5-2023

POTENTIAL FOR BIOACCUMULATION AND BIOTRANSFORMATION OF BIFENTHRIN AND 4, 4'-DDT THROUGH SEDIMENT EXPOSURE TO CHIRONOMUS DILUTUS

Eleni K. Robinson
Southern Illinois University Carbondale, elenikrobinson@gmail.com

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by

Eleni K. Robinson

B.S., Southeast Missouri State University, 2019

A Thesis
Submitted in Partial Fulfillment of the Requirements for the
Master of Science Degree

School of Biological Sciences
in the Graduate School
Southern Illinois University Carbondale
May 2023

THESIS APPROVAL

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in the field of Zoology

Approved by:

Dr. Michael Lydy, Chair

Dr. Karolina Kwasek

Dr. Gregory Whitledge

Graduate School
Southern Illinois University Carbondale
March 10, 2023

AN ABSTRACT OF THE THESIS OF

Eleni K. Robinson, for the Master of Science degree in Zoology, presented on March 10, 2023, at Southern Illinois University Carbondale.

TITLE: POTENTIAL FOR BIOACCUMULATION AND BIOTRANSFORMATION OF BIFENTHRIN AND 4, 4'-DDT THROUGH SEDIMENT EXPOSURE TO CHIRONOMUS DILUTUS

MAJOR PROFESSOR: Dr. Michael J. Lydy

The ecological role of chironomids has been described as an abundant and ubiquitous prey item for not only aquatic species, but terrestrial and avian species as well. Global use of pesticides in urban and agricultural applications have introduced a potential threat not only to chironomid populations, but to the individuals that prey on chironomids. Chironomids direct contact with the sediment has resulted in both legacy and current-use pesticides being detected in the individuals throughout their life cycle. The capacity for contaminant uptake and biotransformation among each midge life stage and from larvae to adults, however, is poorly understood. The lipophilic compounds of focus for this thesis include bifenthrin, and p,p'-DDT, along with the biotransformation products, TFP acid, BP alcohol, BP acid, p,p'-DDE and p,p'-DDD. To observe bioaccumulation and biotransformation at each life stage (2^{nd},3^{rd},4^{th}, pupae, and adult), month long exposures were run with a subset of individuals being removed at each life stage and processed to quantify total, parent, and biotransformation product concentrations. Exposures at low concentrations (below literature *C. dilutus* no observable adverse effects concentration) at 25°C produced mean *C. dilutus* total pesticide concentrations ranging from 36.31 to 896.1 μg/kg dw lipid for bifenthrin and from 41.64 to 877.7 μg/kg dw lipid for DDT through all life stages. The 3rd instar contained the highest parent bifenthrin concentration, though this concentration was not statistically different from the concentration in the pupae. The 3^{rd} instar also contained the highest parent concentration of DDT, though concentrations were not

statistically different from concentrations in 2nd to 3rd instar larvae. *C. dilutus* also displayed subsequent biotransformation of DDT to DDE in each instar. By the 4th instar, 87.10% of the total concentration in the midges was DDE and DDD. The biotransformation of DDT to DDD was also observed to occur in sediments and was likely due to direct reductive dechlorination through chemical processes. Chironomid uptake of DDD increased as the parent DDT was degraded to DDD in the sediments over the testing period. This study provides a greater understanding of the bioaccumulation and biotransformation potential in chironomids at each life stage. Initial spiking concentrations were too low, resulting in low body residues and with no method detection limit or reporting limit defined for the study, so these lower measures have limited certainty. Therefore, the implications of this thesis are limited.

ACKNOWLEDGEMENTS

I would like to thank all members of the Lydy Laboratory, my advisor, Dr. Michael Lydy, and Drs. Kara Huff Hartz, Neil Fuller, and Md Habibullah Al Mamun for their continued support, feedback, and assistance. In addition, I would like to thank my fellow graduate and undergraduate students in the lab for their friendship and support throughout my research. Thank you to Dr. Karolina Kwasek and Dr. Gregory Whitledge for serving on my committee and engaging with the ongoing research and writing process. I would also like to express my gratitude to the California Department of Fish and Wildlife Proposition 1 for providing funding for the research in this thesis (CDFW # P1896015).

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CHAPTER 1

INTRODUCTION

Dipterans, such as *Chironomus dilutus*, are emergent aquatic invertebrates with life cycles that include multiple aquatic larval stages (1st through 4th instars), pupa, and flying adult forms (Goertler et al., 2018; **Figure 1**). The Chironomidae taxon has large populations and play a role in nutrient cycling in aquatic ecosystems and are a common prey item for a variety of fishes, amphibians, bats, and waterfowl (Armitage et al., 1995; Reinhold et al., 1999; Martin, 2014; Vesterinen et al., 2020). These sediment-dwelling organisms spend nearly 90% of their life in the benthos feeding and using sediment as a preferred habitat (Watts and Pascoe, 2000; Lydy and Austin, 2004; Martin, 2014). Due to their direct contact with the sediment, chironomids are likely to be exposed to hydrophobic pesticides that have accumulated in sediments over time (Derr and Zabik, 1974; Weston et al., 2013; 2014). Lipophilic pesticides with octanol-water partition coefficients (log K_{ow}) of 3 or more, pose a threat to sediment-dwelling organisms (Klotz, 2001; López-Roldán, 2004; Ensminger, 2012), as highly lipophilic pesticides will bioaccumulate in aquatic species from sediment through the freely-dissolved phase and from dietary and dermal routes of uptake (Scholz, 2012; Katagi and Tanaka, 2016). Lipophilic pesticides of interest for the current study include bifenthrin (log K_{ow} of 6.00), a current use pyrethroid insecticide, and 4,4'-dichlorodiphenyltrichloroethane, referenced as DDT (log K_{ow} of 6.91), a legacy insecticide banned for use by the USEPA in U.S. since 1972 (National Center for Biotechnology Information, 2022a; 2022b; USEPA, 2022). Both compounds are commonly detected within water, sediments and macroinvertebrates in lakes, rivers, flood plains and ecologically sensitive areas (Sommer et al., 2001a; Agency for Toxic Substances and Disease Registry (ATSDR), 2002; Anzalone et al., 2022). Physiological changes during development can

alter an organisms' ability to uptake, biotransform and eliminate these lipophilic contaminants (Cravedi et al., 1995; Buchwalter et al., 2003; Catalan et al., 2004). Over the chironomid life cycle, size, surface area, lipids, and weight vary greatly. These factors seem to influence the rate of bioaccumulation of lipophilic pesticides for each chironomid life stage. Catalan et al. (2004) reported field concentrations of DDT in Chironomidae pupae of 19 μg/kg dry weight (dw) and 5 μg/kg dw in 2ndand 3rd instar larvae, respectively, though there is little information defining the dynamics of uptake and biotransformation among the larval stages. Chironomus larvae have been observed to have a high bioconcentration factor (47,800) in a DDT water exposure over 3 days (Johnson et al., 1971). The bioconcentration of the organophosphate insecticide chlorpyrifos has been observed to decrease from 2nd to 4th instars (Buchwalter et al., 2004). Second instar *C. riparius* accumulated 1.5 to 2.4 times more chlorpyrifos by body weight than the 3rd or 4th instars (Buchwalter et al., 2004). This variation in bioaccumulation potential is thought to be due to the surface-to-volume ratio and differences in biotransformation among the life stages. However, the biotransformation potential of individual chironomid life stages through the adult stage has not been studied (Buchwalter et al., 2004). The potential for adults to retain accumulated lipophilic pesticides is hypothesized to be mostly lost though pupation as dipterans go through significant physiological changes through maturation, showing shifts in metabolic demands and reserves for pupation (Odell, 1998; Merkey, 2011).

Both larval and adult chironomids have been observed to be a common food source and potential source of xenobiotics for a variety of fish, predatory aquatic insects, and bats (Puig-Montserrat et al., 2020; Shadrin, et al., 2021). Gut content studies have found that the dominant prey items in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in northern California are from the taxon Diptera, primarily members of the family Chironomidae (Sommer et al., 2001b;

Goertler et al., 2018). During flooding years, studies have found that dipterans comprise up to 78% of juvenile Chinook diet in the Yolo bypass, dominated by adult chironomids (Sommer et al., 2001a; Anzalone et al., 2022). Therefore, smolt residing in the bypass before they migrate to the ocean could be at increased risk of exposure to hydrophobic pesticides through a dietary route (Newman, 2014; Anzalone et al., 2022). Detected residues in Chinook, macroinvertebrates, and zooplankton within the Sacramento River watershed showed elevated organochlorine pesticides along with elevated bifenthrin concentrations in zooplankton (Anzalone et al., 2022). Both bifenthrin and DDT are commonly detected in juvenile Chinook habitat of the San Francisco Bay Delta and consumption of both larval (Sommer et al., 2001; Kuivila and Hladik, 2008) and adult (Goertler et al., 2018) chironomids has been demonstrated in juvenile Chinook salmon. Eurasian perch (*Perca fluviatilis*)heavily select chironomid larvae during summer months, additionally birds, bats, and dragonflies have been observed to predate on adult chironomids (Brackenbury, 2000; Wagner et al., 2012; Vesterinen et al., 2020).

The goals of the current project were to examine the potential for bioaccumulation and biotransformation of hydrophobic pesticides ^{14}C-labeled bifenthrin and ^{14}C-labeled DDT in multiple life stages of *C. dilutus*. In order to do this, changes in parent compound concentrations in sediments and organisms were analyzed at each chironomid life stage. An optimum testing temperature for chironomid growth was established for the bioaccumulation tests in a preliminary test. This study provides a greater understanding of the bioaccumulation in chironomids and potential for trophic transfer of bifenthrin and DDT through prey species from contaminated sediments.

CHAPTER 2

MATERIALS AND METHODS

2.1 Chemicals

Radiolabeled bifenthrin (^{14}C-ring-labeled, specific activity 41.97 μCi μmol^{-1}, purity ≥ 96%) was obtained from Izotop Institute of Isotopes, Co., Ltd. (Budapest, Hungary) and 4, 4'-DDT (^{14}C-ring-labeled, specific activity 18.7 μCi μmol^{-1} purity ≥ 98%) was purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Radiolabeled stock purities were verified using methods outlined in You et al. (2009). All samples were filtered using Whatman™ polypropylene syringeless filters (0.2 μm pore size). Next, parent compounds were separated from degradation products using an Agilent 1260 high-pressure liquid chromatograph (HPLC) equipped with a fraction collector (Agilent Technologies, Santa Clara, CA, USA) and radioactivity of the fractions were quantified on a Packard TriCarb 2900TR liquid scintillation counter (LSC) (Packard Instrument Company, Meriden, CT, USA). Scintillation cocktail (Ultima Gold) was purchased from Perkin-Elmer (Waltham, MA, USA). Solvents (Optima-grade acetone, and hexane, and HPLC-grade acetonitrile, and water) were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Reagents for lipid analysis including vanillin, 85% phosphoric acid, chloroform and methanol were purchased from Fisher Scientific.

2.2 Test Organisms

Chironomus dilutus were cultured following the methods detailed in U. S. Environmental Protection Agency (1996). Each culture was maintained in a 120-L glass aquarium filled with 80 L of moderately hard reconstituted water (MHRW; Smith et al., 1997) and 8 to 10 cm of shredded paper towel substrate with a sealed fly-space at the top to accommodate adult chironomids. Culture water was maintained at a temperature of 23 ± 1ºC, with a conductivity of

330-360 μS/cm, pH 6-8, and dissolved oxygen levels > 4 mg/L (USEPA, 1996). A 16:8 light:dark photocycle was used and cultures were fed approximately 9 mL of Tetramin slurry each day. Tetramin slurry was made biweekly using 30g of ground Tetramin flakes mixed with 500mL of MHRW (Eisenhauer and Lydy, 1997).

2.3 Determination of C. dilutus Life Stages

An initial experiment was conducted to identify timing of each chironomid life stage under designated laboratory test conditions. Life stages were identified by measuring head capsule width, length, and weight (Watts and Pascoe, 2000; Lydy and Austin 2004). This data was used to inform the duration of later bioaccumulation tests. These tests were conducted in 1-L mason jars with approximately 80 g wet weight (ww) reference sediment (LaRue Pine Hills, IL, USA) free from the pesticides of interest and 750 mL of MHRW with 30 1st instar individuals (< 24 h) / jar. The total organic carbon content of the reference sediment was determined by Midwest Laboratories (Omaha, NE, USA) and was 7.09 ± 0.71% (N=6). Sediments were stored at 4°C and left for 24 h to adjust to testing temperatures. Two tests were conducted at 23°C and 25°C, two jars were sampled at each of the expected life stages for each temperature treatment (estimated from data detailed in the U.S EPA, 1996) and weight, length and head capsule width measures were taken of 30 individuals for each life stage.

2.4 Bioaccumulation Tests - Chironomus dilutus

Multiple measures were taken to observe chironomid bioaccumulation and calculate a biota-sediment accumulation factor (BSAF) or the ratio of lipid normalized concentration in the organism divided by the organic carbon normalized sediment concentration. These measures include standardized weight (dry weight and wet weight), body length, head capsule width (HCW), lipids, and days spent at each life stage for *C. dilutus*. Chironomid bioaccumulation

testing was conducted to understand bioaccumulation and biotransformation capacity of the two pesticides for each chironomid life stage from spiked sediments.

Bioaccumulation experiments consisted of exposing 1st instar *C. dilutus* individually to bifenthrin or DDT dosed reference sediment and MHRW, allowing the larvae to develop to each of the life-cycle stages (2nd, 3rd, 4th, pupae, and adults) and then quantifying body residues at each life stage end point. First instar individuals were used for testing instead of an earlier life stage to ensure reliable counts of individuals in testing, to better observe rates of biotransformation, and increase survival (Norberg-King et al., 2015). Bioaccumulation tests were conducted using a recirculating system with daily 30% water changes in a temperature-controlled room set at 25°C with a 16:8 L:D cycle. All testing chambers had a pre-drilled hole covered with a screen and the adult exposures had fly space cover to avoid loss of individuals during testing.

For each exposure, reference sediment was spiked to levels less than the individual target insecticide's at no observed adverse effect concentrations (NOAEC) chosen from the literature but with high enough activity to read concentrations as determined from preliminary testing. The NOAEC values for chironomids normalized for organic carbon (OC) were 1,200 μg kg^{-1}OC for bifenthrin (Brazner and Kline, 1990) and 670 μg kg^{-1}OC for DDT (Benton et al., 2017). Sediment was spiked in bulk by adding a known amount of radiolabeled pesticide dropwise to sediment in a 5-L stainless steel bowl, while being mechanically mixed for 2 h using a rotating metal blade. After mixing, sediments were stored in the dark for 15 days at 4ºC to age the sediments (OECD 2000; 2010). This aging period allowed the insecticides to reach steady-state conditions with the sediments (Ding et al., 2013). A subset of samples was taken from the bulk spiked sediment at days 0 and 15 to check for uniformity of the insecticide concentrations and to check for any potential degradation prior to introducing test organisms.

Five replicate containers were used for 1st through 2nd, 3rd, and 4th instar exposures and 10 replicate containers were used for the 1st through pupae and adult treatments to ensure adequate numbers of each life stage for quantification. Larvae and pupae treatments were exposed in 1-L jars filled with ~80 g ww dosed sediment and 300 mL of MHRW, while adult treatments were exposed using 2-L tanks with a fly space added, containing ~700 g ww dosed sediment and 4.5 L of MHRW. Five to 10 replicate control jars (2nd instar to adult life stages) were prepared in the same manner as the treatment jars with the exception that the sediments were not dosed with insecticides. During the bioaccumulation study, the room was set on a 16:8 light:dark photocycle, chironomids in each container were fed ~ 0.02 mL/container of Tetramin slurry daily and the mortality and activity of the organisms was monitored (USEPA, 1996). At each life stage timepoint, water chemistry including temperature, pH, ammonia, and conductivity was measured from the control group jars throughout the bioaccumulation testing.

At each life stage end point (5, 12, 18, 23 and 25 days), a subset of 5-10 individuals were removed from the treated containers and used for total radioactivity estimates, while the remaining individuals from the replicates were combined for biotransformation analyses. Total radioactivity and lipids were assessed on control chironomids with 10 individuals used for each analysis, while the remaining control chironomids were used for wet/dry determinations. A 1 mL water sample was taken to determine aqueous insecticide concentrations, while an aliquot of sediment was taken for quantification of total, parent, and biotransformation product concentrations. All methodology was performed under approved radioactive protocols with an approved RCC-3 form.

2.5 Quantification of Pesticide Concentrations

To measure total radioactivity in *C. dilutus*, 5-10 individuals per container were added to a pre-weighed scintillation vial and re-weighed using a Mettler-Toledo XS105 analytical balance (Mettler-Toledo International, Inc., Columbus, OH, USA) to obtain an estimate of wet weight. Following addition of 5 mL of a 50:50 acetone: hexane solution for DDT or 25:75 acetone: hexane solution for bifenthrin, samples were sonicated at 30% amplitude for 10 s on ice in triplicate using a Sonics & Materials VCX400, (Newton, CT, USA). For biotransformation product analysis, a larger subsample of 50 to 60 individuals from pooled replicate vessels were used. These composited samples were added to a pre-weighed scintillation vial, re-weighed as described above and 5 mL of respective acetone: hexane solution added. Following sonication at 30% amplitude for 10 s on ice in triplicate, samples were exchanged to acetonitrile and filtered through a Whatman™ polypropylene syringeless filter (0.2 µm pore size) and concentrated to 100 µL. Parent compound and biotransformation products were separated by reversed-phase chromatography using an Agilent Poroshell 120 EC-C18 column (4.6 x 100 mm, 5 μm particle size) on an Agilent 1260 HPLC equipped with a fraction collector. After addition of 10 mL scintillation cocktail to each collected fraction, samples were incubated in darkness for 24 h to aid in the extraction efficiency, then analyzed using a Packard TriCarb 2900TR LSC.

The biotransformation products of focus for ^{14}C-bifenthrin were cis-3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropanecarboxylic acid (TFP acid, Chemical Abstract Service (CAS) 72748-35-7), 2-methyl-3-biphenylmethanol (BP alcohol, CAS 76350-90-8), and 2-methyl-3-phenylbenzoic acid (BP acid, CAS 115363-11-6) and for DDT the biotransformation products were DDD and DDE (Kaneko et al., 2010; Purnomo et al., 2011). The parent compounds and biotransformation products were determined using the retention times of non-

labeled standards ran individually ran and ran as a mixture to ensure separation. Using a solvent system of 0.1% phosphoric acid acidified HPLC grade water and acetonitrile, peaks of each cold compound were identified by retention time in comparison to a standard, and the retention times were used to make the method to separate radiolabeled compounds. The bifenthrin method used seven fractions, with BP alcohol collected in fraction 4, BP acid and TFP acid in fraction 5, and parent bifenthrin in fraction 6. The method DDT collected six fractions, DDD in fraction 2, DDT in fraction 3, and DDE in fraction 4. Using time-based collection, the bifenthrin method used an eluent gradient from of 0-4 minutes 80:20 acidified water: acetonitrile, 4-6 minutes increasing to 50:50, 6-8 minutes increasing to 30:60, 8-8.5 minutes changing to 50:50, 8.5-12 minutes of 0:100, 12-16 minutes of 0:100 and 16-20 minutes running 80:20. This resulted in retention times of 13.542 minutes for bifenthrin, 9.160 minutes for TFP acid, 8.475 minutes for BP alcohol, and 8.662 minutes for BP acid. The separation method for 4,4' – DDT fractions used an eluent gradient of 0-4.5 minutes of 30:70 acidified water: acetonitrile, 4.5-7.5 minutes of 20:80, 7.5-9 minutes running 0:100, 9-12 minutes of 0:100, 12-16 minutes using 0:100, 16-20 minutes running 70:30. This resulted in retention times of 5.380 minutes for DDD, 8.174 minutes for DDT and DDE at 9.559 minutes.

For total radioactivity analysis of sediments, three replicates of 0.2 g wet sediment were added to a 20 mL scintillation vial and 10 mL of scintillation cocktail was added. Sediment samples were taken at the beginning and end of each experiment. These samples were ultrasonicated on ice at 30% power for 10 s in triplicate, incubated in the dark for 24 h and analyzed on the LSC. For analysis of pesticide degradants in sediments, ~ 1 g of wet sediment was added to a scintillation vial, 5 mL of respective acetone: hexane solution was added and ultrasonicated for 10 s at 30% power in triplicate. All overlying solvent was transferred to a

clean vial and evaporated to ~ 100 μL under a gentle flow of nitrogen and filtered through a Whatman™ polypropylene syringeless filter Mini-UniPrep™ (0.2 μm pore size). Samples were separated by reversed-phase chromatography as described above and quantified by LSC.

2.6 Lipid Content Analysis

Total lipid content of control individuals for *C. dilutus* was evaluated using a colorimetric phosphoric-vanillin method (Van Handel, 1985). Vanillin-phosphoric acid reagent was prepared with 100 mL of near boiling (~92°C) deionized water and mixed with 600 mg of vanillin in a 1-L amber bottle. After addition of 400 mL of 85% phosphoric acid, the solution was stored in darkness to avoid darkening of the solution and was used within 12 h. A 1 mg/mL vegetable oil standard was made dissolving 10 mg of oil in the same volume (in mL) of chloroform (e.g. 0.0102 g oil with 10.2 mL of chloroform). The calibration curve standards were created by placing 10, 25, 50, 100, 200, and 400 μL of the 1 mg/mL vegetable oil standard into culture tubes (in triplicate) and treated the same way as each sample. Three blanks were included with each group of standards. Pre-dried frozen lipid samples were added to culture tubes in triplicate, then 0.5 mL of a 1:1 chloroform:methanol mixture was added to each sample and standard. Each sample and standard culture tube was placed in an 80°C water bath until the 0.5 mL 1:1 chloroform:methanol reagent mixture was fully evaporated. After cooling for approximately 5 min, 0.3 mL of sulfuric was added and the tubes placed back into the 80°C water bath for 15 min. After cooling, 5 mL of vanillin-phosphoric reagent was added and mixed through manual inversion. These samples and standards were developed for 5 min and were allowed to sit for no longer than 30 min before measuring the absorbance at 525 nm. The standard curve was measured first followed by the samples and a blank was included every third measurement. All pesticide data were lipid normalized on a dry weight basis.

2.7 Calculations and Statistics

BSAF values were calculated using average lipid normalized biota concentrations (C_b/f_{lipid}) divided by the average organic carbon content normalize sediment concentrations (C_s/f_{OC})at each life stage. Using average BSAF values for each life stage the associated relative standard error (RSE) and the standard error in the BSAFs were calculated. The RSE was calculated as the uncertainty that is propagated from the standard deviation and average of lipid normalized organism concentrations and organic carbon normalized sediment concentrations (Equation 2).

Equation 1

$$\text{Biota} - \text{sediment accumulation factor (BSAF in kg OC/kg lipid)} = (C_b/f_{lipid})/(C_s/f_{OC})$$

C_b: Lipophilic compound biota concentration

f_{lipid}: Lipid concentration

C_s: Lipophilic compound sediment concentration

f_{OC}: Organic carbon content

Equation 2

$$\text{Relative standard error (RSE)} = \sqrt{\left(\frac{\text{stdev}_{\text{midge}}}{\text{average}_{\text{midge}}}\right)^2 + \left(\frac{\text{stdev}_{\text{sediment}}}{\text{average}_{\text{sediment}}}\right)^2}$$

Equation 3

$$\text{Standard error (SE)} = \text{RSE x BSAF}$$

Statistical significance was determined among each life stage and parent and biotransformation products in exposure studies. A one-way ANOVA and a Tukey's honesty significant difference (HSD) post-hoc test was run for these measures taken in the temperature optimization study, the sediment degradation studies, and the *C. dilutus* bioaccumulation and

biotransformation studies. Lipid normalized and organic carbon dry weight measures were used to calculate BSAF values and statistical significance using ANOVA was determined for each life stage. Assumptions of normality and homogeneity of variance were assessed using Shapiro-Wilk and Levene's tests, respectively. A type I error rate of $\alpha = 0.05$ was used for each test. All statistical analysis was performed using RStudio version 1.3.1073 (R Core Development Team 2013) using libraries ggplot and multicomp.

CHAPTER 3

RESULTS

3.1 Temperature optimization study

An initial study was conducted to determine a good temperature at which to run the bioaccumulation studies. Temperatures of 23 and 25°C were compared to see if faster growth rates could be achieved at the higher temperature. A faster growth rate is preferred since it would allow for shorter experiments. Results showed no detrimental effects on mortality or shifts in growth measurements were due to the increased temperature. Measurements taken (length, head capsule width, and weight) indicated that each life stage develops faster and resulted in larger organisms in the later life stages, so 25°C was chosen for the temperature of the bioaccumulation tests (ANOVA, $F_{2,12} = 5.8$ $p = 0.022$) (**Table 1**).

3.2 Water Quality

All water quality measures during the bioaccumulation tests fell within Organization for Economic Co-operation and Development (OECD) acceptable ranges, with average and standard deviation in temperature of 24.6 ± 0.5ºC, conductivity of 335 ± 16 µS/cm, pH 7.42 ± 0.12, dissolved oxygen levels 7.27 ± 0.05 mg/L and ammonia levels never exceeding 0.25 mg/L throughout testing (OECD, 2010). Water quality measures were taken at setup and at each breakdown (life stage) throughout each test.

3.3 Sediment Concentrations

A literature NOAEC value (1,200 µg bifenthrin $kg^{-1}OC$ and 670 µg DDT $kg^{-1}OC$) was used as a baseline for spiking bifenthrin and DDT into the sediments. Pesticide concentrations of parent compound were measured in the sediments at day 0 and at each determined life stage end point (5, 12, 18, 23 and 25 days). Average total sediment concentrations for bifenthrin and DDT

throughout testing (n=18) were 135.13 ± 36.14 and 47.19 ± 14.86 μg/kg OC, respectively (**Figures 2A; 3A**). No significant differences were found in parent bifenthrin or DDT concentrations in the sediment over the testing period (ANOVA, $F_{2,12} = 0.227$ $p > 0.944$ for ^{14}C -bifenthrin: $F_{2,12} = 0.24$ $p > 0.936$ for ^{14}C -DDT). Totals included parent compound and any biotransformation products that degraded in the sediments during testing. Bifenthrin sediment concentrations remained constant throughout testing with over 97.25% of the total concentration represented as parent bifenthrin (Figure 3A). The DDT in the sediment degraded to DDD over the 14-d testing period, and by the 15th day of the test, the sediment contained mostly DDD (78% was DDD; Figure 2A).

3.4 C. dilutus concentrations

Bioaccumulation of total bifenthrin and DDT were tracked through each life stage to calculate lipid normalized concentrations for each test. Bifenthrin individuals had average percent lipids of 10.29 ± 7.26 for adults, 3.15 ± 1.10 for pupae, 1.90 ± 0.82 for 4th instar, 1.30 ± 0.17 for 3rd instar, and 2.87 ± 0.31 for 2nd instar individuals (**Table 2**). The average percent lipids for each life stage (n=3) were 7.67 ± 1.68 for adults, 6.88 ± 0.73 for pupae, 4.13 ± 0.72 for 4th instar, 5.14 ± 0.60 for 3rd instar and 5.09 ± 0.52 for 2nd instar individuals in the DDT test. Lipid values were calculated using diluted samples for values reaching or exceeding the high standards. This reduced uncertainty for values exceeding the end of the calibration curve.

Mean *C. dilutus* total pesticide concentrations (n=15) ranged from 36.31 to 896.1 μg/kg dw lipid for bifenthrin totals and from 41.64 to 877.7 μg/kg dw lipid for DDT through all life stages (**Table 3**). Total bifenthrin and parent concentrations were highest in the 3rd instar, though were not statistically different from the pupae (p=0.19). The 3rd instar also contained the highest parent bifenthrin, though these body residues were not significantly different from the pupae life

stage (ANOVA, $F_{2,12} = 16.73$ $p = 0.00020$). Similarly, for parent DDT, body residues were highest in the third instar, though these were not significantly different from the second instar (ANOVA, $F_{2,12} = 6.21$ $p = 0.0089$) (Figures 2B; 3B). Additionally, the rate of uptake of DDD increased as the parent DDT was degraded to DDD in the sediments over the testing period (ANOVA, $F_{2,12} = 4.06$ $p = 0.0065$).

3.5 Bioaccumulation/Biotransformation

Total measures account for originally spiked parent compound and any biotransformation products that degraded in the sediments during the tests. Use of a HPLC with fraction collection allowed for separation of parent and biotransformation products to observe bioaccumulation and biotransformation in biota and sediments. Bioaccumulation of bifenthrin was observed in *C. dilutus* and parent compound was not as readily biotransformed to BP acid, BP alcohol, or TFP acid polar metabolites as DDT was observed to be biotransformed to DDE (Figures 2B; 3B). Biotransformation of bifenthrin in chironomids resulted the highest concentration of polar metabolites in the 3rd instar, though these concentrations were not significantly different from the 2nd instar, pupae, or adults.

DDT that was spiked into the test sediments was observed to quickly degrade into DDD (2.63 to 95.83%) by direct reductive dechlorination (Figure 2A), while DDT was biotransformed by *C. dilutus* via dechlorination through biotic processes to DDE (13.33 to 55.56%) (Figure 2B). Total DDT concentrations were highest in the 3rd instar, though not statistically different from 4th instar or pupae ($p=0.015$; $p=0.031$). Parent DDT concentrations were not statistically different from 2nd to 3rd instar ($p=0.015$; $p=0.031$). *C. dilutus* also displayed subsequent biotransformation of DDT to DDE in each instar. By the 4th instar, 87.10% of the total concentration in the midges was DDE and DDD (Figure 2B). The DDT metabolites, DDE, and DDD, were highest in pupae,

though the DDD concentrations were not statistically different from the 4th instar concentration (p=0.16), and DDE concentrations were the highest in the 3rd instar, but was not statistically different from the 4th instar or pupae (p=0.88, p=0.93, respectively).

BSAF values ranged from 0.39 – 4.15 kg OC/kg lipid and 1.81 – 32.5 kg OC/kg lipid for parent bifenthrin and DDT, respectively (Table 4). Each exposure showed accumulation of lipophilic pesticides from sediments to chironomids over the life cycle. Total and parent bifenthrin BSAF values did not vary much as polar metabolites only represented a small portion of the total concentration. Therefore, 3rd instar and pupae contained the highest BSAF values for both totals and parent bifenthrin (Figure 4C; 4D). However, the 3rd instar and pupae were not significantly different from the BSAF value of the 2nd instar in terms of totals (p=0.24, p=0.41, respectively) or parent (p=0.22, p=0.18, respectively) BSAF values. Total DDT BSAF values were highest for the 3rd instar, though not statistically different from the 4th, pupae, or adult life stage (p=0.31, p=0.68, p=0.06, respectively; Figure 4A). Parent DDT BASF values were highest in the pupae, though not statistically different from the 3rd, 4th or adult life stages (p=0.93, p=0.08, p=0.72, respectively). For both parent and total DDT, the largest difference between BASF values was observed from the 2nd to 3rd instars (p=0.04).

CHAPTER 4

DISCUSSION

Bifenthrin and DDT, which are lipophilic insecticides, were observed to bioaccumulate in midges, especially in the 3rd instar life stage. The bioaccumulation process can be impacted by several factors including differences in size and surface area: volume ratio of individuals, sex, lipid levels, and biotransformation potential (Derr and Zabik,1974; Buchwalter et al., 2004; Fuentes et al., 2005). For example, the size of individuals can vary seasonal within and among species affecting the surface area: volume ratio and resulting body residue of lipophilic pesticides in the individuals (Gresens et al., 2012). Different species of chironomids have variable growth rates and sizes and therefore spend differing amounts of time at each life stage. Buchwalter et al. (2004) observed that uptake rates of chlorpyrifos for 2nd, 3rd and 4th instars were influenced by surface area: volume ratios and cuticle thickness and acknowledged that each life stage had varying levels of sensitivity. Additionally, sexual dimorphism was not accounted for in the current study, but can result in differing growth measures including head length measurement and overall body size for each sex and species, each factor potentially impacting bioaccumulation potential (e.g., *Chironomus tepperi* and *Chironomus frommeri*) (Atchley, 1971). Though, Oliver and Danks (1972) observed sex ratios of chironomids using pupal exuviae, and these sex ratios were observed to be at unity or nearly balanced most of the time. The main influence that resulted in skewed sex ratios observed in exuviae was seasonal effects (i.e., temperature) and in some species, parthenogenesis was observed to result in predominately females. Derr and Zabik (1974) found no difference in the rate of uptake of radiolabeled DDE from dead or live chironomids and a positive correlation was found between uptake and cuticle surface area of individuals. This indicated that the primary route of uptake for DDE, in this

instance, was through passive diffusion relying on movement through the tegument to internal lipids. In the current study, bioaccumulation seems to correlate to surface area except the 4th instar levels were lower, though not significantly lower than 3rd instar or pupae levels. However, 4th instar measures for bifenthrin had less certainty due to a smaller sample size (n=14). Therefore, lipid levels and surface area: volume ratios of the individuals are thought to be the primary drivers of hydrophobic contaminant bioaccumulation in chironomids.

Buchwalter et al. (2004) determined that differences in parent: metabolite ratios and basal acetylcholinesterase (biotransformation enzyme activity) among life stages was due to the 4th instar larvae having significantly increased ability to metabolize chlorpyrifos in comparison to the 2nd or 3rd instar larvae. The current study observed similar discrepancies in body residues among each life stage, which may be explained by similar influences (i.e., surface area: volume ratios, cuticle thickness, and varying levels of sensitivity and metabolic potential) though these influences were not directly measured in the current study. Therefore, uptake and metabolic potential at each life stage is variable and will result in differing body residues among species (Fuentes et al., 2005).

Bifenthrin is highly persistent in sediments (environmental half-life = 7 – 18 months) with major biotic degradation products being TFP acid, BP alcohol, and BP acid, though degradation products of bifenthrin are not observed at equally high environmental concentrations as parent compound bifenthrin (Mukherjee et al., 2010; Rogers et al., 2016). This was observed in the current study as bifenthrin remained primarily as parent compound in the sediments and primarily biotransformed within chironomids. As bifenthrin is biotransformed in organisms, the biotransformation products can be eliminated and released into the environment (Derby et al.,

2021). Parent bifenthrin has high photostability, resistance to aqueous hydrolysis, and is stable in anerobic soils.

Alternatively, parent DDT readily degraded in the sediment to DDD once it was placed at 25°C in the flow-through system used for testing in the current study. The aquatic environmental half-life of DDT is 2 - 30 years, due to the low vapor pressure of DDT, it is less likely to be chemically broken down into biotransformation products once in an aquatic environment. (Chattopadhayay and Chattopadhayay, 2015; U.S. EPA, 1989). DDT is thought to degrade via anaerobic microorganisms or through ammonium production through chironomid activity by reductive dechlorination to the metabolite DDD (Häggblom, 1992; U.S. EPA, 2000; Zhirong et al., 2013). Katagi and Tanaka (2016) and Ma et al. (2019) also observed degradation of DDT to DDD to occur in freshly spiked lab sediments within 17 days. Therefore, chironomids were not exposed to DDT alone throughout the testing period in the current study or the aforementioned studies. It is hypothesized that sediments went anaerobic over the time frame of the study, allowing DDT to transform to DDD via reductive dechlorination. Under field conditions, uptake of DDD and DDT with an increased production of DDE would be expected in individuals as enzymes responsible for dechlorination are upregulated to biotransform the DDT. The formation of DDE is commonly observed through biotic processes when an organism is exposed to DDT. Degradation of DDT to DDE has also been observed in sediments, though it is not well understood (Aislabie et al., 2010). Similar degradation of DDT in sediments has been explained through the synergistic action of the aerobic bacteria species *Ralstoni picketti* and white-rot fungus *Pleurotus eryngii*, the production of DDD is via reductive dechlorination. There is also potential for the subsequent biotransform of DDD to DDE through dehydrochlorination,

though this is most commonly observed to be produced in the livers of mammals (Peterson and Robinson, 1964; Morgan and Roan, 1974; Purnomo et al., 2011; 2019).

Exposures at NOAEC allowed for a picture of hydrophobic pesticide uptake while avoiding any compounding effects on chironomids. Althrough, environmentally relevant concentrations are often much higher, with some bifenthrin sediment concentrations have been observed to reach 2900 ug/kg OC (Hintzen et al., 2008) and DDX sediment concentrations have been recorded at 696-43,054, parent DDT from 0-10,363, DDD at 329-8,320, and DDE ranging 249-29202 ug/kg OC (MacDonald et al., 2003; Michelsen, 1992). These hydrophobic compounds have been observed using toxicokinetic models to better detail the fate and movement of these compounds in aquatic environments. Kuo and Chen (2021) used 40 organic chemicals with log K_{ow} values ranging from 1.46 to 7.75 to create a database of toxicokinetic models of each organic compound for chironomids. Equilibrium partitioning has been used to model bioaccumulation and movement of contaminants into food webs using BCF and BSAF values that correlated with experimental data. Aamir et al. (2017) observed trends among BSAF values and the log K_{ow} for hydrophobic chemicals with log K_{ow}'s ranging from 3.7 to 6.8. The measured BSAF values indicated that hydrophobicity is the main driver of an organism's bioaccumulation potential. BSAF values vary substantially for most sediment-dwelling species and can range from 0.02 to 48.47 kg OC/kg lipid for DDT, DDD, and DDE (U.S. EPA, 2007). Kesic et al. (2021) observed BSAF values for DDE (log K_{ow} of 6.51), DDT (log K_{ow} of 6.12) and DDD (log K_{ow} of 6.02), from orchard soil to earth worms, recording average values of 16.1 ± 6.1, 5.59 ± 2.6, and 26.4 ± 7.4 kg OC/kg lipid (U.S. National Library of Medicine, 2022a; 2022b). In the current study, BSAF values for total bifenthrin (log K_{ow} of 6.00) ranged from 0.65 to 5.15 kg OC/kg lipid and 0.36 to 4.26 kg OC/kg lipid for parent bifenthrin. In comparison, BSAF values

ranged from 3.12 to 15.37 kg OC/kg lipid for total DDT and 1.81 to 26.25 kg OC/kg lipid for parent DDT.

This movement of pesticides can be exemplified by calculating BASF values. These values allow for a simplification of the movement dynamics of lipophilic pesticides from sediments to lipids. Crago et al. (2016) observed polychaetas with DDT body residues with BSAF values averaging 0.007 kg OC/kg lipid, well below BASF values observed in the current study (DDT BSAF = 1.81 – 32.5 kg OC/kg lipid). These lower values observed by Crago et al. (2016) were tropically transferred though feeding of the DDT exposed polychaetas to hornyhead turbot. This resulted in an observable biomagnification of DDT recorded as biomagnification factors (BMF). The BMF's were determined by dividing the DDT body concentrations of the turbot by the DDT concentrations presented it the diet (polychaetas) resulting in lipid normalized BMF's ranging 0.91- 4.09 μg/kg, demonstating movement of low body concentrations through trophic transfer.

Sediment-dwelling organisms have been observed to introduce a route of uptake of sediment-bound contaminants through ingestion to higher trophic organisms (Brander et al., 2016). The bioaccumulation of lipophilic pesticides, such as bifenthrin, DDT, and their metabolites have been observed to occur at relatively low environmental concentrations that can introduce a significant route of uptake for species that predate on chironomids (Environmental Health Criteria 83, 1989; Corcellas et al., 2015; Rossi et al., 2020; Magnuson et al., 2022). Anzalone et al. (2022) observed prominent prey items of threatened juvenile Chinook salmon were primarily Amphipoda and adult Diptera over the two-year project. These prey items were analyzed for prevalent pesticides and found elevated DDE and bifenthrin in adult and larval dipterans, indicating a potential source of uptake of DDE through trophic transfer (Anzalone et

al., 2022). Body residues of these macroinvertebrates (primarily dipterans) ranged from 6.10 – 153 μg/kg lipid of bifenthrin, 5.99 – 368 μg/kg lipid for DDT, and 15.4 – 1562 μg/kg lipid for DDE (Anzalone et al. 2022). Concentrations in the current study fell within these detected ranges with average bifenthrin concentrations of 280.4 μg/kg lipid, DDT concentrations of 94.5 μg/kg lipid, and DDE concentrations of 133.6 μg/kg lipid. The juvenile salmon sampled by Anzalone et al. (2022) had detectable concentrations of predominately bifenthrin, DDT, and DDE. These concentrations were used to observe any possible olfactory effects on juvenile salmon through a dietary route. Magnuson et al. (2022) fed bifenthrin spiked diets bifenthrin at concentrations reflecting field macroinvertebrate concentrations determined in Anzalone et al. 2022 and concentrations observed in the current study. This exposure resulted in reduced swimming endurance of exposed juvenile salmon, demonstrating a negative correlation of swimming capacity and juvenile salmon bifenthrin body residues.

Chironomids have high concentrations of crude fat, fatty acids, most essential amino acids, and have a high caloric content compared to other aquatic prey, such as zooplankton and copepods making them preferred prey species (Bogut et al., 2000; Weston et al., 2014; Goertler et al., 2018). In the current study, adults were shown to have the highest percent of total lipids among the various life stages. However, mean percent lipids were not significantly different among the 2nd, 4th, or pupae life stages due to the high variability of measures, as only 5 individuals were measured per life stage. In contrast many mosquito species have been observed to maintain similar lipid content from pupae to adult life stages (Lang 1963). As a prey species, the high body concentrations of amino acids and caloric content of chironomids have been shown to have a positive effect on fish growth, condition, and health (Bogut et al., 2000). Wagner et al. (2012) observed a pelagic food web in the Saidenbach Reservoir (Saxony,

Germany), and found increased predation on these lipid rich chironomids from May to June. During this time-period, Eurasian perch showed selection of pupae over the other life stages for consumption. Pupae represented approximately 20–48% of Eurasian perch diet and were commonly caught, because pupae swim to the surface and prepare for emergence and are easily consumed as they move (Brackenbury, 2000). Birds, bats, and dragonflies have been observed to overlap in their predation selecting lipid-rich adult chironomids. Adult Diptera comprised 20% of each predators' diet, close only to the predation on Lepidopteran species (Vesterinen et al., 2020). Multiple bat species have been observed to be exposed to pesticides in agricultural landscapes through multiple exposure pathways including dermal, oral, and inhalation (Brooks et al., 2017). Chiropteran exposure endpoints are limited to bird and ground-dwelling mammal information (Brooks et al., 2021). However, exposure to legacy compounds have been observed to result in significant population declines in Brazilian free-tailed bat (*Tadarida brasiliensis Mexicana*) over the span of 52 years. Body residues showed high concentrations of DDT in the 1950's and 1960's as populations declined, nearly 4.8 and 2.7 times the amount detected 20 years later. Persistent exposure to organochlorine pesticides in bats can result in transfer of contaminants from mother to young and reproductive disorders among the population (Ecobichon, 2001). A high foraging rate for chironomids observed for select species of fishes and waterfowl therefore can be linked to exposure to non-polar organic contaminants (Straub et al., 2007).

The trophic transfer of bifenthrin, DDT and their metabolites may be impacted by a variety of chironomids behaviors, habitat shift, and chironomid population change. The consumption of chironomids may increase as climate change influences habitats such as shifts in pH, drought, and increasing salinity and reduction of other prey species that may be less resilient

to change. Chironomids tolerant to a broad range of environmental conditions, and can be found in areas of extreme temperature, salinity, and pH (2.8 – 8.2) (Havas and Hutchinson, 1982). Additionally, during periods of receding water levels, chironomid larvae can survive drying (McLachlan, 1970). He et al. (2017) and Chattopadhayay and Chattopadhayay (2015) have observed an additional route of potential uptake to pelagic aquatic species due to chironomid burrowing activity as chironomids resuspend sediments containing hydrophobic contaminants. Contaminants associated with total suspended solids, such as methylmercury (MeHg) were observed by Capman et al. (1980), to be resuspended due to *L. hoffmeisteri* burrowing activity making MeHg available for uptake by pelagic organisms. Along with multiple routes being introduced, the risk of increased trophic transfer is extenuated as organisms become resistant to pesticides over long-term exposures.

There are several uncertainties and issues with the study design of this thesis that need to be discussed. The main uncertainty associated with the thesis is the low spiking concentrations used to spike the sediments for the bioaccumulation tests. Spiking concentrations in this study were below the NOAEC reported for bifenthrin and DDT for chironomids to insure the survivorship of individuals with no observable adverse effects on the test population. However, this resulted in low ^{14}C-labeled bifenthrin and ^{14}C-labeled DDT body residues in the chironomids. This is exacerbated as parent compound was biotransformed within chironomids, dividing total measures further into 2-3 fractions, parent compound and multiple metabolites. Additionally, the 4th instar measures for the bifenthrin exposure reduced statistical power as mortality was high for this group so the sampling was of a much smaller group of individuals (n=14) in comparison to the other life stages (n= 50-60). The degradation of parent compound DDT to DDD in sediments after 17 days reduced the amount of available parent compound for

uptake, therefore reducing available activity in the parent fraction of body residues. This is a repeatedly observed phenomenon in freshly spiked laboratory sediments that is not fully understood.

The uncertainties found in the current study pertain to these detection limits, as these limits were not well defined leading to multiple indeterminate results as body residues were low. Supplemental changes to the study that would allow for an increase of statistical power and certainty would include increasing the spiking concentrations or sample size as, using sediments treated to restrict microbial growth to avoid the degradation of DDT in sentiments, as well as establishing the method detections limits (MDL) and reporting limits (RL) to determine values that are significantly different from the blank measures (Harris, 2010). A signal detection limit in dpm (disintegrations per minute) can be calculated using $n \geq 7$ measures at a low spiking concentration (1 to 5 times the detection limit) at the time of experimentation and computing the standard deviation of these measures. Having no defined MDL or RL resulted in uncertainty in the measures as MDL and RL calculated during the experiment could differ from post experimental estimates. Non-instrumental limitations including low specific activity of stocks used, low exposure levels, and no differentiation for sexual dimorphism or any differences among male and female individuals' weight, length, or lipids were not accounted for in the study. All measurements were made from a random sample of chironomids. To better observe chironomid pesticide uptake dynamics, observing uptake at the egg casing life stage of chironomids and making distinctions between sexes would create a full picture of chironomid life stage uptake. For example, Katagi and Tanaka (2016) observed that egg mass concentrations of DDT and DDE in *C. tentans* contained nearly 30% of the total observed uptake.

CHAPTER 5

CONCLUSIONS

In conclusion, uptake of both bifenthrin and DDT was observed in each aquatic chironomid life stage. The 3rd life stage had the highest total bioaccumulation of lipophilic pesticides from sediments, though 3rd instar total bifenthrin concentrations were not statistically different from pupae concentrations, and total DDT concentrations were not statistically different from 4th or pupae concentrations. Chironomids exposed to bifenthrin spiked sediments showed uptake of parent bifenthrin with limited biotransformation to polar metabolites. Throughout testing, the degradation of DDT to DDD was observed in sediment within 15 days, resulting in DDD uptake in chironomids. Additionally, detectable biotransformation of DDT to DDE in chironomids was observed. Adult chironomids were observed to have lower total concentrations of parent and biotransformation products than pupae, likely due to the biological changes and loss of mass during pupation. Comparatively, adults had concentrations 3-5 times lower than the pupae in the bifenthrin and DDT tests. These results lend themselves to a better understanding of potential route of sediment exposure to benthic species as well as the bioaccumulation and biotransformation differences at each life stage for species that go through pupation. Lower body residues recorded in the current study have decreased statistical confidence, as no method detection limit or reporting limit was defined prior to the experiment. Without the prior calculation of a detection limit, calculated concentrations from low measured activity have higher degree of uncertainty. Body residues and BSAF values determined in the current study were comparable to other studies observing trophic transfers of lipophilic pesticides from prey species to higher order species. This could introduce a potential risk of exposure through trophic transfer to predatory species feeding on chironomids, though residues observed in this study have

a lower degree of certainty due to low spiking levels, low body residues, and the lack of a defined detection limit. This species rich and commonly occurring freshwater invertebrate family is responsible for a portion of secondary production and overall energy flow in aquatic ecosystems, therefore bioaccumulation of lipophilic pesticides by midges could have impacts on aquatic ecosystems, interrupting trophic cascade, and increasing the risk of exposure to contaminants. Exposures therefore could result in population effects in chironomids as well as potential movement of lipophilic pesticides through the food web from benthic aquatic species over time.

EXHIBITS

Table 1. Life cycle growth tests were performed to ensure health through testing, compare growth to literature, establish methodology and quantify lipids for bioaccumulation and biotransformation normalization including standard deviation of the data set and statistical significance for each life stage among each temperature. (ANOVA, $F_{2,12} = 5.8$ $p = 0.022$) HCW = head capsule width

C. dilutus life stage 23ºC				
	Days	Body Length (mm)	Weight (g)	HCW (mm)
1st	0-5	0.61 ± 0.26	0.005 ± 0.002	0.15 ± 0.019
2nd	5-10	3.3 ± 0.48	0.010 ± 0.009	0.16 ± 0.018
3rd	11-18	5.1 ± 0.56	0.025 ± 0.0026	0.25 ± 0.013
4th	19-31	8.3 ± 0.46	0.05 ± 0.011	0.58 ± 0.02
Pupae	32-34	8.2 ± 0.75	0.006 ± 0.16	2.1 ± 0.35
Adult	34-36	7.7 ± 0.46	0.0022 ± 0.00087	1.1 ± 0.33

C. dilutus life stage 25ºC				
	Days	Body Length (mm)	Weight (g)	HCW (mm)
1st	0-3	0.71 ± 0.18	0.005 ± 0.001	0.15 ± 0.023
2nd	3-6	5.6 ± 0.28	0.011 ± 0.002	0.28 ± 0.017
3rd	6-15	12.5 ± 0.29	0.035 ± 0.002	0.34 ± 0.025
4th	15-19	15.5 ± 0.57	0.06 ± 0.012	0.44 ± 0.028
Pupae	19-27	9.25 ± 0.62	0.0063 ± 0.0001	2 ± 0.36
Adult	27-30	9.25 ± 0.75	0.0024 ± 0.0003	1.1 ± 0.33

Table 2. Average percent lipid dry per life stage and lipid normalized data for bifenthrin and DDT chironomid total concentration

Bifenthrin		
life stage	mg/g dry weight lipid	% lipid dry
2nd	28.70 ± 3.08	2.87 ± 0.31[ab]
3rd	12.98 ± 1.70	1.30 ± 0.17[a]
4th	19.02 ± 8.23	1.90 ± 0.82[ab]
Pupae	31.46 ± 10.98	3.15 ± 1.10[ab]
Adult	102.92 ± 39.08	10.29 ± 7.26[b]
DDT		
life stage	mg/g dry weight lipid	% lipid dry
2nd	50.89 ± 5.16	5.09 ± 0.52[ab]
3rd	51.41 ± 6.01	5.14 ± 0.60[ab]
4th	41.28 ± 7.20	4.13 ± 0.72[a]
Pupae	68.81 ± 7.31	6.88 ± 0.73[ab]
Adult	76.72 ± 16.81	7.67 ± 1.68[b]

Table 3. Sediment (μg/kg OC) and *C. dilutus* (μg/kg lipid) concentrations (n=3) of totals, parent and biotransformation products averages (± one standard deviation). nd = not detectable

Sediment				
	Total	DDT	DDD	DDE
Day 0	44.07 (±11.68)	44.07[a] (±11.68)	nd[c] (*)	nd[e] (*)
Second	50.64 (±26.30)	48.64[a] (±25.27)	1.33[c] (±0.02)	0.67[fg] (±0.35)
Third	42.18 (±10.01)	8.23[b] (±1.97)	32.92[d] (±0.44)	1.03[g] (±0.24)
Fourth	52.87 (±8.32)	6.37[b] (±1.00)	44.59[d] (±0.35)	1.91[f] (±0.30)
Pupae	53.54 (±26.05)	2.23[b] (±1.09)	51.31[d] (±0.89)	nd[e] (*)
Adult	46.07 (±4.12)	1.88[b] (±0.17)	43.96[d] (±1.26)	0.23[ef] (±0.02)

C. dilutus				
	Total	DDT	DDD	DDE
Second	158.02[a] (±103.34)	87.80[ad] (±57.41)	nd[e] (*)	70.22[ad] (±45.92)
Third	648.14[b] (±268.56)	216.03[ac] (±89.51)	216.03[ac] (±89.51)	216.03[ac] (±89.51)
Fourth	417.69[b] (±113.07)	53.88[ad] (±14.59)	188.63[ac] (±51.06)	175.18[ac] (±47.42)
Pupae	562.97[b] (±137.21)	72.62[a] (±17.70)	308.73[bc] (±75.24)	181.62[ac] (±44.26)
Adult	184.67[a] (±35.21)	41.98[d] (±8.00)	117.52[ac] (±22.40)	25.19[f] (±4.80)

Sediment			
	Total	Bifenthrin	Polar metabolites
Day 0	149.97 (±12.97)	149.97 (±12.97)	nd[a] (*)
Second	105.70 (±29.08)	104.25 (±28.68)	1.45[b] (±0.40)
Third	136.30 (±0.10)	133.55 (±0.09)	2.75[c] (±0.002)

Fourth	169.44 (±33.66)	169.44 (±33.66)	nd[a] (*)
Pupae	100.10 (±23.03)	97.33 (±22.40)	2.76[c] (±0.64)
Adult	149.26 (±53.71)	148.54 (±53.45)	0.72[ab] (±0.26)

C. dilutus			
	Total	Bifenthrin	Polar metabolites
Second	334.67[ab] (±53.79)	260.30[cd] (±41.84)	74.36[ef] (±11.95)
Third	702.25[b] (±211.29)	554.43[d] (±166.81)	147.82[f] (±44.48)
Fourth	133.14[a] (±2.20)	114.11[c] (±1.88)	19.03[e] (±0.31)
Pupae	478.60[b] (±84.06)	414.80[d] (±72.86)	63.80[e] (±11.21)
Adult	97.60[a] (±95.94)	58.56[c] (±57.56)	39.04[e] (±38.38)

Table 4: Biota-sediment accumulation factors for bifenthrin and DDT (total and parent) as average (± one standard error) kg OC/kg lipid. BSAF values are calculated using average midge and sediment concentrations from each life stage, the standard error calculated by multiplying the BSAF value and the relative standard error in BSAF.

Life Stage	Bifenthrin		DDT	
	Total	Parent	Total	Parent
Second	3.17[ab] (±1.01)	2.50[ab] (±0.80)	3.12[a] (±2.61)	1.81[a] (±1.51)
Third	5.15[b] (±1.55)	4.15[b] (±1.25)	15.37[b] (±7.34)	26.25[ab] (±12.54)
Fourth	0.79[a] (±0.16)	0.67[a] (±0.13)	7.90[ab] (±2.47)	8.46[ab] (±2.65)
Pupae	4.78[b] (±1.38)	4.26[b] (±1.23)	10.51[ab] (±5.72)	32.53[b] (±17.70)
Adult	0.65[a] (±0.68)	0.39[a] (±0.41)	4.01[ab] (0.84)	22.33[ab] (±4.70)

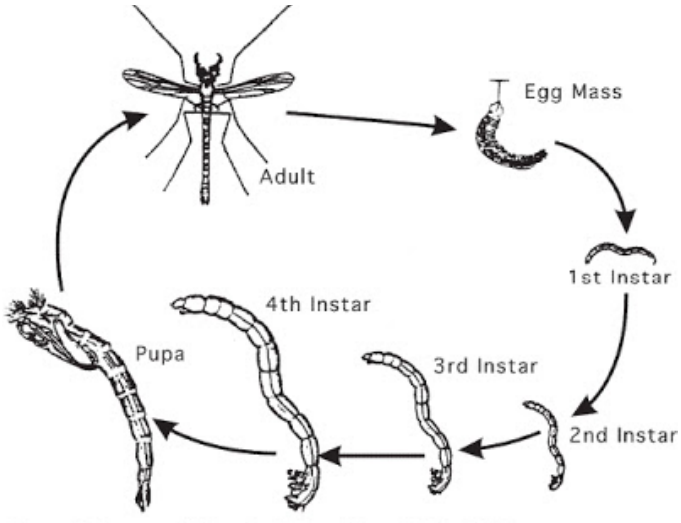


Figure 1. Life cycle of chironomids taken from Walker (1987)

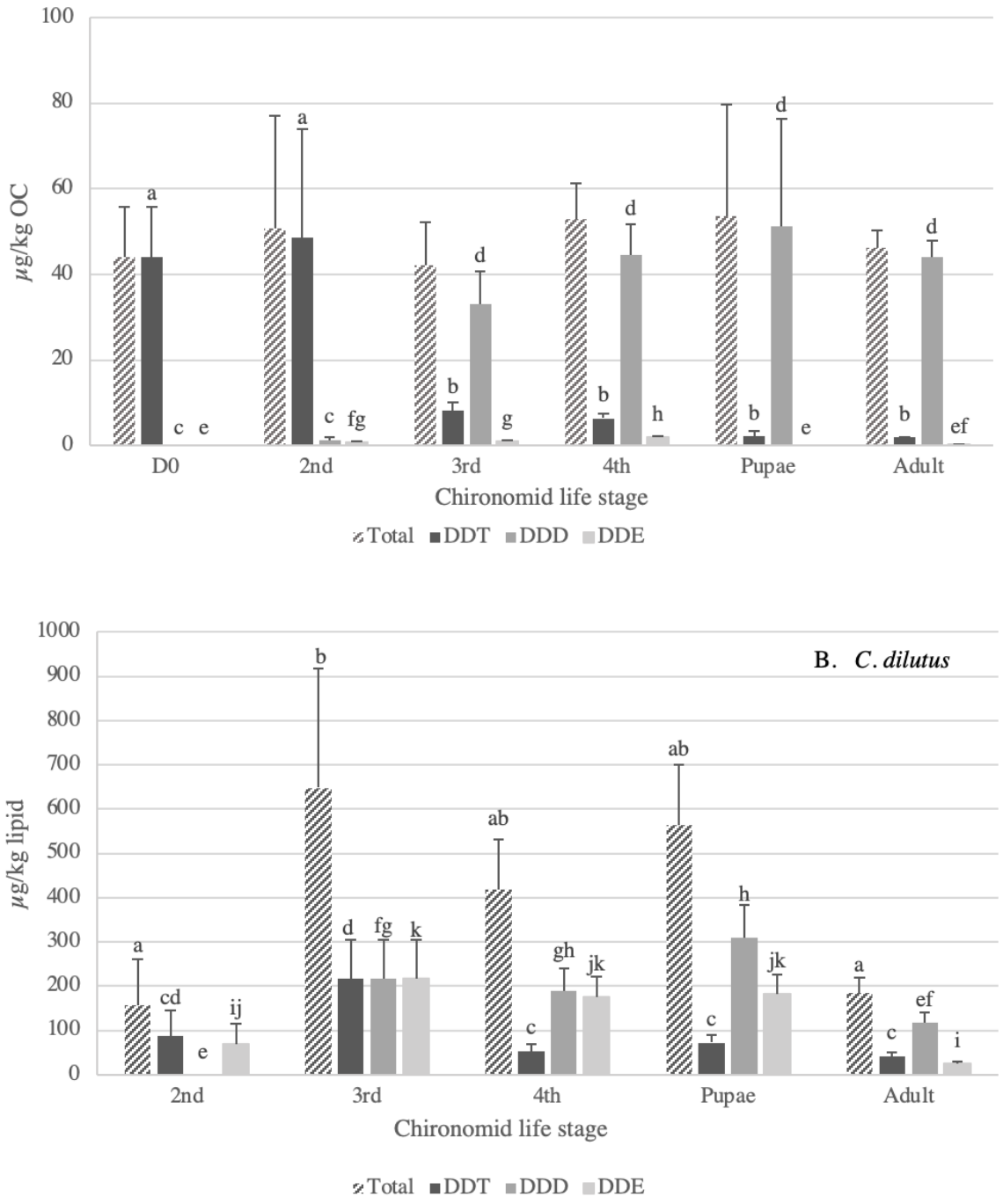


Figure 2. Sediment and *C. dilutus* concentrations for DDT throughout the testing period measured as total radioactivity and as DDT, DDD, and DDE (n=3). A. Sediment concentrations (measured as totals) were not statistically different throughout the testing period (n=3, $F_{2,12}$ = 0.24; $p > 0.936$). DDT and DDD concentrations were statistically different and the differences among concentrations for a specific compound are designated with different letters; B. Chironomid concentrations were statistically different with differences among concentrations for a specific compound designated with different letters (DDT totals: n=10; $F_{2,12} = 6.265$; $p > 0.00864$). Standard deviation indicated by error bar for each measurement.

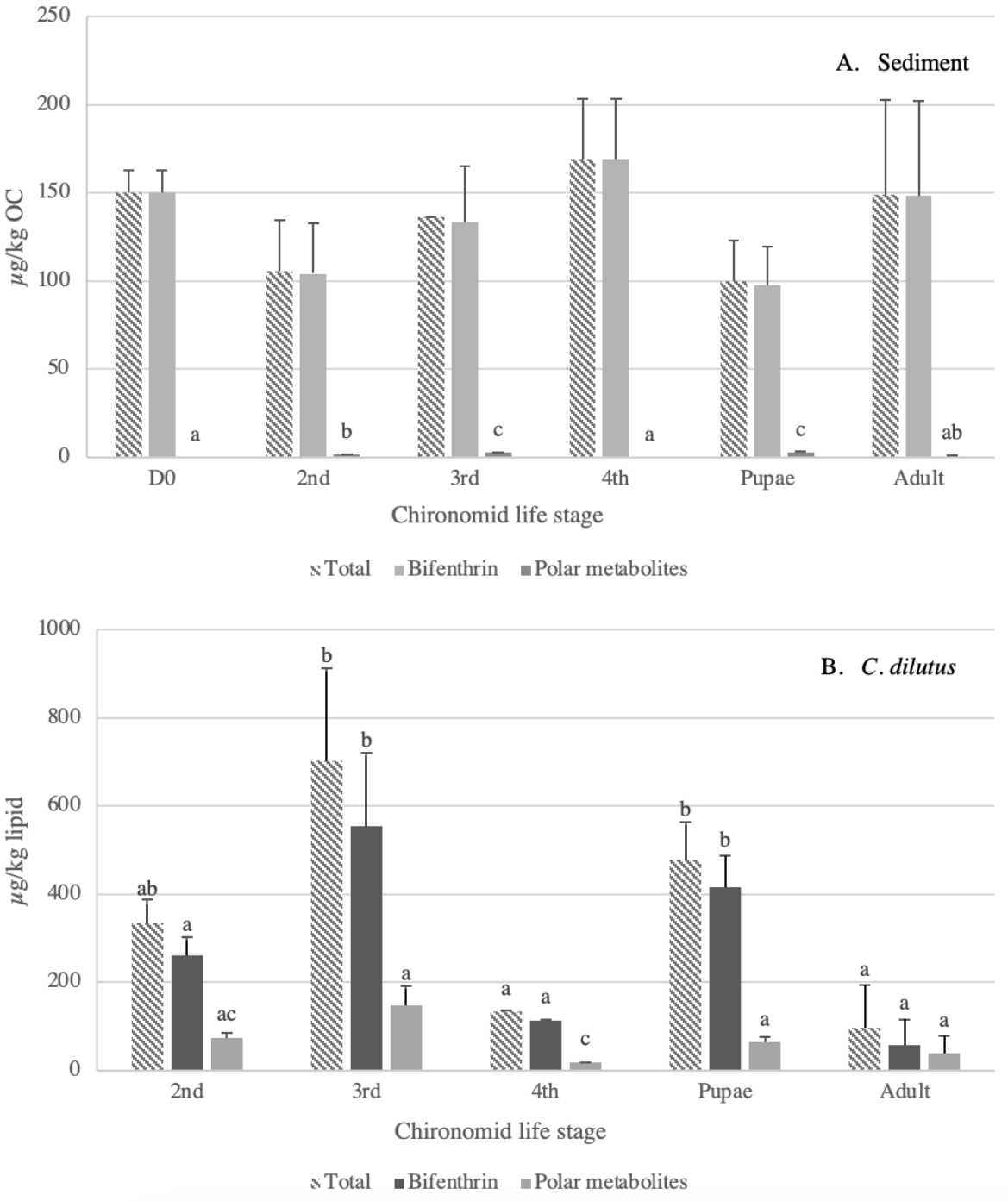
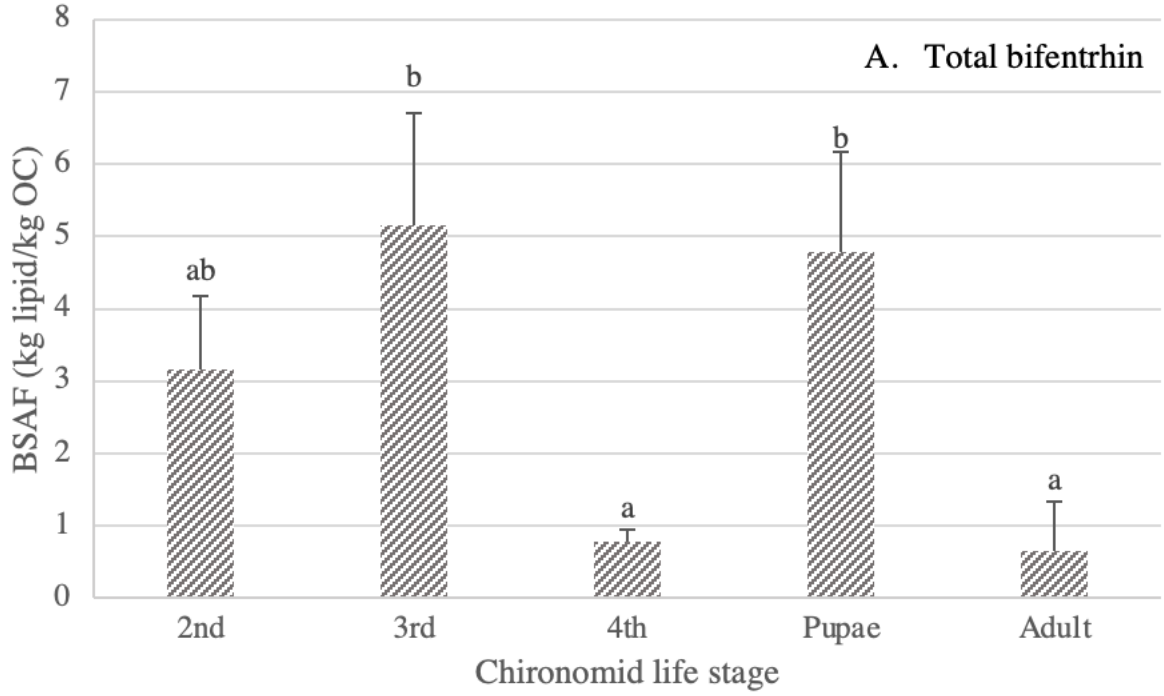
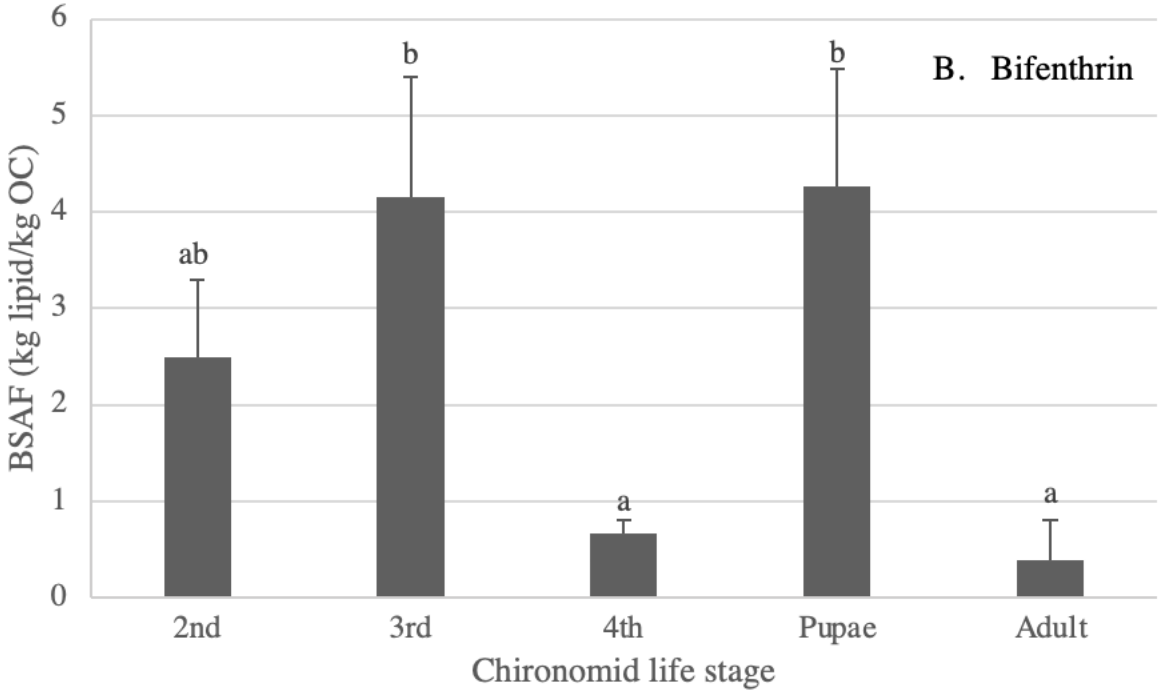


Figure 3. Bifenthrin sediment concentrations though testing exposure are measured as totals and bifenthrin plus polar metabolites. A. Sediment total or bifenthrin concentrations were not statistically different over time (n=3, $F_{2,12} = 0.227$; $p > 0.944$) and significance of polar biotransformation products are indicated by different letters. B. Bifenthrin and polar metabolite concentrations in chironomids are tracked over each life stage and significance for total, bifenthrin, and polar metabolites are marked by different letters (bifenthrin totals: n=10, $F_{2,12}$ =14.79; $p > 0.000334$). Standard deviation indicated by error bar for each measurement.


A. Total bifentrhin
BSAF (kg lipid/kg OC)
8
7
6
5
4
3
2
1
0
ab
b
a
b
a
2nd
3rd
4th
Pupae
Adult
Chironomid life stage


B. Bifenthrin
BSAF (kg lipid/kg OC)
6
5
4
3
2
1
0
ab
b
a
b
a
2nd
3rd
4th
Pupae
Adult
Chironomid life stage

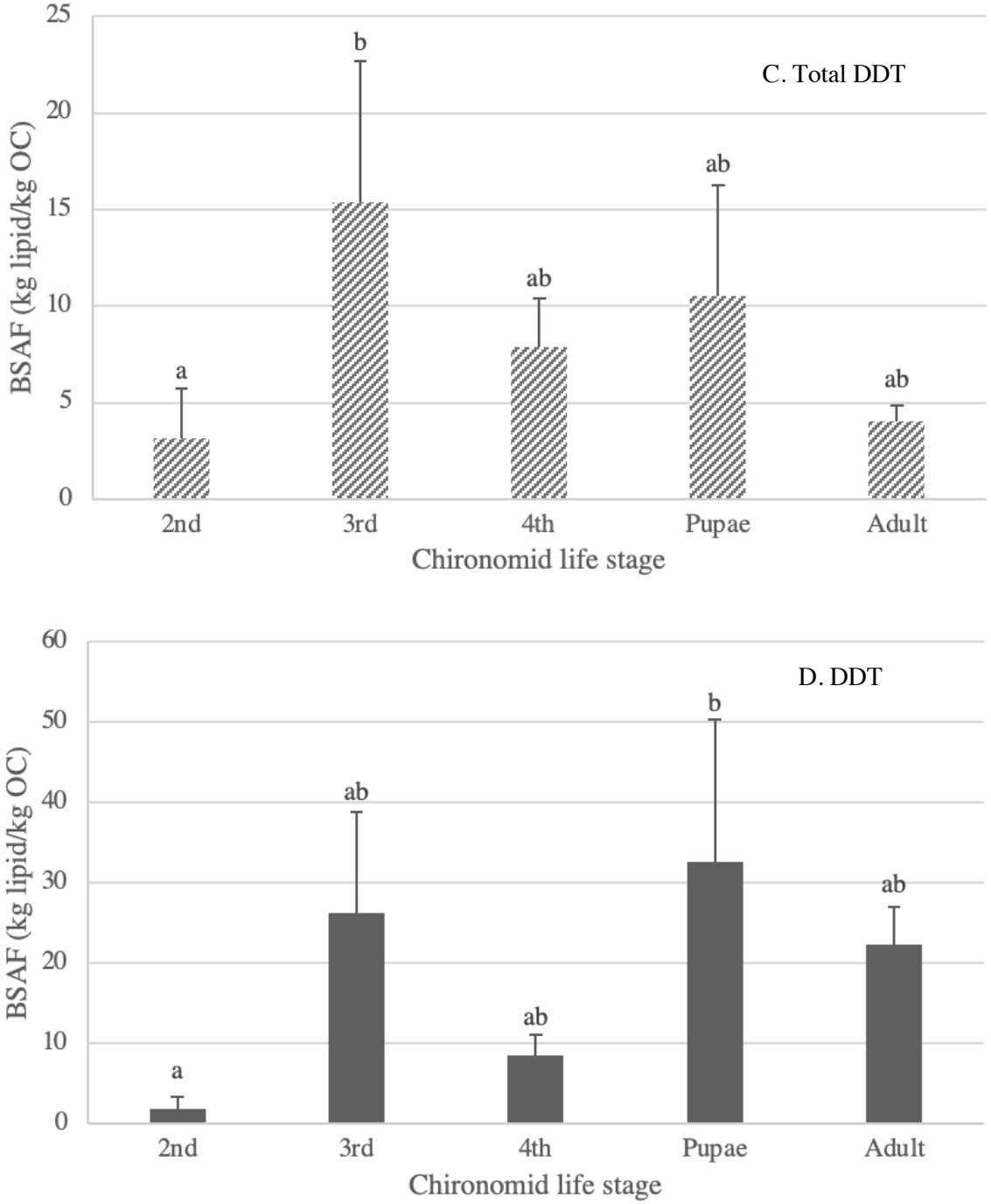


Figure 4. Biota-sediment accumulation factor (BSAF) of A. chironomid for totals through bifenthrin testing, B. parent compound only, C. totals through DDT testing, and D. and parent DDT only (n=3). Significance of each life stage is marked using different letters and standard deviation are indicated using error bar for each BSAF.

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VITA

Graduate School
Southern Illinois University Carbondale

Eleni K. Robinson

elenikrobinson@gmail.com

Southeast Missouri University
Bachelor of Science, Marine Biology and Mico/Celluelar/Molecular/Biotechnology, May 2019

Thesis Paper Title:

Potential for Bioaccumulation and Biotransformation of Bifenthrin and 4, 4'-DDT Through Sediment Exposure to *Chironomus dilutus*

Major Professor: Dr. Michael J. Lydy

Publications:

Fuller, N., Huff Hartz, K. E., Johanif, N., Magnuson, J. T., Robinson, E. K., Fulton, C. A., Poynton, H. C., Connon, R. E., Lydy, M. J. (2021). Enhanced trophic transfer of chlorpyrifos from resistant *Hyalella azteca* to inland silversides (*Menidia beryllina*) and effects on acetylcholinesterase activity and swimming performance at varying temperatures. *Environmental Pollution*, *291*. DOI: 10.1016/j.envpol.2021.118217 PMID: 34583267

Presentations:

Eleni K. Robinson, Neil W. Fuller, Kara E. Huff Hartz, and Michael J. Lydy. Potential for Trophic Transfer of p,p'-DDT and Metabolites to Juvenile Chinook Salmon (*Oncorhynchus Tshawytscha*) from Consumption of *Chironomus dilutus*: SETAC North America 42nd Annual Meeting, (2021). Portland, Oregon.

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