

Fig. 2. Mean ± standard error lipid-equivalent mass (mg neutral lipid + mg 5% lean dry protein) in *Hexagenia* spp. across different life stages and by sex from three collection locations. Nymphs from Colchester Harbour (ON, Canada) were not collected.

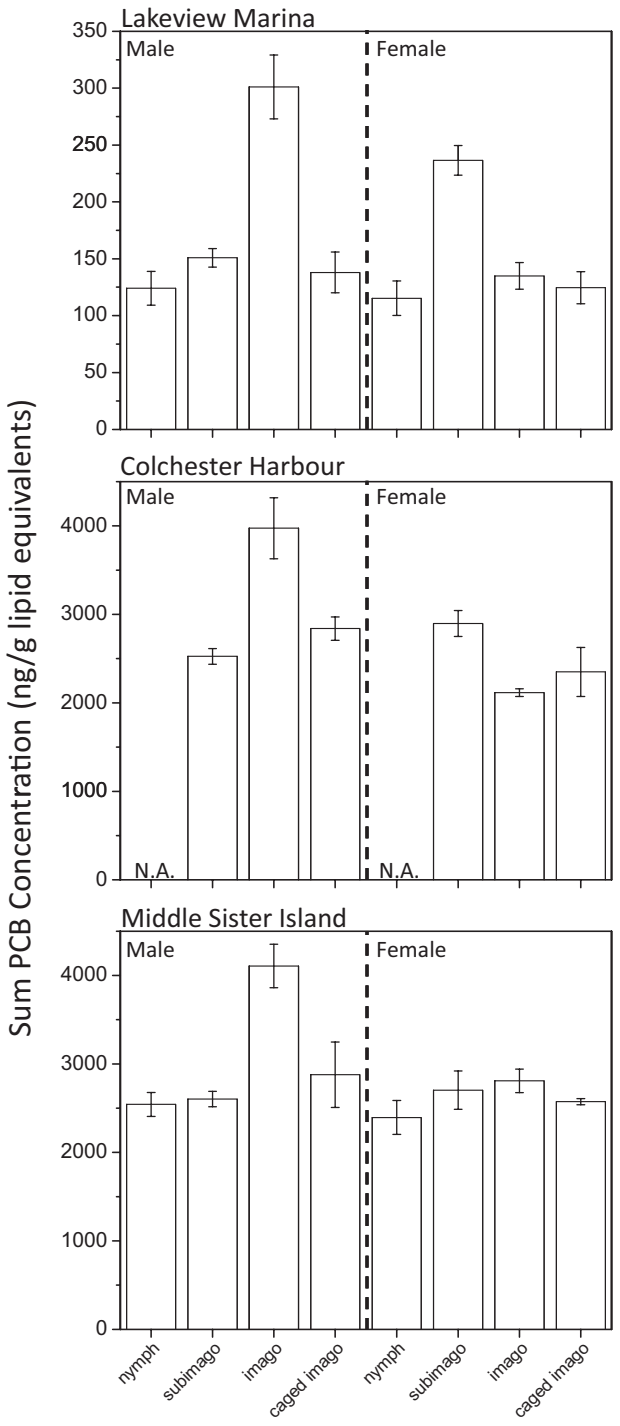

Fig. 3. Mean ± standard error sum polychlorinated biphenyls (ng/g lipid equivalents) in *Hexagenia* spp. across different life stages and by sex from three collection locations. Nymphs from Colchester Harbour (ON, Canada) were not collected.

equivalent sum PCB concentrations for males, no significant changes in the sum PCB mass of animals were noted between the subimago to imago life stage within a given sex and site ($p > 0.5$; ANOVA among all tests between life stages by site and sex). This indicates that PCB elimination did not occur from emerged mayflies during reproductive activity.

Linear regression analysis indicated no significant trends in bioamplification factors with chemical K_{OW} ($p > 0.05$) for the individual sex and site data sets, except for male mayflies from Lakeview Marina, which exhibited an increasing trend of BAmFs with chemical K_{OW} (Fig. 4). The across-site mean BAmF for all congeners for male imago/subimago was 1.91 ± 0.18 and ranged from 1.58 ± 0.05 to 2.43 ± 0.20 between individual sites. For females, the mean BAmF for the imago/subimago was close to a value of unity at 0.97 ± 0.09 and ranged from 0.72 ± 0.03 to 1.22 ± 0.13 between sites. For imago/nymph life stages, the mean BAmFs were 2.05 ± 0.38 for males and 1.17 ± 0.05 for females.

DISCUSSION

Field-collected male mayflies demonstrated an overall weight loss (apart from water) between subimagos and imagos, whereas females did not. Sartori et al [21] found a similar relationship between male and female mayflies (*Siphlonurus* spp.). Male imagos lost as much as half their lipid equivalents mass between the subimago and the sexually mature imago life stage. A much weaker but nonsignificant decline in lipid equivalents was observed for females. It should be noted that female imagos were not checked to identify if egg deposition had occurred, which could alter the proximate composition.

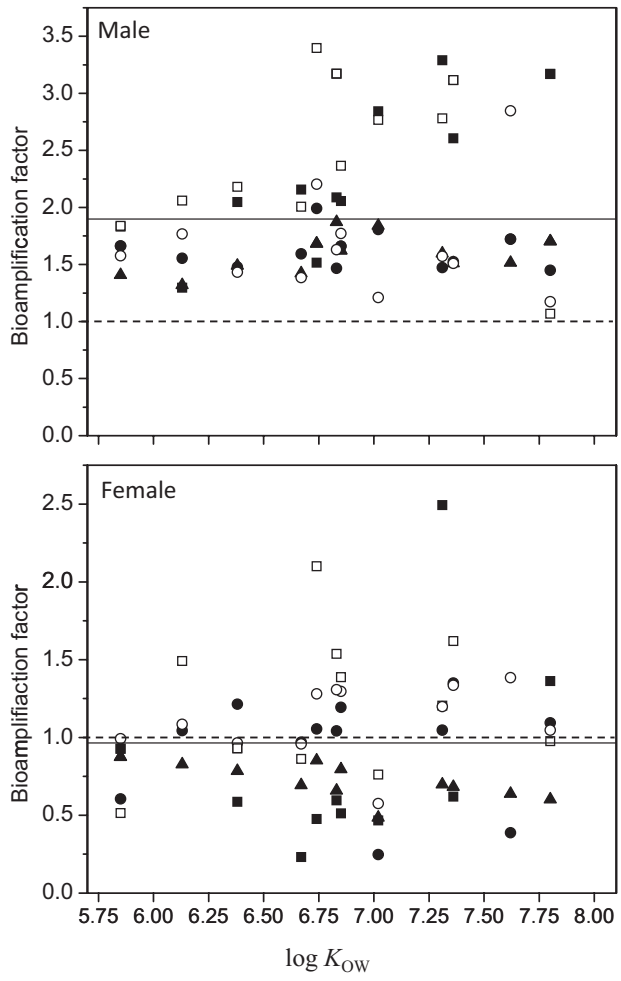

Fig. 4. Bioamplification factors determined for individual polychlorinated biphenyl congeners for Lakeview Marina imago/subimago (■) and imago/nymph (□), Colchester Harbour imago/subimago (▲), and Middle Sister Island imago/subimago (●) and imago/nymph (○). Solid line represents mean bioamplification factor across sites and congeners for males (1.90 ± 0.18) and females (0.97 ± 0.13). Dashed line represents theoretical equilibrium bioamplification factor of 1.0. All sites are located in Ontario, Canada.

Also, neither male nor female imagos were completely exhausted when they were collected, and it is anticipated that greater declines in lipids in the late imago life stages could occur. The different patterns of lipid utilization between field collected males and females are consistent with other mayfly studies [21,31]. The large difference in lipid content between males and females on emergence has been hypothesized to be related to reproductive flight costs required by males during mating swarms [21].

The caging studies allowed the testing of reproductive flight costs as a causal mechanism of energy utilization and lipid depletion observed for males. Caged male and female imagos exhibited nonsignificant dry weight and lipid equivalents loss over a period of time equivalent to the time required for field males to complete their adult life cycle. This supports the conclusion that the lipid losses experienced in field-collected males were largely due to activity, most likely related to flight costs, rather than differences in basal metabolism between the sexes.

The major objective of the present study was to quantify bioamplification in emerged mayflies and compare the magnitude of bioamplification for POPs between sexes and across study locations. The term *bioamplification* was defined as the process by which an organism loses body weight and chemical partitioning capacity faster than it can eliminate contaminants. Although several studies have provided empirical observations of bioamplification of POP compounds [14–20], few attempts have been made to formalize a descriptive term and definition for this bioaccumulation mechanism to distinguish it from bioconcentration and biomagnification [14]. Macdonald et al. [37] referred to this process as ''solvent depletion,'' to describe the physical mechanism of concentration when lipids are lost from an animal. However, solvent depletion as a descriptive term does not portray the major causative physiological (e.g., bioenergetic constraints) and physiological/ecological interactions (e.g., food resource limitations) that result in solvent depletion, nor does it provide a unique and meaningful keyword term for use in ecotoxicology. Alternatively, the word bioamplification has close associative ties with the words bioconcentration and biomagnification, which have very specific meaning in ecotoxicology, particularly when applied to hydrophobic, nonionic organic chemicals. However, it is acknowledged that the word bioamplification has been used in other contexts within the field and was first published by Potter et al. [38] to describe the increase of mercury up a food chain. It is specifically noted that the above definition is now more widely accepted under the term biomagnification and, to the authors' knowledge, the word bioamplification has yet to be applied to POP compounds outside the proposed definition presented above.

In the present study, congener-specific PCB bioamplification was observed in field-collected male imagos. Bioamplification of PCBs was mostly independent of chemical K_{OW} because the elimination of PCBs by emergent mayflies approximated zero. Consequently, the magnitude of bioamplification approached the magnitude of the difference in PCB-partitioning capacity of organisms between life stages. Although significant bioamplification of PCBs was demonstrated for males, the estimates of bioamplification factors established in the present study may not reflect maximum bioamplification factors achieved in spent male or female imagos, because it was impossible to verify and collect mature animals at the very end of their life span.

As expected, caged male mayflies, prevented from flight, exhibited lower to negligible bioamplification compared to field-collected mayflies. One exception was female subimagos from Lakeview Marina, which had unusually high PCB concentrations compared to other female samples, including caged imagos, collected from this site. Given that there were no exceptional differences in lipid or other proximate contents of subimago females from this sample set, the high subimago sum PCB concentration observed in females at this site was likely a result of sampling artefacts at this location due to high spatial heterogeneity of sediment contamination. Drouillard et al. [39] reported spatial variation in PCB concentrations greater than sixfold between the U.S. and Canadian sites of the Detroit River. Due to the constriction of the river channel at the Lakeview Marina location, it is possible that field collected emergent mayflies came from both sides of the Detroit River and thus integrated the high spatial variability of sediment contamination in this region of the Huron-Erie corridor.

Emerged mayfly life stages have been used as biomonitors to detect spatial gradients of sediment contamination [24]. However, differences in the lipid content of male emergent mayflies compared to females can result in different wet-weight PCB concentrations between the sexes. The differences in wet-weight PCB concentration between female and male subimagos occurs as a result of lipid differences accumulated by mayfly nymphs. However, site-specific sex-based differences in PCB concentrations are reduced when concentrations are expressed on a lipid-normalized or lipid-equivalent basis. Last-instar nymphs showed no sex differences in lipid-equivalent PCBs at either site. Drouillard et al. [12] demonstrated that time to steady state for mayflies exposed to sediment-associated POPs was approximately 30 d, a short period of time compared to the aquatic nymph duration of two years. Given the above, and lack of sex-based differences in lipid-equivalent PCB concentrations of black-wing pad nymphs, it is concluded that mayflies, regardless of sex, achieve steady state with the sediments at the time of emergence [12].

If the goal of interpreting contaminants in adult mayflies is to establish spatial gradients in sediment contamination (e.g., use as biomonitors), then it is recommended that mayflies be separated by life stage and sex to insure greater precision in spatially resolved contaminant levels across sites. Subimago males may prove to be the best life stage to monitor because their higher wet-weight concentration means a greater likelihood of detecting POP residues. Alternatively, if subimagos are not to be sorted by sex, then lipid-normalized data are recommended because there is less bias between the sexes compared to wet-weight POP concentrations. Imago life stages will be more strongly influenced by bioamplification processes that will contribute additional variability in samples, particularly when sexes are mixed into sample pools. When using the imago life stage for contaminant biomonitoring purposes, it may be best to utilize female samples to reduce variation of sample concentrations related to individual differences in bioamplification factors.

The risk of sediment contaminant exposure to wildlife consumers of emergent mayflies will likely be underestimated if bioamplification is not considered as part of the exposure assessment. Using the highest BSAF reported by Drouillard et al. [12] of 9.4 for mayfly nymphs and an average bioamplification factor of two in male adults, the combined BSAF · BAmF can be as high as 18.8 above that of sediments for animals that feed predominantly on male imagos. However, the time required to achieve steady state for wildlife that consume emergent insects likely exceeds the approximate two- to four-week period over which mass emergence occurs.

Thus, it is difficult to quantify additional risk to wildlife consumers on the basis of potential change in steady-state biomagnification factors because emergent insects reflect only a small component to the annual integrated exposures of wildlife to seasonally changing diet items. A more realistic assessment may involve model scenarios that estimate the change in total daily intake (TDI) of PCBs that occur pre- and postemergence among wildlife species that exhibit sex- and life stage–biased feeding during such emergence events.

Here, it is demonstrated that animals that preferentially feed on male imagos would achieve higher TDIs compared to animals that feed on subimago life stages (see Supplemental Data for sample calculations). For example, a predator at Colchester Harbour that requires 100 calories to support its daily energy needs and feeds primarily on male imagos would need to consume 69.1% more mayflies and 13.1% greater biomass compared to wildlife having the same energy demands but that feed on subimagos. In this scenario, the predators feeding on Colchester Harbour male imagos would increase their PCB TDIs during the emergence period by 27.0%. Across the three sites, the average increase in wildlife PCB TDIs for animals feeding exclusively on male imagos during the emergence period was estimated to be $36.1 \pm 18.7\%$ compared to wildlife feeding exclusively on male subimagos. Given that imago and subimago life stages show distinct differences in the timing of their availability to predators, it is likely that the above TDI scenarios have some basis in reality. Imagos commence their mating swarms at dusk and are more likely to be consumed by avian consumers such as tree swallows, which are most active at this time of day and capture prey in flight. If male imagos do in fact spend a greater amount of time in active flight defending aerial territories in the mating swarms, then it is possible that aerial feeders, such as tree swallows, will exhibit both stage- and sex-biased feeding and include more male imagos within their diets. Subimagos generally arrive from offshore sites approximately two hours after sunset and remain suspended on vegetation during the next day. These animals are more likely to be consumed by late night feeders (e.g., bats) or a wide variety of diurnal birds including gleaning house wrens, robins, starlings, and other insectivores. These animals will ultimately be exposed to lower mass transfer of PCBs compared to aerial dusk-feeders.

It should be noted that the above simulations demonstrate the potential impacts of bioamplification in emergent insects to wildlife TDIs but do not directly quantify the overall contribution of sediment-terrestrial transfer related to such diet shifts. To estimate the latter, further research to quantify seasonal diet selectivity and diet-specific PCB concentrations would be needed to calculate the overall contribution of emergent insects relative to other diet items to the annual PCB load and bioaccumulation rate in an indicator wildlife species. While such calculations are beyond the scope of the present study, past research on tree swallows has indicated that emergent insects reflect an important contribution to the overall PCB load of these animals [5,6]. Therefore, further research documenting the role of emergent insects to sediment-terrestrial transfer and the effect of bioamplification to this transfer process is warranted.

SUPPLEMENTAL DATA

Tables S1-S2. (151 KB DOC).

Acknowledgement—The present study received financial support from a Natural Sciences and Engineering Research Council (NSERC) grant to K. Drouillard and NSERC-CGS-D scholarship to J.M. Daley. Jan Ciborowoski provided logistical sampling support for mayfly nymphs at Colchester Harbour and Aaron Fisk provided nitrogen content analysis of samples.

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