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MICROCOSM EVALUATION OF THE BIOACCUMULATION OF ORGANOCHLORINE PESTICIDES FROM SOILS IN THE NORTH SHORE RESTORATION AREA AT LAKE APOPKA

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Prepared by

MARIA S. SEPÚLVEDA[1], TIMOTHY S. GROSS[1,2], SHANE D. RUESSLER[2], AND JESSICA A. GROSSO[1],

[1]Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610;
[2]USGS-BRD, Florida Integrated Science Center, Center for Aquatic Resource Studies, Gainesville, FL, 32653; and

For

The Department of Water Resources, St. Johns River Water Management District, Palatka, FL, 32178.

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ABSTRACT

During 1998 - 1999, a significant bird mortality event occurred after flooding of a former agricultural property on the north shore of Lake Apopka, Florida (North Shore Restoration Area, NSRA). High organochlorine pesticide (OCP) tissue concentrations from dead birds suggest these compounds may have contributed to the mortality event. The objective of this study was to determine biota sediment accumulation factors (BSAFs) of different OCPs in two biota species (Eastern mosquitofish, *Gambusia holbrooki* and calico crayfish, *Orconectes immunis*) in relation to varying levels of OCP and total organic carbon (TOC) content of soils collected throughout the NSRA. Biota was exposed in 700-L tanks to inundated sediments, with three replicates per treatment. We tested four levels of soil OCPs (based on toxaphene values: controls, below detection limit; low < 7,000 µg /kg; medium >12,000 - < 30,000 µg /kg; and high > 22,000 µg /kg) and three levels of soil TOC (low < 10 %; medium > 18 and < 26 %; and high > 38 %). Twelve treatments were established (November, 2001), and test organisms were sampled at regular intervals (weeks 0, 2, 4, 8, 12, and 16). Site ZSS0767 also served as an additional microcosm ("Hot Spot"), with very high soil OCPs (mean toxaphene of 174,000 µg/kg) and TOC (38 %). Crayfish appeared to have adapted less than mosquitofish to captive conditions, and showed a decline in lipid content over the course of the study. Lipid contents in crayfish were about half to those measured in mosquitofish. Regardless of species, over 98% of all the OCPs bioaccumulated consisted of toxaphene > 4,4'-DDE > 4,4'-DDT > 4,4'-DDD > dieldrin. This pattern of bioaccumulation matched the distribution of OCPs in soils. Overall,

mosquitofish tended to bioaccumulate higher concentrations of OCPs compared to crayfish. For some of the treatments, mosquitofish and crayfish had body burdens of OCPs that fell within survival threshold values previously reported for other freshwater fish species. This is a significant finding because it would suggest that these species are less likely to die if exposed to relatively high concentrations of OCPs. Sublethal effects, however, should be potentially examined in future studies.

In both species OCP concentrations in tissues increased significantly during the first two weeks of experiment, and remained more or less constant until the end of the study (week 16). After 8 weeks of exposure, animals were considered to have reached steady state, and only these OCP values (i.e. those attained between weeks 8 and 16) were used for calculating BSAFs. Overall, BSAFs were higher (1.5 to 2.6 times) in mosquitofish compared to crayfish. Log K_{ow} was not a driving factor in the BSAFs attained. The OCPs with highest BSAFs in this study were metabolites of DDT, namely 4,4'-DDE and 4,4'-DDD. The reason behind the higher BSAFs for metabolites as compared to that of parent compounds remains unknown at this time.

Since BSAFs are calculated using soil TOC values, estimations of BSAFs based on soil TOC values would be inherently biased because of the autocorrelation between dependent and independent variables. Thus, we used the ratio of the OCP in tissue to that in soil (Accumulation Potential or AP) as a way to evaluate the effect of soil TOC on bioaccumulation. Regardless of species, there was a significantly negative relationship between soil TOC and AP for most of the OCPs studied. Similarly to other studies, BSAFs were variable and in many instances were high and exceeded the theoretical limit of 1 – 2. This range in BSAFs across studies could limit the use of this model as

the only method for screening the bioaccumulative potential of sediments, and strengthens the need for determination of specific BSAF values on an analyte by analyte, and species by species basis as optimal. Nevertheless, the BSAFs reported in the present study, fall within ranges previously reported for other species and, despite all of the uncertainties, these values can still be used for estimating exposure of OCPs by fish-eating birds inhabiting NSRA flooded marshes. Furthermore, BSAFs in the present study were fairly consistent (regardless of percent TOC) which strengthens its use as a predictive model for determining bioaccumulation in biota at a wide range of sites within the NSRA.

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CHAPTER 1
INTRODUCTION

Background and Justification

During the fall of 1998 and winter of 1999, a significant bird mortality event occurred on the former agricultural property on the north shore of Lake Apopka, an area now known as the North Shore Restoration Area (NSRA). An estimated 676 birds died during this event, including migratory (American white pelicans, *Pelecanus erythrorhynchos*) and federally endangered (woodstorks, *Mycteria americana*) bird species. In addition, hundreds of additional birds ingested unknown quantities of organochlorine pesticides (OCPs) that could have potentially impacted their long-term reproductive success.

Following this mortality event, the U.S. Fish and Wildlife Service (USFWS) and the Department of Justice (DOJ) initiated an investigation into the cause of the bird mortality and the person(s) or entity(ies) potentially responsible. Additionally, the St. Johns River Water Management District (SJRWMD) conducted a multi-year independent investigation to determine the likely cause(s) for these mortalities. Recently, it was concluded that the majority of these deaths were due to OCP toxicosis. Organochlorine pesticides from these former farmland soils had contaminated fish that were later consumed by birds.

The study reported here, forms part of a series of projects funded by the SJRWMD that aim to better understand the bioaccumulation of OCPs by fish-eating birds inhabiting NSRA-flooded marshes. Specifically, more data is needed on soil and tissue OCP concentrations and on the biotic and abiotic factors affecting rates of bioaccumulation in potential prey species (invertebrates and vertebrates living in contact with sediments from the NSRA). This information is needed for the calculation of Biota Sediment Accumulation Factors (BSAFs), which in turn will help in modeling avian exposure to OCPs

Organochlorine pesticides of potential concern in the NSRA are toxaphene, 4,4'-DDT and its metabolites 4,4'-DDE and 4,4'-DDD, and dieldrin. In addition, the NSRA soils are uncommon in that they contain a high level of total organic carbon (TOC). Biota sediment accumulation factors in areas with similar high organic matter contents have rarely been studied, nor has environmental fate modeling for OCP been evaluated in these or similar high organic conditions. It is, therefore, unclear whether the magnitude of bioaccumulation reported for other extensively studied areas could be directly and validly applied to the NSRA. In addition, there are almost no data on BSAFs for toxaphene.

Biota Sediment Accumulation Factor (BSAF)

Definition

Throughout this report, the BSAF is defined as the relative concentration of a chemical in the tissues of an organism compared to the concentration of the same chemical in the sediment. For this calculation, tissue and soil chemical concentrations need to be normalized by the lipid and TOC contents present in biota and soil, respectively. The reasoning behind this normalization relates to the theory of

equilibrium partitioning. The essence of this theory is that chemical concentration in sediment organic matter is a better predictor of bioaccumulation and biological effects than chemical concentrations on a dry weight basis in the sediment (Di Toro et al., 1991).

Applicability and Variability of BSAFs

The BSAF model has been suggested as a simple tool to predict bioaccumulation of hydrophobic organic compounds in fish and other aquatic biota from measured concentrations in sediment. In fact, the US EPA has adopted its use for the establishment of sediment quality guidelines (US EPA, 1994). Based on the equilibrium partitioning theory, the movement of hydrophobic compounds between the two carbon pools (lipids in tissues and organics in soil) should be independent of sediment type, biota species, or type of hydrophobic chemical. Therefore, in theory, bioaccumulation rates calculated based on this equation could be applied over a wide range of environmental conditions and biota types.

Currently, however, there is still debate as to how applicable BSAFs are across different species and environmental conditions. In a recent study, Tracey and Hansen (1996) compared BSAF values obtained from laboratory and field studies on 27 different species (bivalves, pelagic and benthic fish species). They found that BSAF values for various species were similar both within and among habitat groups, and concluded that the sum of total exposures from all routes must have been similar across species inhabiting different environments. Other studies, however, have reported considerable degree of variation in BSAFs among chemicals, species, and locations. For instance, in fish and benthic invertebrates with similar feeding type, BSAFs can vary considerably. In oligochaetes, BSAFs of lipophilic compounds have ranged from 5.5 to 22.7 (Markwell

et al., 1989); in mussels and crustaceans from 2.0 to 21 (Van der Oost et al., 1996); and in mayflies BSAFs can range from 0.2 to 1.0 (Gobas et al., 1989).

Several factors may be responsible for the observed variations in BSAFs. Factors such as hydrophobicity of a chemical and its concentration in sediment; differences in metabolism and lipid composition across species; effects of biomagnification and food webs; and differences in sediment and organic composition have all been reported to affect BSAFs (Wong et al., 2001). In relation to the latter factor, there is information suggesting that high TOC in sediments can result in lower BSAFs. For example, Ferraro et al. (1990) reported that bivalves inhabiting polluted sediments containing "high" organic content (> 3.7 %) had lower BSAF values (< 2) compared to bivalves collected from sediments with "low" TOC (< 0.86%). A similar trend was described by Lake et al. (1990) in that invertebrates collected from high TOC sediments had lower BSAFs of PCBs. These authors hypothesized that this decline in BSAFs with increased TOC in sediment was a reflection of an increased sorption of contaminants by the organics present in the sediments. In a laboratory study, Nebeker et al. (1989) observed a decreased toxicity of spiked DDT soils to *Hyalella azteca* when the organic content of the soils was increased (from 3.0 to 10.5% TOC). It is important to mention, however, that the majority of BSAFs reported in the literature have been developed from sediments containing TOC at levels ~ 10% or below. The present study constitutes the first to evaluate BSAFs under much higher TOC conditions (~ 40%).

Other Important Definitions

Related to BSAFs, are the terms *bioconcentration*, *bioaccumulation*, and *biomagnification*. Although similar, these represent distinct events defined as follows: *Bioconcentration* is the process by which there is a net accumulation of a chemical

directly from water into aquatic organisms; *Bioaccumulation* is the accumulation of chemicals in tissues of organisms through any route (respiration, ingestion, or direct contact); and *biomagnification* is the result of the processes of bioconcentration and bioaccumulation by which tissue concentrations increase as the chemical passes up through two or more trophic levels (US EPA, 2000a).

Another important term that needs definition is that of *steady state*. An organism is said to be in steady state when the rates of uptake and elimination of a particular chemical are equal. In other words, it is assumed that under these conditions, lipid-normalized tissue concentrations remain constant over time. The steady state represents the highest accumulation potential of a chemical in an organism for a given lipid content.

Routes of Exposure

Routes of exposure to OCPs differ depending on the organism. For most benthic organisms, exposure to dissolved chemicals in sediment pore water appears to be the predominant route of exposure (Oliver, 1987). Soil ingestion, however, has also been considered an important route of exposure in some benthic species (Harkey et al., 1994). For upper-trophic level species, ingestion is one the principal routes of exposure rather than bioconcentration from water. Since these kinds of compounds generally exhibit low water solubility, they tend to concentrate in the lipid fractions of biological tissues leading to biomagnification in long-lived top predators (Suedel et al. 1994). More recently, uptake of lipophilic compounds through the gills and skin is one of the principal routes of exposure in several fish species (Ueno et al., 2002; Barber, 2003; Nichols et al., 2004).

Factors Affecting Bioavailability of Chemicals

A wide range of biotic and abiotic factors are known to influence the bioavailability of chemicals in sediments. The following is a brief summary of some important factors affecting bioavailability of chemicals.

a) Physical factors:

Chemicals mix in sediments through several physical processes. The most important forces are turbulence and bioturbation which compete with sedimentation to determine the depth at which a contaminated sediment will be found. Another important physical process is that of diffusion. Chemicals diffuse between different concentration gradients established both within and between sediment pore waters and the overlying water column.

b) Chemical factors:

There are several important chemical characteristics that influence bioavailability. The most important ones are molecular size and polarity. These will largely determine the extent and type of association with particles (e.g. degree of sorption, desorption, and precipitation). Large, nonpolar chemicals (such as OCPs) have low water solubility and a strong tendency to be associated with dissolved organic matter, and thus are less bioavailable.

The octanol/water partition coefficient (K_{ow}) is an important predictor of bioavailability for nonionic organic chemicals. The K_{ow} is the ratio of a chemical's concentration in an n-octanol phase to its concentration in water at equilibrium. The log K_{ow} of a substance represents its likelihood to complex or sorb to organic carbon. This coefficient can be related to the organic carbon-water partition coefficient or K_{oc}.

In theory, bioaccumulation can be predicted when the log K_{ow} of a chemical lies between 3 and 6 (Thomann, 1989). Uptake efficiency increases with increasing log K_{ow}; however when K_{ow} is over 6 uptake efficiency starts to decrease. In addition, biomagnification through the food chain is unlikely to occur for chemicals with a log K_{ow} of less than 5 (Thomann, 1989).

c) Biological factors:

The amount of chemical bioaccumulation will depend on the bioavailability of contaminants as well as on species-specific uptake and elimination processes. These in turn will depend on the organism's lipid content, its size, growth rate, gender, diet, and ability to metabolize or transform a given contaminant (US EPA, 2000a). In addition, environmental factors (such as temperature and dissolved oxygen, DO) can indirectly affect the uptake of chemicals by altering the metabolic rate and growth of organisms. For instance, low water temperatures can lead to decreased food consumption, and thus a decreased uptake of chemicals (Spiragelli et al., 1983). In contrast, low DO concentrations can lead to increased ventilation rates and increased rates of chemical uptake.

Study Objectives

Due to the general lack of information regarding BSAFs in sediments from the NSRA, the main objective of this study was to quantify site-specific BSAFs for OCPs found in soil/sediments in different locations within the NSRA. In this study we tested the effects of sediment quality, species, and chemical type on BSAFs through the development of the following hypotheses:

Null Hypothesis 1: BSAFs will not be affected by the amount of TOC present in soils.

Alternative Hypothesis 1: BSAFs will change with increasing TOC contents.

Null Hypothesis 2: BSAFs will not be affected by the animal model or species tested. In other words, a benthic invertebrate (crayfish) and a pelagic fish (mosquitofish) will have comparable BSAFs.

Alternative Hypothesis 2: BSAFs will be affected in crayfish because of their higher contact with the sediment and their slightly higher position in the food chain compared to mosquitofish, and thus the possibility of biomagnification.

Null Hypothesis 3: BSAFs will not be affected by the type of OCP. NSRA soils are contaminated with over 30 different OCPs, and we hypothesized all of them will behave similarly in terms of bioaccumulation in biota.

Alternative Hypothesis 3: BSAFs will be affected in relation to the K_{ow} of each OCP.

CHAPTER 2
MATERIAL AND METHODS

General Description of Experimental Design

The main objective of this study was to determine the bioaccumulation rates of different organochlorine pesticides (OCPs) from soils throughout the NSRA in eastern mosquitofish (*Gambusia holbrooki*) and calico crayfish (*Orconectes immunis*) in relation to different levels of total organic carbon (TOC) and OCPs in soils. A total of thirteen microcosm treatments with three replicates each were established (November, 2001), and test organisms sampled at regular intervals (weeks 0, 2, 4, 8, 12, and 16). Water and sediments from each tank were also analyzed for OCP contents, as well as for other quality parameters. A summary on some physicochemical and ecotoxicological characteristics of the OCPs measured in this study are presented in Table 2-1.

Identification of NSRA Soils for Setting up the Microcosm Treatments

The soils utilized for setting the microcosm tanks were collected from multiple locations throughout the NSRA (Figure 2-1). The experimental design consisted of testing three levels of TOC (low, medium, and high) and four levels of OCPs (none to very low, low, medium, and high). Since the concentrations of OCPs with highest potential for concern in the NSRA (toxaphene, 4,4'-DDT-metabolites, and dieldrin) are highly correlated, a simple OCP derived matrix was considered sufficient rather than separate matrix designs for each of the three pesticides. Since suitable soils do not exist in the NSRA to fill each block of the matrix (e.g. low TOC but high OCP), soils were mixed with different amounts of sand (see below) or clean peat (i.e. not

contaminated with OCPs) to reflect these matrix types/conditions. In addition, a set of replicate microcosms was included to test soils from a high OCP (Hot Spot) site in the NSRA, which was outside the matrix design.

Soils were selected to represent this matrix design based upon previous evaluations of soil OCP and TOC (see Table 2-2 for a summary of OCP and TOC levels for each site/soil sample and Figure 2-1 showing site locations in the NSRA). In general, site selections were based upon approximate total soil OCP [total 4,4'-DDT, dieldrin, and toxaphene residues (µg/kg)] and soil TOC. Because typically, soil samples with high toxaphene levels have high levels of dieldrin and total 4,4'-DDT (and, similarly, low toxaphene levels correspond to low dieldrin and total 4,4'-DDT levels), sites/soils were classified for OCP concentrations based on the following toxaphene values: low OCP - $\leq$ 7,000 µg /kg; medium OCP - $\geq$12,000 and $\leq$ 30,000 µg /kg; and high OCP - $\geq$ 22,000 µg /kg. Sites/soils were also classified for TOC as follows: low TOC - $\leq$ 10 %; medium TOC - $\geq$ 18 and $\leq$ 26 %; and high TOC - $\geq$ 38 % (Table 2-2). A total of 8 NSRA sites were identified as appropriate for this matrix design, however, soil for the medium TOC/low OCP type had to be diluted with sand (Edgar Sand Plant, Palatka, FL) to create the necessary OCP and TOC levels (Table 2-3). In addition, two of these sites were utilized to create the low TOC/medium OCP and low TOC/high OCP soil types. The low TOC/medium OCP microcosm was created by diluting soil from site ZES0587 with sand. Similarly, the low TOC/high OCP microcosm involved the dilution of soil from site ZSS0767 with sand. The control treatments (i.e. no OCP present) were also created in the laboratory by mixing different amounts of peat (Traxler Peat, Florahome, FL) with sand. (Table 2-3).

Site ZSS0767 also served as an additional high OCP microcosm (Hot Spot) not listed in the original experimental matrix. This site represents an additional soil sample that is very high in OCP and in TOC. Soil characteristics for site ZSS0767 are as follows: 4,800 µg /kg sum of 4,4'-DDT and derivatives; 1,670 µg /kg dieldrin; 174,200 µg/kg toxaphene; and 38 % TOC (Table 2-2).

Collection and Preparation of Soils

A bulk soil sample (~ 2.0 m^3) was collected from each of the eight sites identified on the NSRA for production of appropriate laboratory microcosms (see Figure 2-4 for a timeline of events). Soil collections began with the low OCP sites and proceeded to the high OCP sites. Each sampling site consisted of an area of approximately 4 m x 4 m. First, each area was prepared for collection by manual removal of weeds and roots. Soil was then collected to a depth of 15 - 20 cm using a backhoe front-loading excavator. After a mixing period of 25 min with a front-loading excavator, soils were placed into two 120 cm diameter polyethylene tanks (~ 0.8 m^3/tank for each site). Tanks were then sealed with polyethylene sheeting and netting prior to transport or storage. One tank from each soil type was stored at the NSRA for future use as needed. The other tank was transported to the USGS/Ecotoxicology Program Facilities (USGSEPF) in Gainesville. Before and following sample collection at each site, the front-loading bucket for the excavator and the container tanks were first cleaned/decontaminated with soap (20% Alconox) and water, and later rinsed with de-ionized water and isopropyl alcohol.

Soil types and mixing ratios are listed in Tables 2-2 and 2-3. For eight of the 13 microcosm types, soils were utilized directly from only one of the two collection tanks. Soils representing each treatment were mixed thoroughly for 30 min using a cement

mixer. The cement mixer was thoroughly cleaned using pond water between treatments. Each soil type was then manually distributed to three 120 cm round, 75 cm tall polyethylene microcosm tanks to ensure homogenous distribution of soil to each tank. Soils were placed at a depth of 20 cm within each tank, and all microcosms were established in a three-day period (13 tanks or one complete replicate/day).

For several of the microcosm types, soils were mixed with sand or peat to produce the appropriate levels of TOC and OCP residues. For the control tanks, Traxler peat was mixed with sand so as to create low, medium, and high TOC/NO OCP treatments. Traxler Peat/sand ratios were calculated based on an average TOC of 42% in the Traxler peat, and aiming for an overall TOC value of 42, 21, and 6% for the high, medium, and low TOC treatments, respectively (Table 2-3). These values correspond to the approximate average TOC concentrations found in the study soils classified as high, medium, and low TOC. For the remaining treatments, soil was removed from a site tank and placed into four microcosm tanks (three for subsequent microcosm analyses and one for storage) and sand added at an appropriate mass per mixing ratios. Soil/sand mixtures were thoroughly mixed using a cement mixer for 30 min to ensure adequate homogeneity.

Initiation and Maintenance of Microcosms

Microcosms (total of 39 tanks: three replicates/treatment or sediment type times 13 treatments) were constructed using 790 L round polyethylene tanks (dimensions: 120 cm internal diameter x 75 cm height). Tanks were labeled to represent each of the 13 treatments, and with an A, B, or C letter to represent the three replicates. Each tank was filled with a sediment layer of 20 cm and was filled with 45 cm of pond water. Tanks were equilibrated for four weeks prior to the introduction of

test species. Tanks were placed in an outdoor concrete area (10 m x 13 m) that was covered with a transparent roof (Figure 2-2). This allowed sunlight to reach the tanks and prevented excess water from rain. In order to minimize the potential effects of gradients in sunlight and temperature, tanks were randomly arranged throughout this concrete pad. However, after placement of the tanks, it was noticed that some tanks were receiving extra shading from a tree close by. There was concern that an increase in shade could affect overall productivity by affecting water temperature. This prompted for a repositioning of the tanks, which took place on December 6 (Figure 2-3). Specifically, tanks 1 through 18 and 19 through 21 were repositioned using two pallet jacks. Tanks 1 through 18 were moved to the center of the enclosure, and tanks 19 through 21 were moved to the east side of the enclosure (Figures 2-2 and 2-3).

Water levels were checked daily, and pond water added as needed to account for possible losses due to evaporation. To avoid colonization by insects and frogs, each tank was covered with a black piece of shade cloth clipped to the lips of the tank. Tanks were checked on a regular basis for the presence of vascular plant growth, and any plants seen were removed. In order to provide cover and habitat for fish and invertebrates, a set amount of artificial substrate (*Hydrilla*-like plastic aquatic plants, 5/tank) were added to each tank. Artificial plants also helped increase the surface area for algae growth. Each tank was also inoculated with 1 L of zooplankton inocula collected from ponds located at the USGSEPF to provide a more realistic food-chain microcosm.

Monitoring of Water Quality Parameters

Dissolved oxygen (DO), temperature, pH, and conductivity were checked weekly during the course of the experiment. Turbidity was measured every other week. In addition, productivity of each microcosm was measured a total of six times during the course of the study (weeks 0, 1, 3, 8, 9, and 12). All water quality measurements were taken between 8 and 9 AM at a depth of about 10 cm.

Temperature and DO were measured using a YSI Inc., model 55 (Yellow Springs, OH); conductivity was measured using a YSI Inc., model 30; and pH was measured using a water-resistant hand-held pH meter (Oakton, Vernon Hills, IL). Turbidity was measured with a handheld Aquafluor^{TM} fluorometer and turbidometer (Turner Designs, Fresno, CA). Primary productivity was measured using the light and dark bottle method (Czaplewski and Parker, 1973). Briefly, water samples from each microcosm tank containing phytoplankton were distributed between two 1 L glass bottles, one of which was clear and the other covered with aluminum foil, preventing light from penetrating inside the bottle. The bottles were then incubated *in situ* (i.e. inside the microcosm tank at a depth of approximately 20 cm) for one hour. Using the portable DO meter described above, oxygen concentrations were measured in both bottles both at the beginning and at the end of the incubation period. Photosynthesis will have occurred in the clear bottle, thereby adding dissolved oxygen to its water. Light cannot enter the opaque bottle, so no photosynthesis will have occurred there. In both bottles the phytoplankton (and any zooplankton contaminants) will have carried on respiration (the reverse reaction of photosynthesis) and thereby removed oxygen from the water. A measurement of net photosynthesis (photosynthesis in excess of respiratory needs) can be obtained by measuring the gain in oxygen concentration in

the light bottle. Net photosynthesis is equated with net oxygen evolved and is obtained by subtracting the oxygen content of the water before incubation from the oxygen content of the light bottle following incubation. A measurement of gross photosynthesis can be obtained by adding the amount of respiratory oxygen to the net oxygen evolved. Respiratory oxygen is calculated by subtracting the oxygen content of the dark bottle after incubation from the oxygen content of the water before incubation. Gross primary productivity of was calculated as:

$$P_G = 2\,[V_c\,(C_{fc} - C_{ic}) + V_d\,(C_{ic} - C_{fc})]/A$$

where:

P_G = gross production $mg/O_2/m^3/d_{12h}$,

V_c = volume of clear chamber (1 L),

V_d = volume of dark chamber (1 L),

C_{ic} and C_{fc} = initial and final DO concentrations,

A = substrate area, m^2 (for a 700 L microcosm tank = 1.1 m^2).

Test Organisms Stocking and Maintenance

After a four-week equilibration period, tanks were stocked with test organisms. In this study, bioaccumulation of OCP was evaluated using both benthic (calico crayfish) and pelagic (mosquitofish) organisms. Both species represent critical food-chain components for the bioaccumulation of OCP for higher trophic species, such as wading birds.

Mosquitofish were obtained from a clean pond located at the USGSEPF. Crayfish were purchased from a commercial vendor (Carolina Biological, Burlington, NC). Organisms were acclimated in the laboratory for at least two weeks prior to the start of the study. Tanks were stocked with similar size fish and crayfish, and with an

approximate sex ratio of 1:1 for each species. Mosquitofish and crayfish weighed an average of 0.15 g (range of 0.1 – 0.25 g) and 15 g (range of 3 – 20 g), respectively. Each tank was stocked with 50 g of mosquitofish (approximately 300 individuals) and with 20 crayfish (approximate total biomass of 350 g, which corresponds to an overall stocking density of 687 g/m^3).

Sampling of Test Organisms for Chemical Analyses and Measurements

Tables 2-4 and 2-5 summarize the sampling of biota through the course of this study. Prior to the start of the experiment, a representative sample of each test species (at least 20 g including a minimum of two organisms) were submitted to EN CHEM Laboratories Inc. (Madison, WI) to verify that OCPs were at non-detectable concentrations. In addition, the same day tanks were stocked, five representative samples of each test organism were submitted for chemical analysis to represent time 0. Mosquitofish (minimum of 5 g total wet weight for each collection) were subsequently collected from each tank at 2, 4, 8, 12, and 16 weeks. Crayfish were also collected at 2, 4, 8, 12, and 16 weeks, but only between one and two individuals were submitted for chemical analysis each time. Animals were collected at random from each tank, total fresh weight recorded, and wrapped in aluminum foil, labeled, and stored at –80ºC until submitted to the laboratory for analysis. Samples were sent to the laboratory within a week after being collected.

Sampling of Water and Sediment for Chemical Analyses

Collection, storage, and manipulation of sediments and water followed USEPA standards (USEPA, 1996). In brief, sediment samples (200 g from the top 5-cm layer) were collected from each tank for OCP analyses at the initiation and completion of the study (weeks 0 and 16) using core samplers which consisted of a 120 cm x 5 cm

diameter PVC sampler. Disruption of sediment was kept to a minimum. Sediment samples (~ 200 g) were stored in 250 mL pre-cleaned amber glass jars. In addition, water samples (1 L) were also collected from each tank at 0 and 2 weeks and stored in 1 L pre-cleaned amber glass bottles. Water samples were not filtered. After collection, sediment and water samples were stored at 4ºC until submission to EN CHEM Laboratories for analysis of OCPs.

Chemical Analyses in Biota, Soils, and Water

Test organisms were screened for a total of 24 OCPs (including, but not limited to: dieldrin, aldrin, 4,4'-DDT and metabolites, toxaphene, chlordane, nonachlor, and endosulfan) as described in (EN CHEM 2000a, 2000b). Briefly, 20 g of biota samples were frozen with liquid nitrogen, and then mixed with 40 g of anhydrous sodium sulfate. Samples were then extracted with methylene chloride (~150 mL) for a minimum of 16 hr in a soxhlet extractor (40 mm internal diameter x 250 mm length). After concentration of the Soxhlet extract, a portion was analyzed for lipid content and the rest was cleaned up by Gel Permeation Chromatography (GPC) (SW846 Method 3640A). After GPC clean-up, the solvent was exchanged to hexane and concentrated to either 5 or 10 ml. A portion of this GPC extract was further cleaned up by the Florisil method (SW846 Method 3620B) and another portion underwent sulfuric acid clean-up (SW846 Method 3665A). The Florisil-cleaned extract was analyzed for general OCP contents with a Gas Chromatograph (GC) (Hewlett-Packard 5890 or 6890) equipped with and Electron Capture Detector (ECD). The sulfuric acid-cleaned extract was analyzed specifically for Toxaphene. Toxaphene was identified and quantified as described below. Percent lipid and moisture content was also determined from these samples as described in EN CHEM (1999). Throughout this report, OCP concentrations in whole mosquitofish and

crayfish carcasses are given in µg/kg (ppb) based on wet weight. In some instances, these concentrations are also presented as lipid-normalized values.

The same suite of OCPs was measured in soil and water samples. For soils, a 20 g sample was homogenized and mixed with 60 g of anhydrous sodium sulfate, then extracted with methylene chloride/acetone (~80 mL) using a sonifier (Branson 450 Sonifier) for a total of 15 min (SW846 Method 3550B). An aliquot of the extract was then cleaned up by GPC, solvent exchanged to hexane, then concentrated to 10 ml. Similar to biota samples, separate portions of the GPC extract were further cleaned up by the Florisil and the sulfuric acid methods. The Florisil-cleaned extract was analyzed for general OCP contents and rthe sulfuric acid-cleaned extract was analyzed specifically for Toxaphene. Toxaphene was identified and quantified as described below (for more details, see En Chem, 1998). For water, 1 L of sample was serially extracted 3 times with methylene chloride/acetone (~60 mL) at a neutral pH using a 2000 mL Teflon-stopcock separatory funnel. The methylene chloride/acetone extracts were then concentrated, solvent exchanged to hexane, and the extract cleaned up by the Floriisil and the sulfuric acid methods. The Florisil-cleaned extract was analyzed for general OCP contents and the sulfuric acid-cleaned extract was analyzed specifically for Toxaphene. Toxaphene was identified and quantified as described below (see (EN CHEM, 2000c). Portions of extracted samples were analyzed for OCP contents as described above. Sediments were also examined for percent TOC, and total volatile solids (TVS) (DB Environmental Laboratories, Inc., Rockledge, FL). For TOC analyses, soil samples were dried (85 - 90ºC), finely ground using a mixer mill, and weighed into tin or silver capsules. Capsules were then inserted via an autosampler (AS 200) into a

Carbon Nitrogen Sulfur (CNS) analyzer combustion chamber (Carlo-Erba NA 1500 Series 2 Elemental Analyzer). Inside the chamber, samples were oxidized in an oxygen-rich environment, causing the release of gases which were then chromatographically measured on a thermal conductivity detector. Similarly to what was described under biota, OCP concentrations in water and sediment are given in ppb (µg/kg for sediment and µg/L for water). In contrast to biota, sediment OCP concentrations were calculated based on dry weight.

Since toxaphene is really a complex "mixture" made out of several hundred different chlorinated camphene congeners, its quantification is complex and varies greatly across different laboratories. In the present study, toxaphene was measured by EN CHEM following the "Apopka Method". The sulfuric acid extract was analyzed for toxaphene using five calibration standards (Restek). Toxaphene was identified by matching at least four peaks with that of the toxaphene standard pattern. This comparison was based on both retention time and relative peak height. The concentration of toxaphene in the sample was then quantified using the total area under the curve above a straight base line procedure as described in Method 8081A, Section 7.6.1.3.2. The area of large single peaks not matching the toxaphene pattern (i.e., peaks greater than three times the highest toxaphene standard peak that occur at approximately the same retention time) was then subtracted from the total area under the curve.

Quantification of Phytoplankton and Zooplankton Abundance and Species Composition

Tanks were sampled at intervals of approximately 15 d for evaluation of natural populations of phyto and zooplankton. The abundance and species composition of

zooplankton was measured from a composite of two samples per tank on each sampling date. Each sample consisted of five subsamples constituting a combined volume of 5 L of water column (four collected adjacent to the tank walls at equidistant points, and the fifth sample collected near the center). Subsamples were collected by dropping a PVC tube (water sampler, approximate dimensions of 5 cm in diameter and 120 cm long) through the water column, stoppering the bottom of the tube, and pouring the tube's content trough a 80 µm Wisconsin Plankton Net (Wildco, Buffalo, NY) attached to a 20-mL glass vial. Zooplankton was then preserved in 20 mL plastic cups using 10 % buffered formalin until identification and counting.

In the laboratory, samples were poured from the plastic cups trough a funnel into a 50 mL plastic conical tube. Deionized water was used to wash out any remaining sample from the original containers. Deionized water was then added to each conical tube to the 40 mL mark to ensure that enough volume existed within each tube for proper separation during the centrifugation process. Samples were centrifuged (Chermile centrifuge) at 3,000 rpm for 20 min. After centrifugation, the supernatant was checked in a subset of vials to ensure that no zooplankton was present. Since the supernatant was negative every time, the volume of each conical tube was reduced to 5 mL using a disposable pipette for an easier access to the pellet containing all the zooplankton. Prior to sampling, the tube was hand-shaken, and a 1-mL sample collected with a micropipette. The 1-mL sample was then placed into a Sedgewick Rafter cell microscope slide (Graticules®, Pyser-SGI Limited, Kent, UK) marked with 100 cubic mm squares. The slide was placed under a light microscope (4x to 40x) and all of the zooplankton present was counted an identified.

Phytoplankton abundance was determined every other week by measuring chlorophyll *a* concentration by *in vivo* fluorescence with a Turner Designs Aquafluor™ fluorometer and turbidometer.

Statistical Methods

All statistical analyses were conducted using the Statistical Analysis System (SAS) version 9.0. Prior to statistical analyses, data sets were checked for normality (PROC UNIVARIATE). If the assumption of normality was not met, data sets were log-transformed. Unless otherwise specified, significance was declared at $p < 0.05$.

Throughout this report, statistical analyses were focused on a subset of OCPs. These included: alpha and gamma-chlordanes; 4,4'-DDE, 4,4'-DDD, and 4,4'-DDT; dieldrin; endosulfan II; and toxaphene. The decision to focus mainly on these 8 OCPs (out of 24 analyzed) was mostly based on historical information on soil OCP concentrations measured in NSRA. Because the remaining 16 OCPs have been historically found at relatively low concentrations in these soils, it was expected concentrations in tissues would follow the same trend. With a few exceptions, this was indeed what happened, and most of these OCPs were found in tissues at very low or non-detectable concentrations (see Chapter 6).

Chapter 3: Water Quality Parameters

Water quality parameters were compared across treatments and time using a 2-way repeated measures ANOVA, followed by a Tukey's multiple comparison test. The interaction between treatments and time was also tested.

Chapter 4: Soil TOC and OCP values

Soil TOC and OCP concentrations were compared across treatments using a 2-way ANOVA followed by a Tukey's multiple comparison test. In addition, soil TOC and

OCP concentrations were compared between weeks 0 and 16, by treatment. This chapter also presents a limited amount of data collected from water. No statistics were performed on this data.

Chapter 5: Zooplankton and phytoplankton data

The concentration of chlorophyll *a* was compared across treatments and time using a 2-way ANOVA. The interaction between treatments and time was also measured. Regression analyses between soil TOC and OCP and total numbers of zooplankton measured in water were also performed.

Chapter 6: Biota lipid and OCP values

The effect of treatment on total amount of biota (mass) recovered was tested using regression analyses. In addition, the effect of biota type and treatment on both OCP lipid-normalized and OCP non-lipid normalized tissue values were evaluated using a 2-way ANOVA. Regression equations were developed between tissue OCP lipid-normalized values and soil OCP-TOC normalized values.

Chapter 7: Biota Sediment Accumulation Factors

In this study, BSAF is defined as: “the ratio of a substance’s lipid-normalized concentration in tissue of an aquatic organism to its organic carbon normalized concentration in sediment, in situations where the ratio does not change substantially over time, both the organism and its food are exposed, and the surface sediment is representative of average surface sediment in the vicinity of the organism” (US EPA, 2000a).

BSAF was calculated using the following formula:

$$BSAF = (C_t/f_l)/(C_s/f_{oc})$$

where C_t is the OCP concentration(µg/kg, wet weight) in crayfish or mosquitofish; f_l is the lipid fraction in tissue; C_s is the OCP concentration (µg/kg, dry weight) in soil; and f_{oc} is the TOC fraction in soil.

Since one of the assumptions for the calculation of BSAFs is that of steady state, only the mean OCP tissue values attained between weeks 8 and 16 were included in these analyses. The sediment TOC-normalized OCP concentrations used in calculating BSAFs were the average of samples taken at the beginning and end of the experiment (mean of weeks 0 through 16). In addition, all OCP values with a “U” qualifier (non-detect values) were excluded from these analyses.

Table 2-1. Summary of physicochemical and ecotoxicological information on the OCPs measured in this study. Sources: (US EPA, 2000b and 2002).

Analyte	CAS Number	Log Kow	Log Koc	Environmental Half-Life
alpha-Chlordane	5103719	6.32	6.21	283 d – 3.8 y
gamma-Chlordane	5103742	6.32	6.21	283 d – 3.8 y
4,4'-DDD	72548	6.10	6.0	2.0 – 15.6 y
4,4'-DDE	72559	6.51	6.65	2.0 – 15.6 y
4,4'-DDT	50293	6.83	6.71	2.0 – 15.6 y
Aldrin	309002	6.5	5.0	-[a]
Dieldrin	60571	5.2	5.28	175 d – 3 y
Endrin aldehyde	7421934	4.8	4.03	-
Endrin ketone	53494705	4.99	3.98	-
Endosulfan I	959988	3.83	4.34	7 – 238 d
Endosulfan II	33213659	3.83	4.34	7 – 238 d
Endosulfan sulfate	1031078	3.66	4.5	7 – 238 d
Heptachlor epoxide	1024573	4.98	3.7	-
Methoxychlor	72435	5.08	4.63	30 d – 1 y
Oxychlordane	27304138	5.48	3.91	-
cis-nonachlor	5103731	6.35	5.16	-
trans-nonachlor	39765805	6.35	5.16	-
Toxaphene	8001352	5.50	5.41	1 – 14 y

[a] No data available.

Table 2-2. Microcosm soil types, site/sources, and attained selected organochlorine pesticides (µg/kg dry weight) and total organic carbon (TOC, %) concentrations for subsequent microcosm studies. Given are means ± SD of the three replicates, by week of sampling. Sites were identified from the NSRA to best fit the matrix experiment. Missing matrix soil types were produced using native soils and clean peat or sand mixes.

Mixture	Source of Soil	Analyte	Attained Concentration	
			Week 0	Week 16
Low TOC/Low OCP (L/L)	DES0163	DDTR[a]	- [b]	42 ± 41
		Dieldrin	9 ± 12	23 ± 37
		Toxaphene	513 ± 140	1,000 ± 1,000
		TOC	10 ± 0.6	10 ± 0.9
Low TOC/Medium OCP (L/M)	ZES0587 MIX	DDTR	1,300 ± 288	953 ± 161
		Dieldrin	3 ± 1	11 ± 3
		Toxaphene	1,200 ± 180	880 ± 147
		TOC	3 ± 0.7	0.4 ± 0
Low TOC/High OCP (L/H)	ZSS0767 MIX	DDTR	56 ± 32	39 ± 8
		Dieldrin	6 ± 5	15 ± 4
		Toxaphene	1,700 ± 493	1,600 ± 436
		TOC	2 ± 0.08	1 ± 0.9
Medium TOC/Low OCP (M/L)	ZWS0484 MIX	DDTR	74 ± 8	41 ± 4
		Dieldrin	51 ± 7	23 ± 17
		Toxaphene	1,500 ± 153	977 ± 387
		TOC	10 ± 0.2	9 ± 3
Medium TOC/Medium OCP (M/M)	ZWS0480	DDTR	1,100 ± 140	713 ± 172
		Dieldrin	413 ± 103	270 ± 176
		Toxaphene	10,500 ± 2,300	9,700 ± 1,200
		TOC	16 ± 2	18 ± 0
Medium TOC/High OCP (M/H)	ZES0669	DDTR	2,300 ± 364	2,000 ± 121
		Dieldrin	160 ± 44	127 ± 15
		Toxaphene	13,300 ± 3,200	12,700 ± 1,500
		TOC	23 ± 0.8	23 ± 3
High TOC/Low OCP (H/L)	DES0260	DDTR	191 ± 71	113 ± 31
		Dieldrin	53 ± 46	26 ± 21
		Toxaphene	5,900 ± 600	4,700 ± 100
		TOC	44 ± 2	45 ± 2
High TOC/Medium OCP (H/M)	ZSS0963	DDTR	1,000 ± 728	937 ± 256
		Dieldrin	1,200 ± 474	860 ± 120
		Toxaphene	45,300 ± 17,200	39,700 ± 7,400
		TOC	39 ± 2	40 ± 0.3
High TOC/High OCP (H/H)	ZES0587	DDTR	60,000 ± 8,900	48,900 ± 19,400
		Dieldrin	548 ± 446	590 ± 141
		Toxaphene	50,000 ± 4,600	39,000 ± 5,300
		TOC	49 ± 2	48 ± 3
High TOC/High OCP (Hot spot)	ZSS0767	DDTR	5,000 ± 792	4,500 ± 1,600
		Dieldrin	1,800 ± 153	1,500 ± 1,600
		Toxaphene	193,300 ± 25,200	155,000 ± 76,000
		TOC	39 ± 3	37 ± 2
High TOC/NO OCP (Control; H/NO)	Traxler Peat	DDTR	-	3 ± 5
		Dieldrin	1 ± 0.08	3 ± 0.6
		Toxaphene	62 ± 3	177 ± 68
		TOC	40 ± 2	39 ± 3
Medium TOC/NO OCP (Control, M/NO)	Traxler/Edgar Sand	DDTR	-	1 ± 1
		Dieldrin	1 ± 0.02	1 ± 0.05
		Toxaphene	22 ± 1	31 ± 2
		TOC	5 ± 0.6	3 ± 0.7
Low TOC/NO OCP (Control, L/NO)	Traxler/Edgar Sand	DDTR	-	40 ± 56
		Dieldrin	1 ± 0.02	1 ± 0.003
		Toxaphene	18 ± 0.8	23 ± 0.3
		TOC	1 ± 0.2	1 ± 0.3

[a] DDTR = sum of 4,4'-DDT, 4,4'-DDD, and 4,4'-DDE.
[b] Not reported.

Table 2-3. Microcosm soil types, site/sources, and mixing ratios with sand and soil to produce the necessary soil types for subsequent microcosm bioaccumulation analyses.

Mixture	Source of Soil	Sand Added (Ratio of soil: sand)
Low TOC/Low OCP	DES0163	NM[a]
Low TOC/Medium OCP	ZES0587	20:80
Low TOC/High OCP	ZSS0767	10:90
Medium TOC/Low OCP	ZWS0484	50:50
Medium TOC/Medium OCP	ZWS0480	NM
Medium TOC/High OCP	ZES0669	NM
High TOC/Low OCP	DES0260	NM
High TOC/Medium OCP	ZSS0963	NM
High TOC/High OCPHigh	ZES0587	NM
TOC/High OCP (Hot spot)	ZSS0767	NM
High TOC/NO OCP (Control)	Traxler Peat	NM
Medium TOC/NO OCP (Control)	Traxler/Edgar Sand	50:50
Low TOC/NO OCP (Control)	Traxler/Edgar Sand	15:85

[a] Not mixed.

Table 2-4. Summary of crayfish sampled for OCP analyses, by treatment and sampling date. The range of weights summarized represent single crayfish, with exception of week 16 in which all remaining animals were sampled.

		Range of Weights (g)					
Tank Source	Treatment[a]	**Week 0** Nov 5 - 7 2001	**Week 2** Nov 19 - 21 2001	**Week 4** Dec 3 - 5 2001	**Week 8** Jan 2 - 4 2002	**Week 12** Jan 28 - 30 2002	**Week 16** Feb 25 - 27 2002
Holding Tank		-[b]	-[c]	-[c]	-[c]	-[c]	-[c]
2, 23, 32	**HS**	-[c]	16 - 19	15 – 18	15[d]	9[d]	0[e]
6, 14, 29	**H/H**	-[c]	13 - 18	13 – 16	7 - 16	0[e]	10[f]
13, 20, 28	**H/M**	-[c]	13 - 23	13 – 16	15 - 22	14 - 20	52 – 80[g]
1, 26, 31	**H/L**	-[c]	16 - 19	12 – 20	18 - 22	12 - 21	65 – 49[g]
9, 24, 39	**H/NO**	-[c]	14 - 17	15 – 22	14 - 29	13 - 20	40 - 62[g]
10, 15, 38	**M/H**	-[c]	14 - 18	14 – 17	15 - 18	15 - 21	11 – 76[h]
4, 22, 30	**M/M**	-[c]	16 - 24	16 – 18	14 - 19	12 - 19	68 – 109[g]
12, 21, 36	**M/L**	-[c]	13 - 22	10 – 16	16 - 18	16 - 18	46 – 80[g]
8, 18, 35	**M/NO**	-[c]	13 - 16	12 – 19	15 - 20	11 - 16	43 – 80[g]
7, 16, 34	**L/H**	-[c]	14 - 21	13 – 16	15 - 17	16 - 24	32 – 83[g]
11, 17, 33	**L/M**	-[c]	15 - 17	16 – 19	13 - 15	14 - 15	10 – 26[i]
3, 19, 37	**L/L**	-[c]	12 - 20	15 – 16	13 - 18	16 - 19	42 – 77[g]
5, 25, 27	**L/NO**	-[c]	15 - 16	12 – 18	11 - 15	14 - 19	41 – 92[g]

[a] Refer to Figure 2-2 for a definition of the different treatments tested.
[b] Five crayfish were collected at random from the holding tank prior to stocking and were individually analyzed to represent background OCP concentrations. Weight of sample was not recorded.
[c] No sampling done.
[d] Only one crayfish found in tank 23.
[e] No crayfish found.
[f] Only one crayfish found in tank 6.
[g] All remaining crayfish collected and submitted for chemical analyses. Assuming a 15 g overall body weight/crayfish, these samples included a range of 1 to 7 crayfish.
[h] Only one crayfish found in tank 38.
[i] Only one crayfish found in tank 17.

Table 2-5 Summary of mosquitofish sampled for OCP analyses, by treatment and sampling date. The range of weights summarized represents a pool sample of approximately 30 mosquitofish, with exception of week 16 in which all remaining mosquitofish were sampled.

		Range of Weights (g)					
		Week 0	**Week 2**	**Week 4**	**Week 8**	**Week 12**	**Week 16**
Tank Source	Treatment[a]	Nov 5 - 7 2001	Nov 19 - 21 2001	Dec 3 - 5 2001	Jan 2 - 4 2002	Jan 28 - 30 2002	Feb 25 - 27 2002
Holding Tank		-[b]	-[c]	-[c]	-[c]	-[c]	-[c]
2,23,32	**HS**	-[c]	0[d]	0[d]	0[d]	0[d]	0[d]
6,14,29	**H/H**	-[c]	5.2 - 5.3	5.1 - 5.3	4.3 - 5.2	5.1 - 5.6	3.3[e]
13,20,28	**H/M**	-[c]	5.1 - 5.5	5.1 - 5.3	5.2 - 5.4	3.8 - 5.7	4.2 - 6.0[f]
1,26,31	**H/L**	-[c]	5.1 - 5.2	5.3 - 5.3	5.2 - 5.6	5.1 - 5.6	4.9 – 21[f]
9,24,39	**H/NO**	-[c]	5.2 - 5.3	5.2 - 5.5	5.3 - 5.6	5.2 - 5.3	3.8 - 7.4[f]
10,15,38	**M/H**	-[c]	5.2 - 5.5	5.2 - 5.4	5.2 - 5.4	5.2 - 5.4	5.9 – 23[f]
4,22,30	**M/M**	-[c]	5.1 - 5.3	5.3 - 5.4	5.2 - 5.4	5.1 - 5.2	2.6 – 13[f]
12,21,36	**M/L**	-[c]	5.1 - 5.3	5.0 - 5.3	5.1 - 5.4	5.0 - 5.5	6.3 – 13[f]
8,18,35	**M/NO**	-[c]	5.1- 5.3	5.2 - 5.6	5.1 - 5.4	4.8 - 5.2	3.0 - 9.0[f]
7,16,34	**L/H**	-[c]	5.0 - 5.4	5.1 - 5.3	0[c]	0[c]	1.2 - 4.1[f]
11,17,33	**L/M**	-[c]	5.1 - 5.6	5.1 - 5.5	5.1 - 5.6	5.1 - 5.6	1.9 - 4.5[f]
3,19,37	**L/L**	-[c]	5.1 - 5.3	5.2 - 5.4	5.5 - 5.8	5.2 - 5.3	8.8 – 23[f]
5,25,27	**L/NO**	-[c]	5.0 - 5.4	5.3 - 5.5	5.3 - 5.5	5.3 - 5.7	5.6 - 8.8[f]

[a] Refer to Figure 2-2 for a definition of the different treatments tested.
[b] Five mosquitofish samples were randomly collected from the holding tank prior to stocking and were individually analyzed to represent background OCP concentrations. Weight of sample was not recorded.
[c] No sampling done.
[d] No mosquitofish found.
[e] Mosquitofish found only in tank 29.
[f] All remaining mosquitofish collected and submitted for chemical analyses. Assuming a 0.15 g overall body weight/mosquitofish, these samples included a range of 8 to 154 mosquitofish.

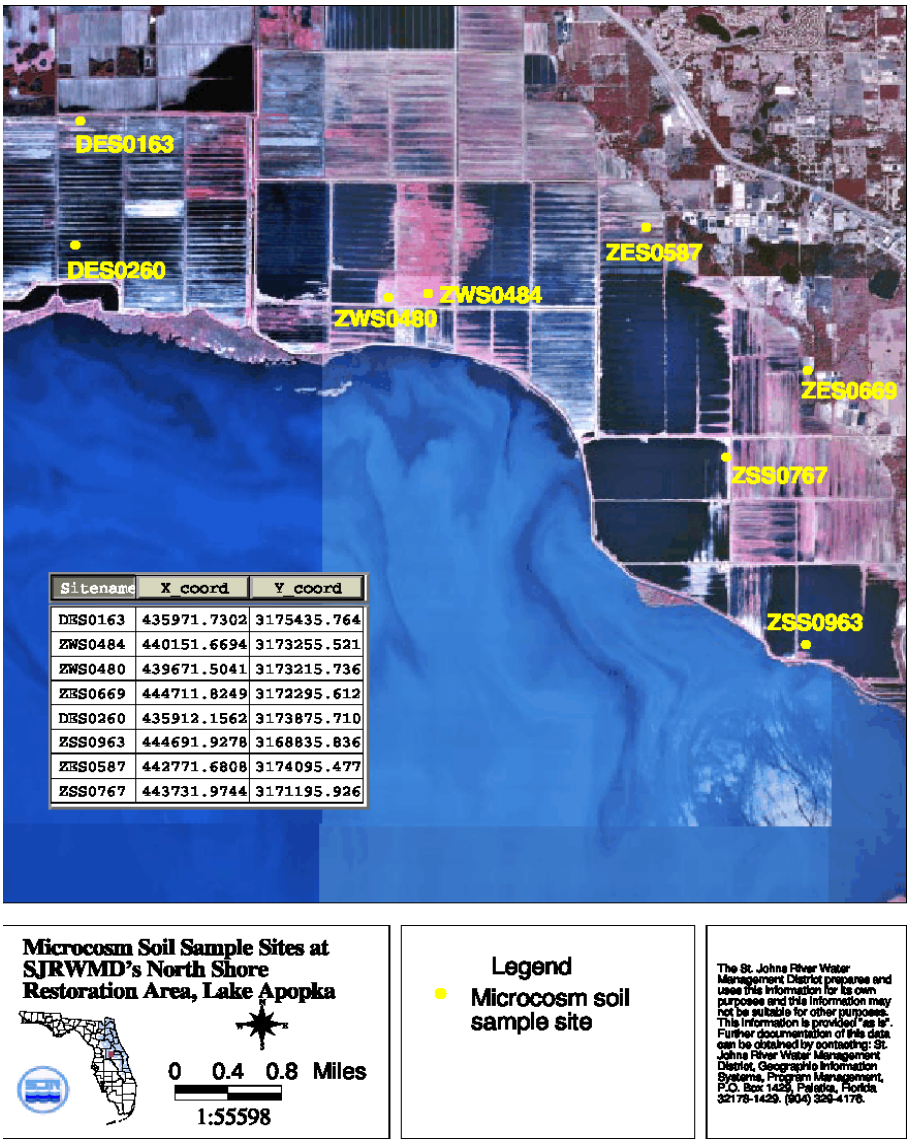
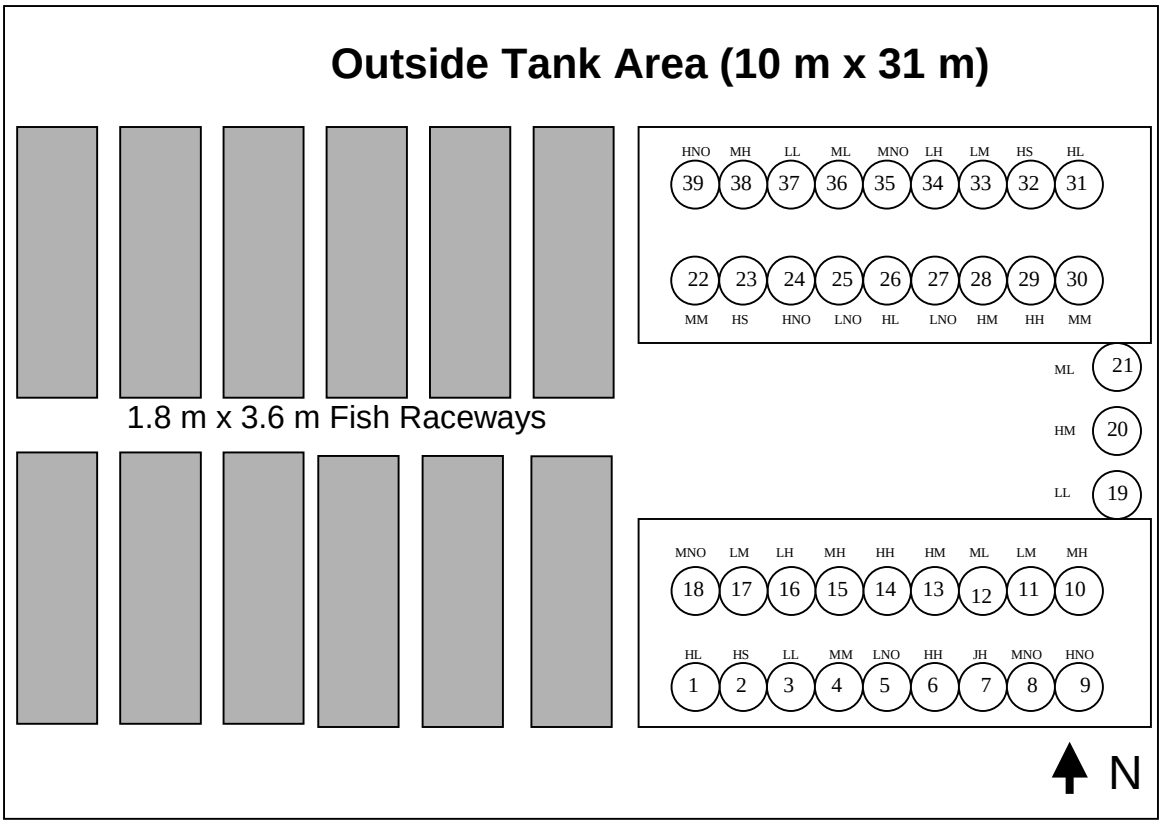
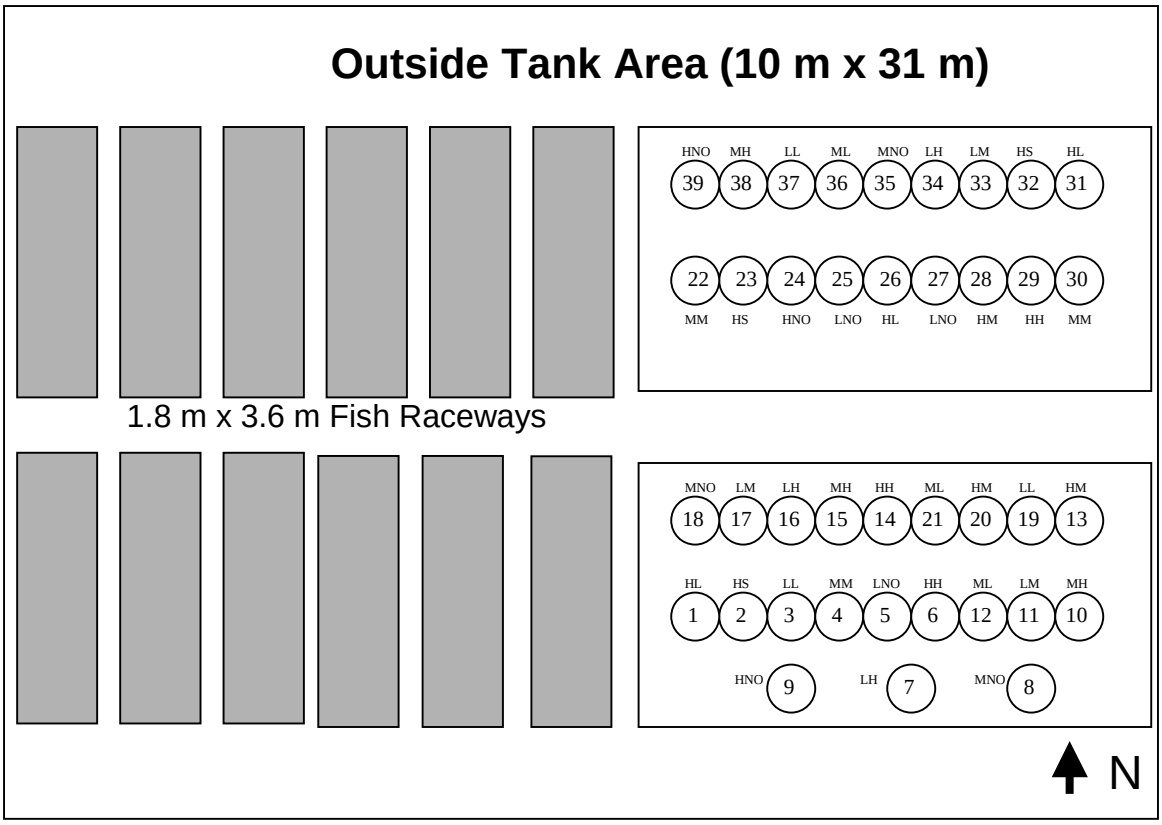


Figure 2-1. Map of the NSRA and sampling sites. Eight sampling sites were identified as appropriate for microcosm analyses based upon OCP and TOC evaluations.



List of Treatments: **HS =** Hot Spot**; L/NO** = Low TOC, No OCP; **L/L** = Low TOC, Low OCP **L/M** = Low TOC, Medium OCP; **L/H** = Low TOC, High OCP; **M/NO** = Medium TOC, No OCP; **M/L** = Medium TOC, Low OCP; **M/M** = Medium TOC, Medium OCP; **M/H** = Medium TOC, High; **H/N0** = High TOC, No OCP; **H/L** = High TOC, Low OCP; **H/M** = High TOC, Medium OCP; **H/H** = High TOC, High OCP.

Figure 2-2. Diagram of the original outside tank area at the USGS- Center for Aquatic Resource Studies, Gainesville, FL. Microcosm tanks are represented by opened circles, with each tank representing a treatment (13 treatments x 3 replicates, total of 39 tanks). The facility is covered with a 90% light transmittance roof.



List of Treatments: **HS =** Hot Spot**; L/NO** = Low TOC, No OCP; **L/L** = Low TOC, Low OCP **L/M** = Low TOC, Medium OCP; **L/H** = Low TOC, High OCP; **M/NO** = Medium TOC, No OCP; **M/L** = Medium TOC, Low OCP; **M/M** = Medium TOC, Medium OCP; **M/H** = Medium TOC, High; **H/N0** = High TOC, No OCP; **H/L** = High TOC, Low OCP; **H/M** = High TOC, Medium OCP; **H/H** = High TOC, High OCP.

Figure 2-3. Diagram of the outside tank area at the USGS- Center for Aquatic Resource Studies, Gainesville, FL, after repositioning of tanks on December 6, 2001. Microcosm tanks are represented by opened circles, with each tank representing a treatment (13 treatments x 3 replicates, total of 39 tanks). The facility is covered with a 90% light transmittance roof.

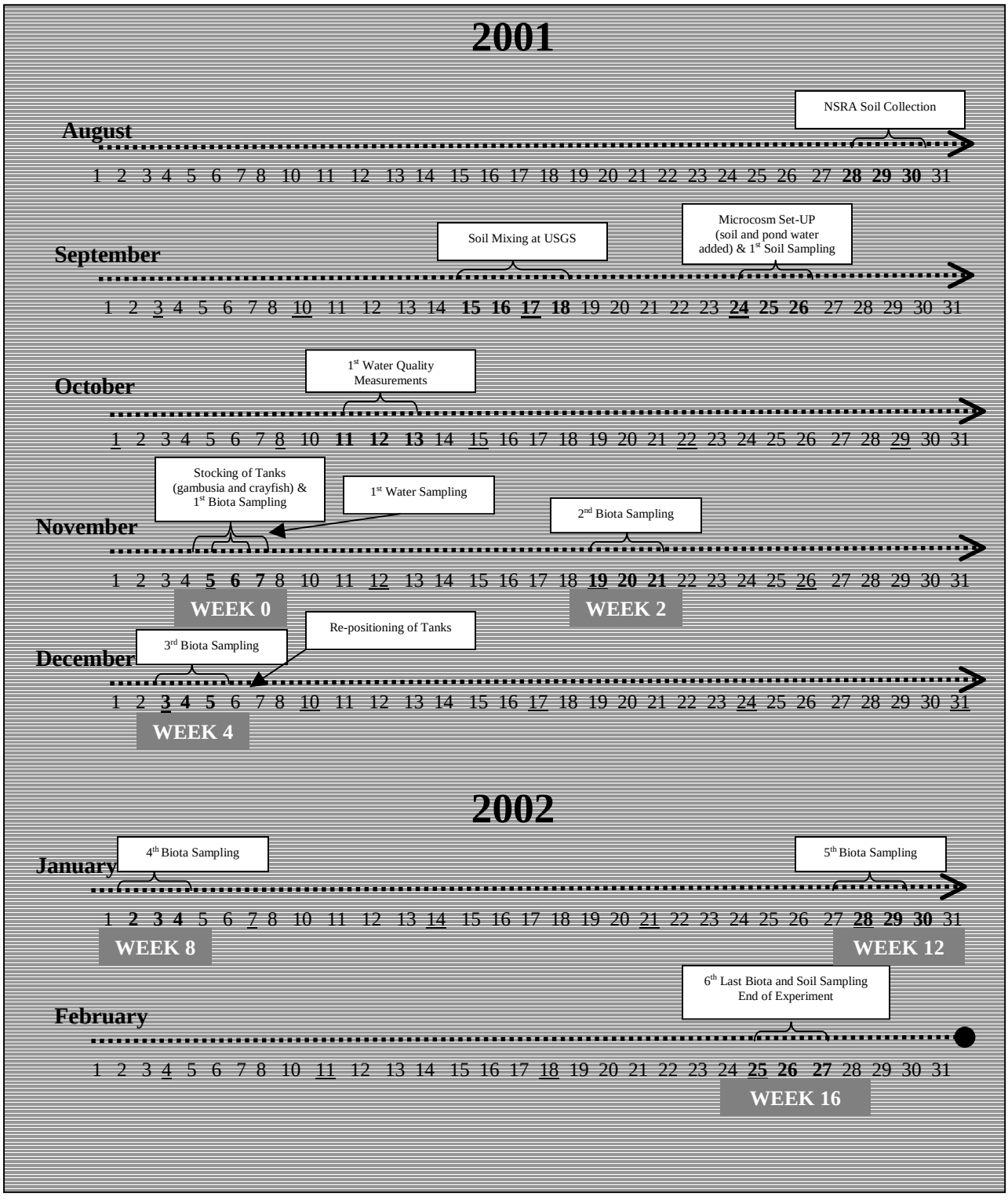


Figure 2-4. Timeline of events, from soil collection at NSRA in August 2001, to last biota sampling from microcosm tanks in February 2002. Collection days are in bold, and Mondays are underlined.

CHAPTER 3
RESULTS AND DISCUSSION: ASSESSMENT OF WATER QUALITY PARAMETERS

This chapter will summarize the water quality parameters results measured during the microcosm tank study, and discuss these data in relationship to possible treatment and temporal effects. If available, these parameters will also be compared to literature values in terms of minimum requirements for mosquitofish and crayfish.

With the exception of Figure 3-1, all the graphs presented in this chapter represent the mean ± standard deviation (SD) of the three replicates measured per treatment, by week. This approach was justified since there were no differences in water quality parameters across replicates (Figure 3-1).

As described in Chapter 2, with the exception of turbidity and productivity, water quality parameters were measured once a week, beginning the second week of October, 2001. This date corresponded to approximately two weeks after tanks were filled with soil and pond water (see Figure 2-4).

Water Temperature

Results on water temperature are presented in Figure 3-2. There were no treatments effects on water temperature. Regardless of treatment, there were significant changes in water temperature over time, with highest and lowest values observed during the fall (October) and winter months (mid December to early January), respectively. Water temperature showed intermediate values during November and from late January until the end of the study (late February).

The minimum and maximum temperatures (3 – 28 °C) registered during the course of the study, were within acceptable limits for mosquitofish (Jessica Noggle, University of Florida, Gainesville, personal communication).

Dissolved Oxygen

Results on dissolved oxygen (DO) are presented in Figure 3-3. Similarly to what was observed with temperature, DO values varied significantly over time. This temporal change, followed an almost opposite trend to what was observed with temperature, with lowest and highest values observed during the fall (October) and winter months (mid December to early January), respectively. This was expected, since it is well known that higher water temperatures will reduce the DO saturation concentration, and vice versa.

Treatment had a significant effect on DO concentrations. Overall, DO concentrations were highest in tanks with high sand content (> 85%) and thus low TOC content (L/H, L/M, L/L and L/NO; TOC 2 - 22%), and lowest in treatments with high TOC in soils (H/H, M/H, H/M, and HS; TOC 23 – 48%). The low organic content in soils probably resulted in a decreased biological oxygen demand (BOD) which was translated in higher oxygen concentrations in water. An exception to this high TOC/low DO pattern, was observed in treatments H/NO and M/NO, which had high to intermediate DO concentrations instead. This could have been related to the fact that in both of these treatments, TOC in soil was “artificially” increased in the laboratory by addition of commercial peat soil (50 – 100 % content). This would suggest that the muck soils from the NSRA have a much higher biological activity compared to the commercial peat. In addition, the achieved TOC in treatment M/NO was significantly lower than predicted (4 vs. 21%).

Despite these treatment differences, the range of DO concentrations registered during the course of this study (1 - 14 mg/L) fell within comparable ranges reported for mosquitofish (2 – 13 mg/L in Florida habitats, Noggle, unpublished data). Mosquitofish are known to tolerate low DO and anoxic conditions, because of their ability to gulp air from the water surface (Dawes, 1991).

pH

Results on water pH are presented in Figure 3-4. In this study, pH was affected by both time and treatment. During the first month of the study, and prior to the addition of mosquitofish and crayfish, pH was constant over time, and differences between treatments were already evident. pH in treatments H/NO, H/H, and M/H were close to neutrality, and were between 8 and 9 in the remaining treatments. Interestingly, immediately after stocking (week 0), the difference in pH across treatments was maintained, but intensified. For instance, pH in H/NO, H/H, and M/H decreased to acidic conditions (approximate range of 5 to 7), with the remaining treatments showing alkaline conditions (pH > 7). This decline was most evident from mid November through early January. By the end of the study, however, pH in these tanks had increased almost to pre-stocking conditions. This “stocking effect” on pH was probably due to changes in conductivity (see below).

The overall range in pH (5 – 10) fell within tolerable limits for both of the species studied. For mosquitofish, our laboratory has registered a range of 4 to 8 pH in North-Central Florida Rivers and creeks (Noggle, unpublished data).

Conductivity

Results on conductivity are presented in Figure 3-5. Similarly to what was observed with pH, conductivity was affected by time and treatment, and at the time of

stocking, there was a sharp decrease in conductivity in all treatments, probably due to a soil disturbance. In fact, pH and conductivity were positively correlated and thus, behaved very similarly over the course of the study (R = 0.6, $p < 0.0001$). Temporal changes in conductivity were evidenced by a constant increase over time for all treatments except H/NO, H/H, M/H, and M/NO. These treatments were also the ones with lowest pH (Figure 3-4). An increase in conductivity over time could also be related to increased evaporation of water contained in the microcosms. Water evaporation is unlikely to have affected water quality parameters in this study, however, because water levels were maintained constant by addition of pond water every two weeks.

The range of conductivities found in this study (92 – 581 µS/cm) is compatible with values found in mosquitofish habitats in North-Central Florida (33 – 2,321 µS/cm, Noggle unpublished data) and to tolerable ranges reported for crayfish (Gallaway and Hummon, 1991).

Turbidity

Results on turbidity are presented in Figure 3-6. Overall, turbidity tended to increase with time, from 3 ± 1 NTU (mean ± SD) to 9 ± 6 NTU. Changes in turbidity during the course of the study were less prominent in certain treatments (HS, H/H, H/M, M/M, and M/L). This temporal change in turbidity was probably due to changes in the amount of phytoplankton in water (measured as Chlorophyll *a*) since both parameters were positively correlated (R = 0.5, $p < 0.0001$). See Chapter 5 for a more detailed discussion on changes in Chlorophyll a over time.

The range of turbidity observed in this study (1 – 31 NTU) falls within reported turbidity ranges in Florida streams and Rivers known to contain mosquitofish (1 – 50 NTU) (Noggle, unpublished data).

Primary Productivity

Results on turbidity are presented in Figure 3-7. Primary productivity in this study was measured by the light and dark bottle method described in Chapter 2. As can be seen from the data, this parameter was extremely variable across treatments and time. In fact, in almost all cases the standard deviation was higher than the mean. This high variation could have been due to methodological factors, such as an insufficient incubation time prior to measurements of DO in the bottles and an inappropriate correction for phytoplankton metabolism. The high variation of this parameter, coupled with the lack of agreement with all remaining water quality parameters measured, does not justify its use in future similar studies.

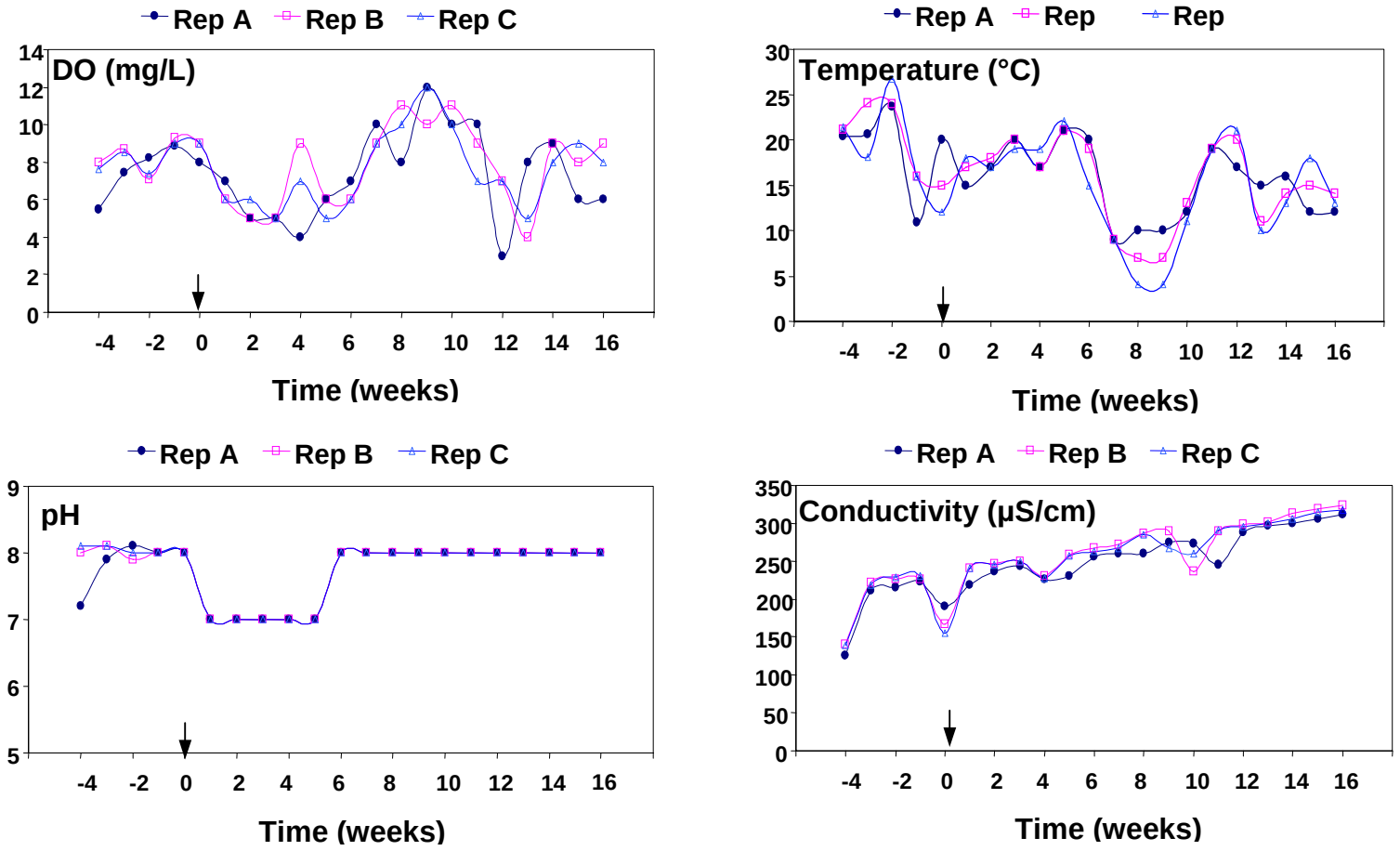


Figure 3-1. Comparison of water quality parameters among replicates, by week, all treatments combined. The arrow (week 0) indicates the time microcosms were stocked with mosquitofish and crayfish (November, 5 – 7, 2001).

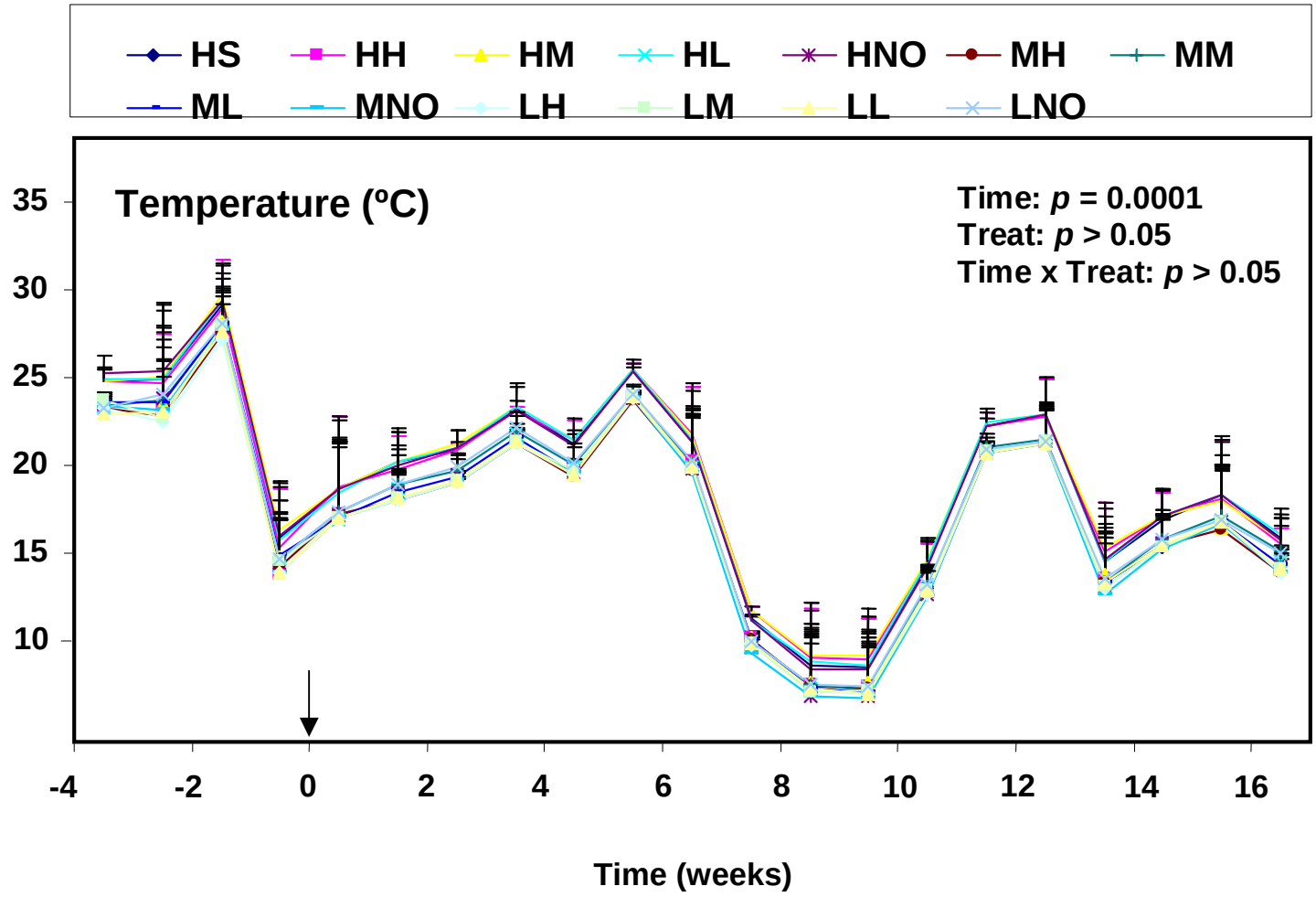


Figure 3-2. Changes in water temperature over the course of the microcosm study, by treatment. Plotted are averages ± SD of the three replicates/treatment. The arrow (week 0) indicates the time microcosms were stocked with mosquitofish and crayfish (November, 5 – 7, 2001). Results of the 2-way repeated measures ANOVA are also shown.

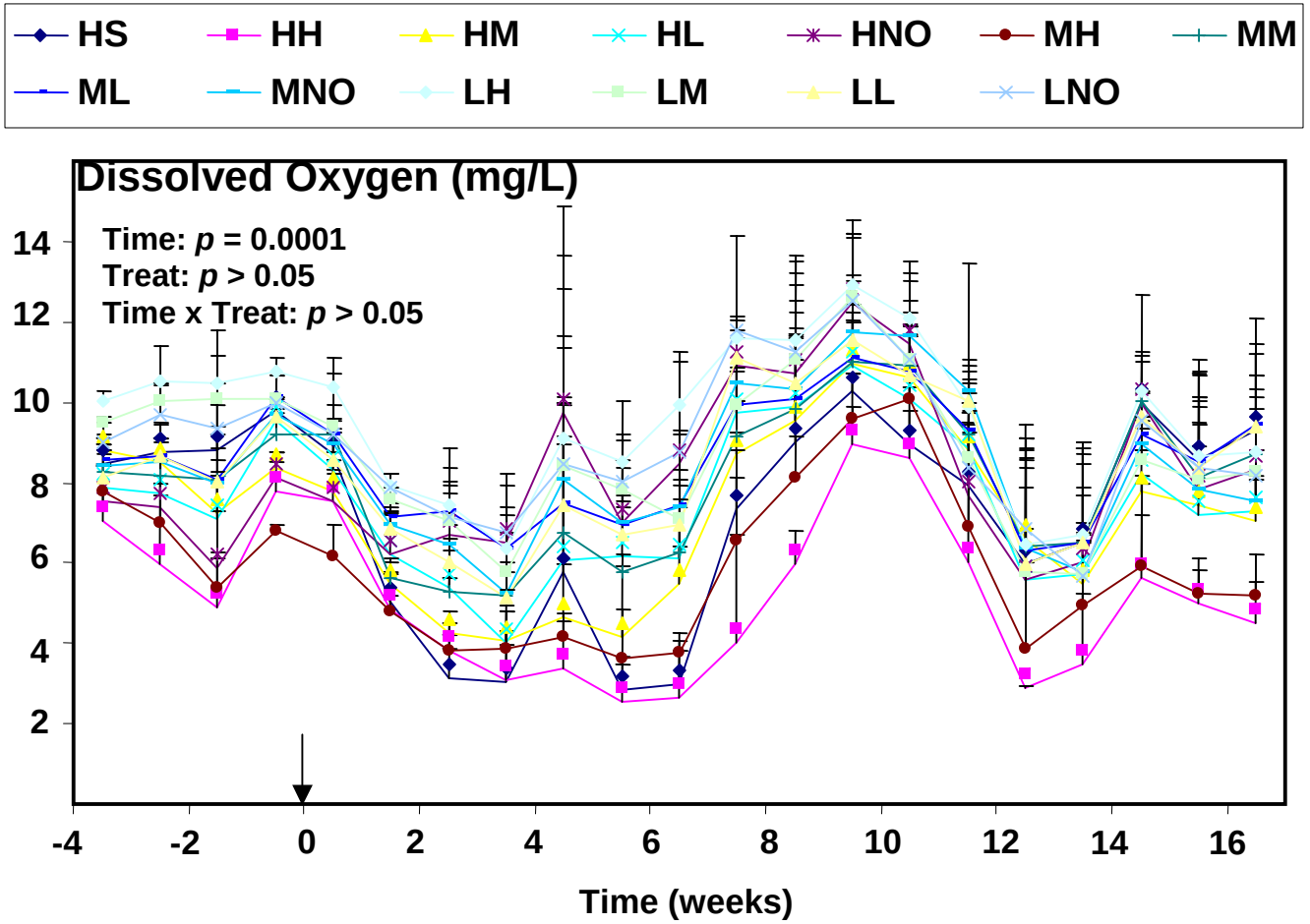


Figure 3-3. Changes in dissolved oxygen over the course of the microcosm study, by treatment. Plotted are averages ± SD of the three replicates/treatment. The arrow (week 0) indicates the time microcosms were stocked with mosquitofish and crayfish (November, 5 – 7, 2001). Results of the 2-way repeated measures ANOVA are also shown.

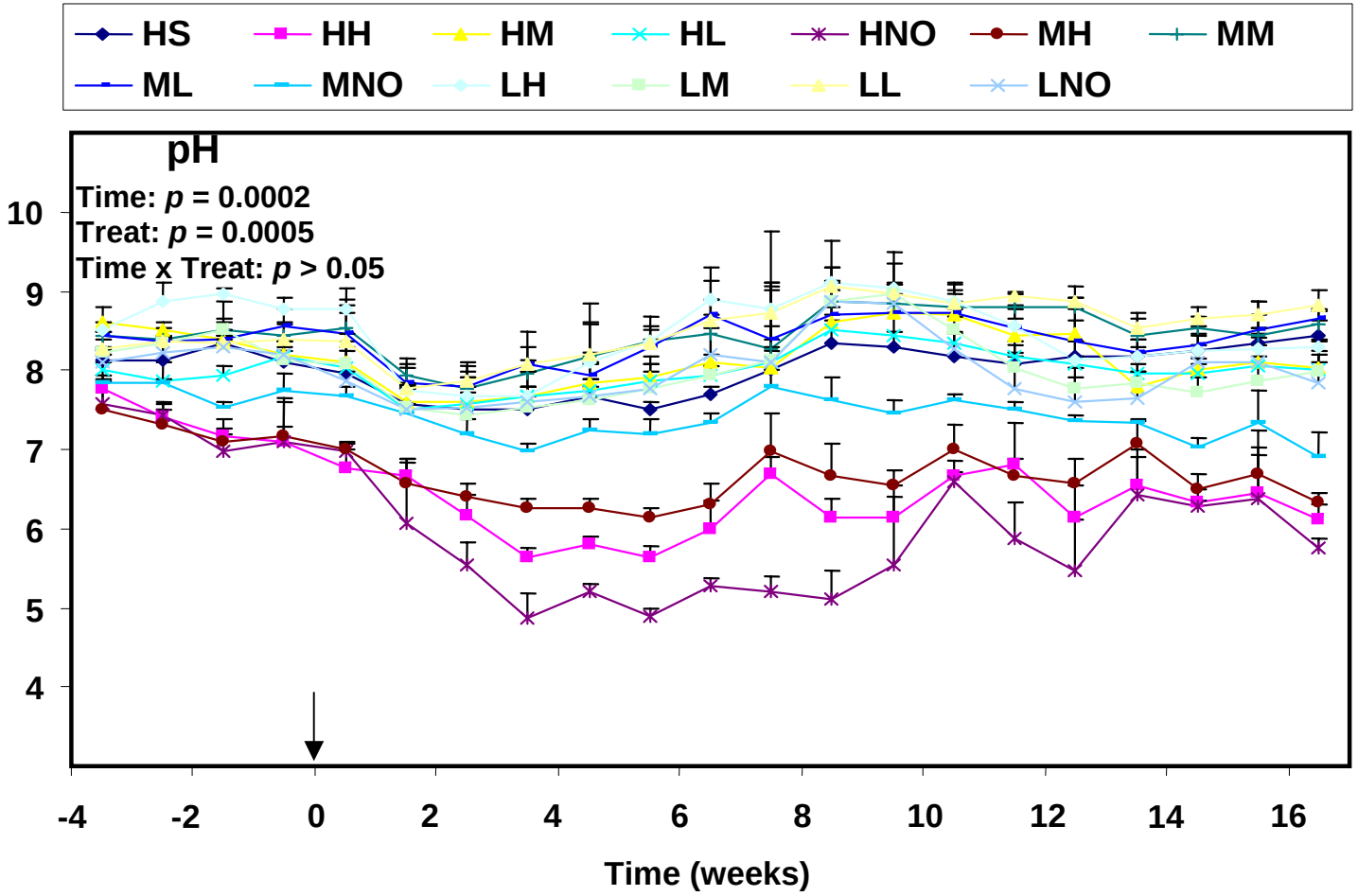


Figure 3-4. Changes in pH over the course of the microcosm study, by treatment. Plotted are averages ± SD of the three replicates/treatment. The arrow (week 0) indicates the time microcosms were stocked with mosquitofish and crayfish (November, 5 – 7, 2001). Results of the 2-way repeated measures ANOVA are also shown.

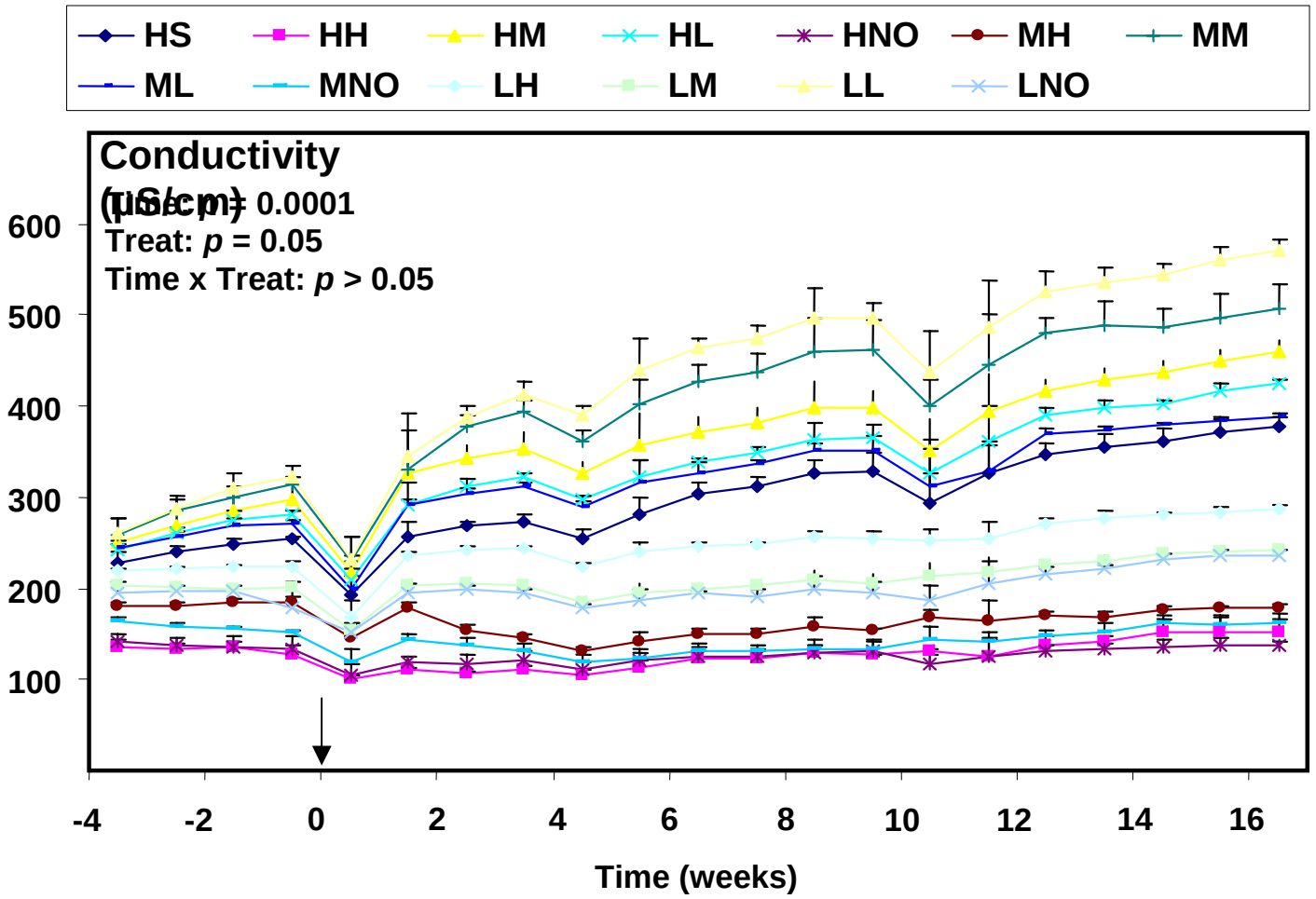


Figure 3-5. Changes in conductivity over the course of the microcosm study, by treatment. Plotted are averages ± SD of the three replicates/treatment. The arrow (week 0) indicates the time microcosms were stocked with mosquitofish and crayfish (November, 5 – 7, 2001). Results of the 2-way repeated measures ANOVA are also shown.

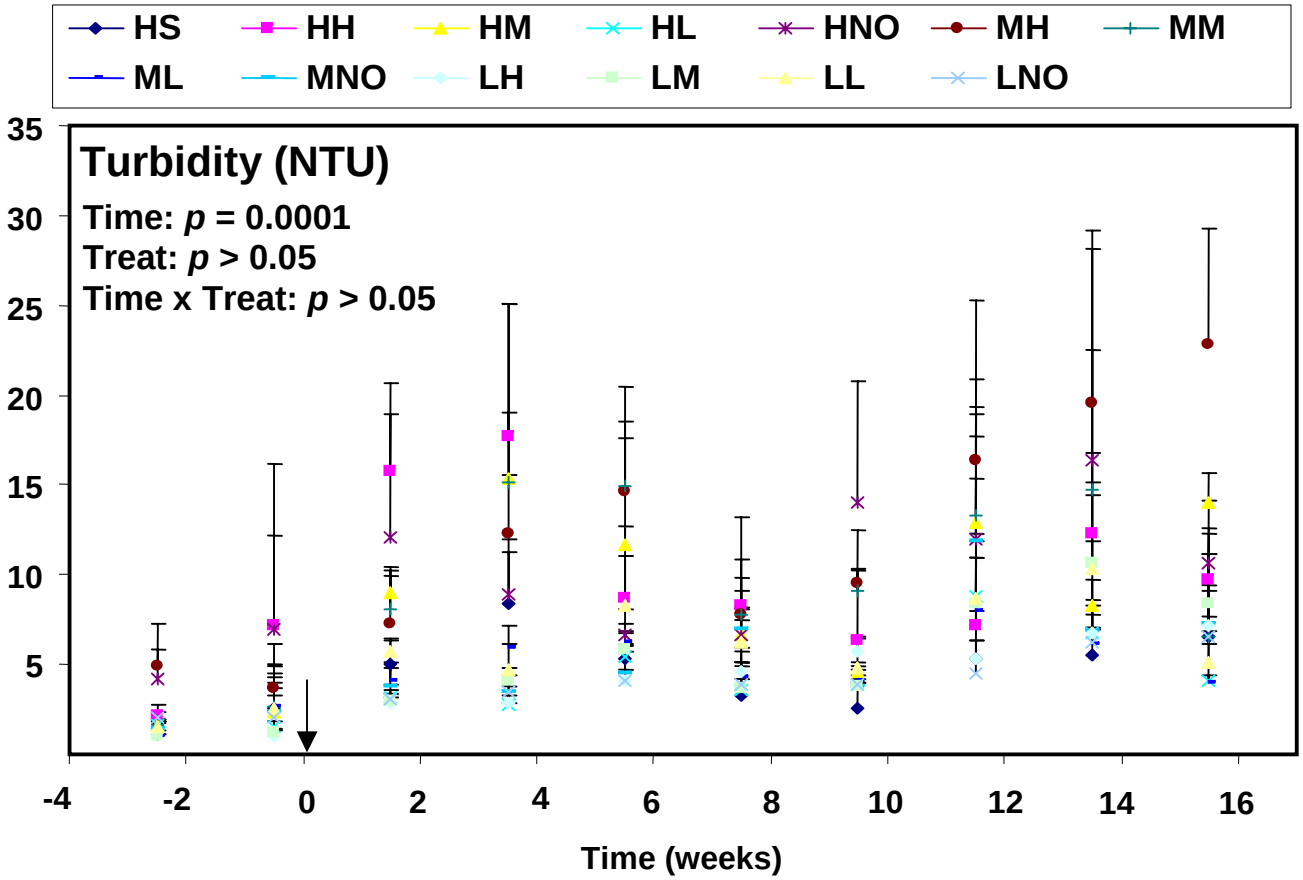


Figure 3-6. Changes in turbidity over the course of the microcosm study, by treatment. Plotted are averages ± SD of the three replicates/treatment. The arrow (week 0) indicates the time microcosms were stocked with mosquitofish and crayfish (November, 5 – 7, 2001). Results of the 2-way repeated measures ANOVA are also shown.

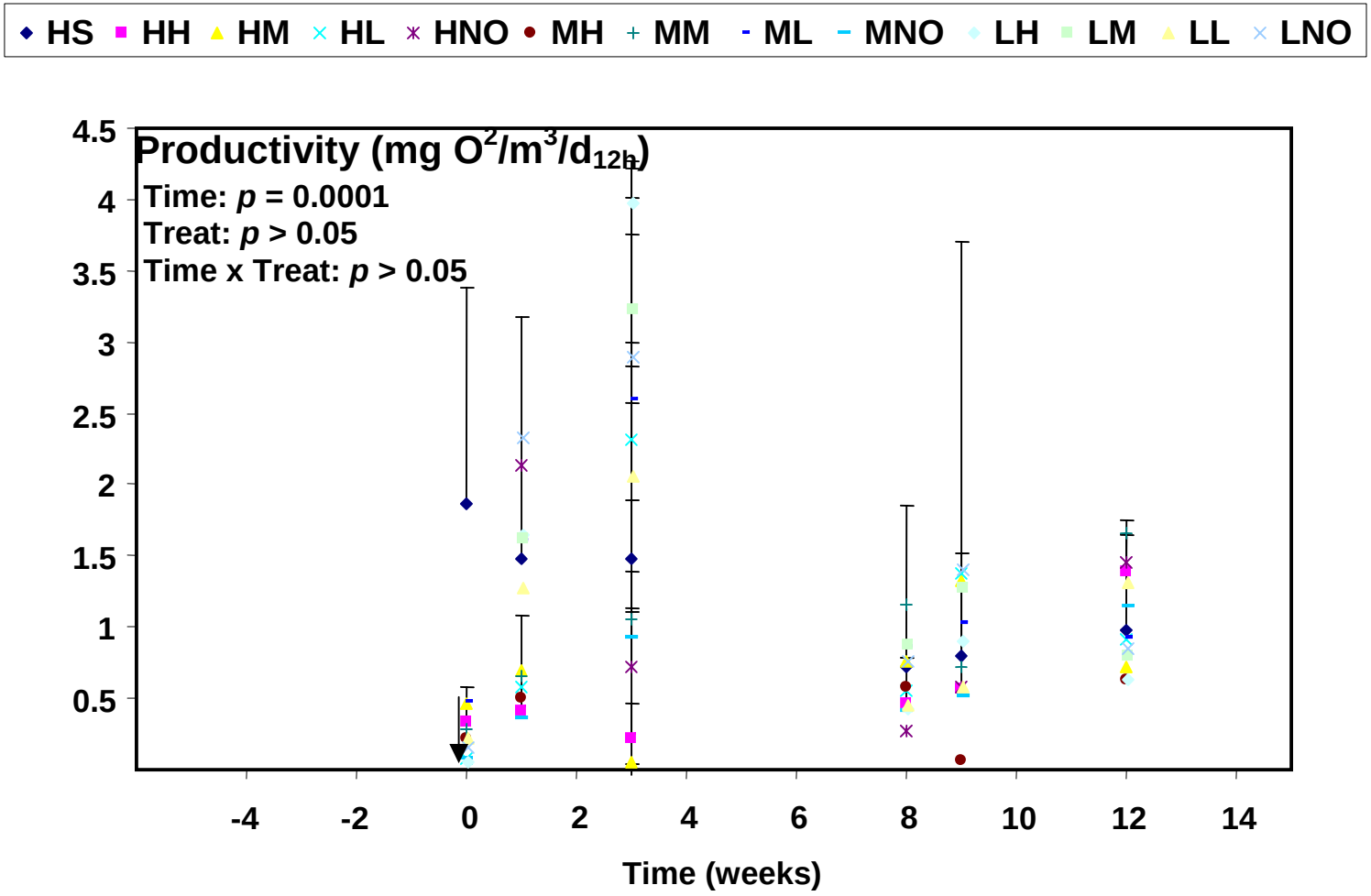


Figure 3-7. Changes in productivity over the course of the microcosm study, by treatment. Plotted are averages ± SD of the three replicates/treatment. The arrow (week 0) indicates the time microcosms were stocked with mosquitofish and crayfish (November, 5 – 7, 2001). Results of the 2-way repeated measures ANOVA are also shown.

CHAPTER 4
RESULTS AND DISCUSSION: CHEMICAL ANALYSES OF SOIL AND WATER

This chapter will summarize results on TOC and OCP concentrations in the different soils used for the microcosm treatments. These concentrations were compared among values obtained at the beginning (week 0) and at the end of the experiment (week 16), and to predicted soil concentrations based on prior studies at the NSRA. In addition, this chapter will summarize a limited dataset on water OCP values from these treatments tanks (weeks 0 and 2). For these analyzes, we have focused only on a subset of chemicals (see Chapter 2 for an explanation of why these were chosen). However, data on additional OCPs measured in soil and water are summarized in Tables 4-1 through 4-4.

All the graphs presented in this chapter show the mean ± SD of the three replicates measured by week and treatment. In addition, a dotted line was added to each graph to indicate the soil average method detection limit (MDL) for each chemical.

Soil Chemical Data

Total organic content and OCP concentrations in microcosm soils are presented in Figure 4-1 and Figures 4-2 to 4-9, respectively. There were very few differences in concentrations between weeks 0 and 16. Treatments M/NO and L/M had significantly higher TOC concentrations at week 0 in relation to week 16 (M/NO: 5 vs. 2 % and L/M: 31 vs. 13%, for weeks 0 and 16,

respectively) (Figure 4-1). Since both of these treatment soils were the result of mixing peat and sand in the laboratory, it is possible that the observed discrepancies in TOC values were due to incomplete mixing and/or homogenization prior to analysis. It is important to keep in mind however, that OCP concentrations from these two soil types did not differ among samplings.

In relation to OCPs, most of the differences between weeks were observed in treatment H/L (71 in week 0 vs. 43 µg/kg in week 16 for gamma-chlordane, Figure 4-3; 493 vs. 353 µg/kg for 4,4'-DDE, Figure 4-5; 52 vs. 25 µg/kg for dieldrin, Figure 4-7; and 5,900 vs. 4,700 µg/kg for toxaphene, Figure 4-9). In addition, treatment M/M had higher concentrations of 4,4'-DDT (853 vs. 423 µg/kg, Figure 4-6) at week 0, and in treatment H/M toxaphene was higher at week 16 (45,300 vs. 39,666 µg/kg, Figure 4-9). These differences simply reflect analytical differences rather than significant changes in soil concentrations, therefore, mean values were utilized to calculate BSAFs for all treatments from NSRA soils (DES0260, ZNS0480, and ZSS0963 for treatments H/L, M/M, and H/M, respectively; see Table 2-2 in Chapter 2). Another way of looking at this data would be to ignore individual treatments and group them based on either TOC (High, Medium, or Low) or OCP (Hot Spot, High, Medium, Low, and No) categories (Figures 4-10 and 4-11, respectively). With this approach, there was a significant difference in TOC across the three categories studied (ANOVA, $F = 362$, $p < 0.0001$). The mean ± SD for categories "High", "Medium" and "Low" were: 42 ± 4%, 13 ± 7%, and 3.7 ± 3.8%, respectively (Figure 4-10). These values were right on target in relation to what was predicted prior to the start of

the experiment (> 38, 18 – 26, and < 10% for categories “High”, “Medium”, and “Low”, respectively).

For total OCPs, the category (or treatment) “Hot Spot” contained significantly more pesticides when compared to all other categories (188,800 ± 58,500 µg/kg), and category “High” (43,100 ± 52,500 µg/kg) was significantly higher than categories “Low” (2,700 ± 2,300 µg/kg) and “No” (71 ± 72 µg/kg) (ANOVA F = 45, $p < 0.0001$) (Figure 4-11A). Although category “Medium” (22,400 ± 22,200 µg/kg) contained soils with approximately half the concentration of total OCPs than category “High”, this difference was not significant. This was probably due to the high variation in soil OCP in both of these treatments. Except for the “Hot Spot” treatment, all categories had OCP soil concentrations that fell within predicted values (Hot Spot: 2,800,000; High: > 22,000; Medium: 12,000 – 30,000; and Low: < 7,000 µg/kg).

The percent distribution of the OCPs of interest for each of the above mentioned categories is presented in Figure 4-11B. Excluding categories “Low” and “No” (for which most of the reported values were either below or very close to MDL values), OCPs from highest to lowest in occurrence were: toxaphene > 4,4’-DDT > 4,4’-DDE > 4,4’-DDD > dieldrin > endosulfan II > alpha-chlordane > gamma-chlordane. It is interesting to note the low presence of 4,4’-DDT and derivatives and the higher content of toxaphene in “Hot Spot” soils compared to “High” and “Medium” soils.

Water Chemical Data

Water OCP concentrations in microcosm tanks are presented in Figures 4-12 to 4-19. Because of limited funding, water samples were only analyzed during

the first two weeks post-stocking (which corresponded to approximately 5 weeks post-tank set up; see Figure 2-4). With the exception of gamma-chlordane, water concentrations of OCPs were above detection limit only for treatments H/S and H/H. In addition, 4,4'-DDE and 4,4'-DDT were above MDL values in treatments M/M and L/M, respectively (Figures 4-15 and 4-16). The variation within each treatment was considerable higher in water compared to soils.

An interesting observation was the apparent increase in OCP water concentrations in such a short time period (2 weeks). This is important because it could explain the rapid uptake of pesticides that has been reported to occur in biota inhabiting NSRA sites with high soil OCPs ("First Flush Study", report in preparation). Water OCP concentrations, however, were several thousand times lower compared to soil.

An important consideration to keep in mind, however, is that water samples were not filtered prior to being analyzed for chemical concentrations. Thus, this increase in OCP water concentrations could be due to adsorption of OCPs onto organic material in the water. The distinction among dissolved vs. adsorbed OCPs is important, because the latter forms are not as readily available for bioaccumulation by biota.

Table 4-1. Means (µg/kg) for additional cyclodiene OCPs and for methoxychlor measured in soil, by week and treatment.

Week	Analyte	Hot Spot	H/H	H/M	H/L	H/NO	M/H	M/M	M/L	M/NO	L/H	L/M	L/L	L/NO	Grand Average
0	**Aldrin**	12	23	6.67	2.23	0.72	3.28	3.20	1.23	0.26	1.14	1.77	0.88	0.22	4.37
	Endosulfan I	9.78	20	96	1.85	0.60	2.72	2.67	1.03	0.22	0.96	1.48	0.75	0.18	11
	Endosulfan sulfate	600	570	15	4.75	1.53	6.83	6.83	2.62	0.57	2.42	3.80	1.90	0.46	94
	Endrin	463	37	11	3.65	1.17	5.33	5.15	2.00	0.42	1.83	2.88	1.45	0.35	41
	Endrin aldehyde	27	56	16	5.28	1.70	7.83	7.33	2.90	0.62	2.67	4.13	2.12	0.51	10
	Endrin ketone	27	127	15	5.12	1.65	7.33	7.33	2.82	0.60	2.61	4.07	2.03	0.49	16
	Heptachlor	9.02	18	5.10	1.72	0.57	2.52	2.47	0.95	0.20	0.87	1.37	0.70	0.17	3.36
	Heptachlor epoxide	461	34	47	3.18	1.03	20	4.55	1.77	0.37	3.12	2.53	1.27	0.31	45
	Methoxychlor	145	918	82	28	9.00	41	40	15	3.27	14	22	11	2.70	102
16	**Aldrin**	63	27	22	0.90	1.57	2.72	3.15	0.31	0.37	0.39	0.28	0.35	0.28	9.36
	cis-nonachlor	968	111	170	24	2.60	36	23	3.50	0.60	15	2.37	4.10	0.46	105
	Endosulfan I	315	19	95	15	1.32	1.72	1.93	0.26	0.31	1.19	0.23	0.30	0.23	35
	Endosulfan sulfate	1,870	134	6.17	1.92	10	30	9.68	0.67	0.78	1.63	2.53	0.75	0.60	159
	Endrin	43	37	4.72	1.47	2.53	3.40	3.70	0.51	0.60	0.44	0.45	0.58	0.45	7.61
	Endrin aldehyde	86	55	163	23	3.68	4.82	5.48	5.50	0.85	0.65	0.65	0.83	0.65	27
	Endrin ketone	242	104	64	10	3.58	25	11	2.30	0.83	0.62	0.65	2.18	0.65	36
	Heptachlor	20	18	2.22	0.70	1.22	1.60	1.79	0.24	0.28	0.21	0.21	0.27	0.22	3.63
	Heptachlor epoxide	673	33	33	14	2.25	12	17	1.57	0.52	5.00	1.13	2.96	0.40	61
	Methoxychlor	1,420	1,392	267	11	20	173	72	8.58	4.53	14	21	4.40	3.47	262
	Oxychlordane	457	55	84	5.02	2.87	26	13	0.85	0.67	3.63	0.50	1.42	0.50	51
	trans-nonachlor	515	152	580	53	2.88	89	77	10	0.68	8.87	2.80	8.07	0.50	116

Table 4-2. Means (µg/L) for additional cyclodiene OCPs and for methoxychlor measured in water, by week and treatment.

Week	Analyte	Hot Spot	H/H	H/M	H/L	H/NO	M/H	M/M	M/L	M/NO	L/H	L/M	L/L	L/NO	Grand Average
0	**Aldrin**	0.0037	0.0038	0.0039	0.0040	0.0037	0.0037	0.0037	0.0041	0.0037	0.0037	0.0037	0.0037	0.0038	0.0038
	Endosulfan I	0.0029	0.0030	0.0030	0.0032	0.0029	0.0029	0.0029	0.0032	0.0029	0.0029	0.0029	0.0029	0.0030	0.0030
	Endos. sulfate	0.0067	0.0067	0.0067	0.0072	0.0065	0.0065	0.0067	0.0070	0.0065	0.0065	0.0065	0.0065	0.0067	0.0067
	Endrin	0.0047	0.0047	0.0049	0.0050	0.0047	0.0047	0.0047	0.0051	0.0047	0.0046	0.0047	0.0047	0.0049	0.0048
	Endrin aldehyde	0.0057	0.0057	0.0058	0.0060	0.0057	0.0055	0.0057	0.0062	0.0055	0.0055	0.0057	0.0057	0.0058	0.0057
	Endrin ketone	0.0049	0.0050	0.0052	0.0055	0.0049	0.0049	0.0050	0.0055	0.0050	0.0049	0.0049	0.0049	0.0051	0.0051
	Heptachlor	0.0035	0.0035	0.0036	0.0037	0.0035	0.0034	0.0034	0.0038	0.0034	0.0034	0.0034	0.0034	0.0035	0.0035
	Hepta. epoxide	0.0214	0.0032	0.0033	0.0034	0.0031	0.0031	0.0031	0.0035	0.0031	0.0031	0.0031	0.0031	0.0032	0.0046
	Methoxychlor	0.0350	0.0352	0.0362	0.0378	0.0350	0.0345	0.0348	0.0387	0.0348	0.0343	0.0348	0.0348	0.0358	0.0355
2	**Aldrin**	0.0037	0.0038	0.0038	0.0038	0.0039	0.0036	0.0038	0.0037	0.0038	0.0038	0.0037	0.0037	0.0037	0.0037
	Endosulfan I	0.0029	0.0030	0.0030	0.0030	0.0030	0.0028	0.0030	0.0029	0.0030	0.0030	0.0029	0.0030	0.0029	0.0029
	Endos. sulfate	0.0065	0.0067	0.0067	0.0067	0.0068	0.0063	0.0067	0.0065	0.0067	0.0065	0.0067	0.0067	0.0065	0.0066
	Endrin	0.0047	0.0047	0.0048	0.0048	0.0049	0.0046	0.0049	0.0046	0.0048	0.0047	0.0047	0.0048	0.0047	0.0047
	Endrin aldehyde	0.0055	0.0057	0.0058	0.0057	0.0058	0.0055	0.0058	0.0057	0.0058	0.0057	0.0057	0.0057	0.0057	0.0057
	Endrin ketone	0.0049	0.0050	0.0050	0.0050	0.0052	0.0048	0.0050	0.0048	0.0051	0.0051	0.0049	0.0050	0.0049	0.0050
	Heptachlor	0.0034	0.0035	0.0035	0.0035	0.0036	0.0033	0.0036	0.0034	0.0035	0.0035	0.0035	0.0035	0.0034	0.0035
	Hepta. epoxide	0.0534	0.0032	0.0032	0.0032	0.0033	0.0030	0.0032	0.0031	0.0032	0.0032	0.0031	0.0032	0.0031	0.0070
	Methoxychlor	0.0347	0.0353	0.0357	0.0355	0.0362	0.0338	0.0360	0.0345	0.0357	0.0353	0.0350	0.0352	0.0348	0.0352

Table 4-3. Means of (µg/kg) hexachlorocyclohexane OCPs measured in soil, by week and treatment.

Week	Analyte	Hot Spot	H/H	H/M	H/L	H/NO	M/H	M/M	M/L	M/NO	L/H	L/M	L/L	L/NO	Grand Average
0	**alpha-BHC**	10	20	5.70	1.88	0.62	2.77	2.72	1.05	0.22	0.96	1.50	0.77	0.19	3.73
	beta-BHC	59	74	21	6.95	2.28	10	10	3.90	0.82	3.68	5.75	2.82	0.68	15
	delta-BHC	12	23	6.47	2.17	0.70	3.20	3.12	1.20	0.26	1.12	1.73	0.87	0.21	4.26
	gamma-BHC	14	28	8.00	2.72	0.88	3.98	3.90	1.52	0.32	1.39	2.18	1.10	0.27	5.30
16	**alpha-BHC**	23	20	2.43	0.75	1.33	1.76	1.95	0.27	0.31	0.23	0.23	0.30	0.24	4.02
	beta-BHC	84	74	9.08	2.83	5.03	6.60	7.43	1.00	1.17	0.85	0.87	1.12	0.88	15
	delta-BHC	28	22	2.80	0.87	1.55	2.01	2.27	0.31	0.36	0.26	0.27	0.35	0.27	4.73
	gamma-BHC	33	28	3.58	1.08	1.90	2.58	2.87	0.38	0.45	0.33	0.34	0.43	0.34	5.84

Table 4-4. Means (µg/L) of hexachlorocyclohexane OCPs measured in water, by week and treatment.

Week	Analyte	Hot Spot	H/H	H/M	H/L	H/NO	M/H	M/M	M/L	M/NO	L/H	L/M	L/L	L/NO	Grand Average
0	**alpha-BHC**	0.0027	0.0027	0.0028	0.0029	0.0027	0.0027	0.0027	0.0030	0.0027	0.0026	0.0027	0.0027	0.0027	0.0027
	beta-BHC	0.0028	0.0028	0.0029	0.0030	0.0028	0.0028	0.0028	0.0031	0.0028	0.0028	0.0028	0.0028	0.0029	0.0029
	delta-BHC	0.0029	0.0030	0.0030	0.0032	0.0029	0.0029	0.0029	0.0032	0.0029	0.0029	0.0029	0.0029	0.0030	0.0030
	gamma-BHC	0.0029	0.0029	0.0030	0.0031	0.0029	0.0029	0.0029	0.0032	0.0029	0.0028	0.0029	0.0029	0.0030	0.0029
2	**alpha-BHC**	0.0026	0.0027	0.0027	0.0027	0.0028	0.0026	0.0028	0.0026	0.0027	0.0027	0.0027	0.0027	0.0026	0.0027
	beta-BHC	0.0028	0.0029	0.0029	0.0029	0.0029	0.0027	0.0029	0.0028	0.0029	0.0029	0.0028	0.0029	0.0028	0.0028
	delta-BHC	0.0029	0.0030	0.0030	0.0030	0.0030	0.0028	0.0030	0.0029	0.0030	0.0030	0.0029	0.0030	0.0029	0.0029
	gamma-BHC	0.0029	0.0029	0.0029	0.0029	0.0030	0.0028	0.0030	0.0028	0.0029	0.0029	0.0029	0.0029	0.0029	0.0029

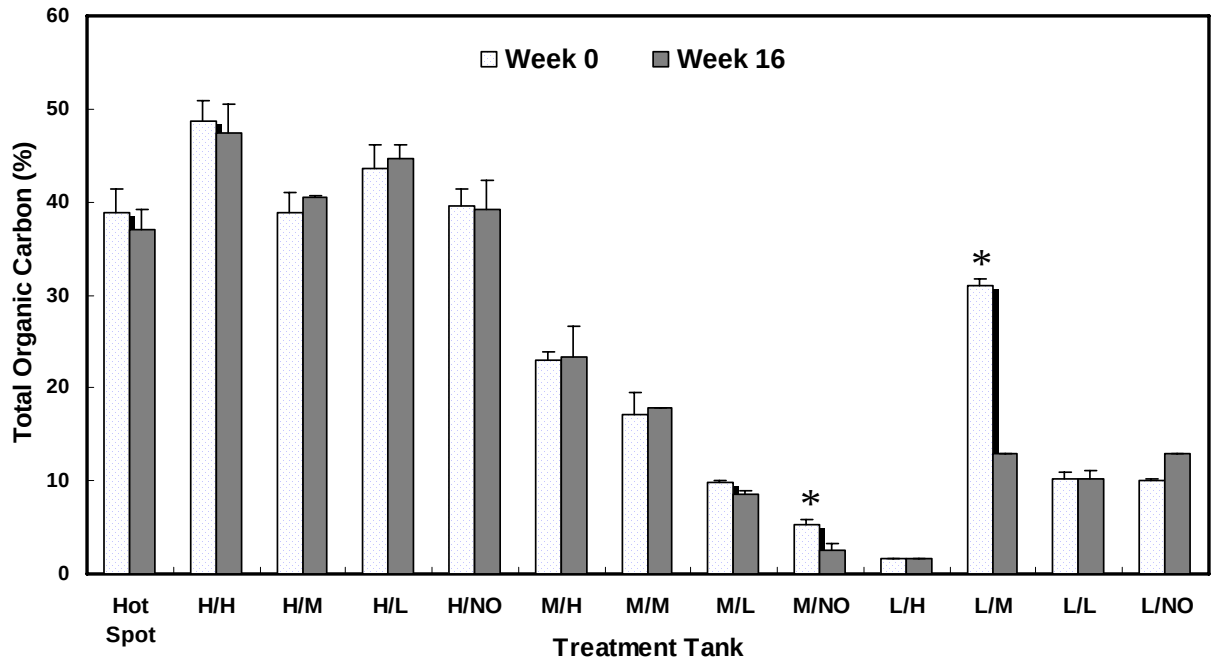


Figure 4-1. Mean ± SD of total organic carbon (TOC, %) in soils collected from each treatment tank at weeks 0 (November 5, 6, and 7, 2001) and 16 (February 25, 26, and 27, 2002). Significant week differences within treatments are also shown (ANOVA, p values: * 0.05 and ** 0.01).

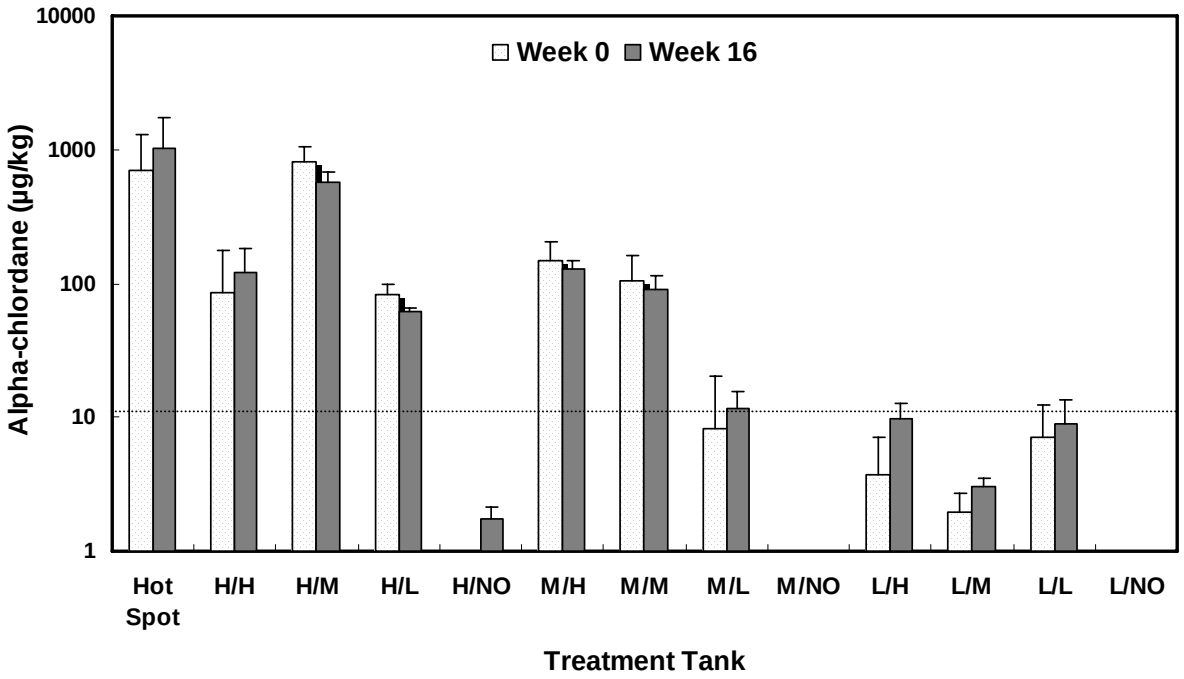


Figure 4-2. Mean ± SD of alpha-chlordane (µg/kg) in soils collected from each treatment tank at weeks 0 (November 5, 6, and 7, 2001) and 16 (February 25, 26, and 27, 2002). There were no significant week differences within treatments. Dotted line shows approximate average of method detection level (MDL) for all soils run (11.4 µg/kg).

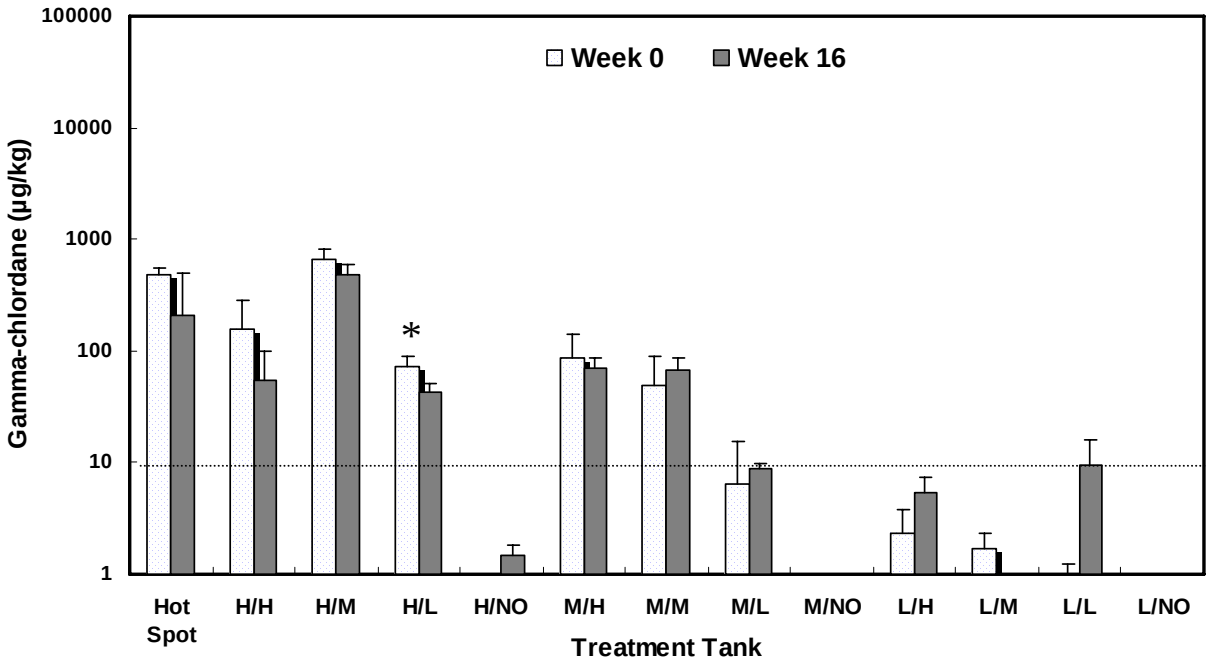


Figure 4-3. Mean ± SD of gamma-chlordane (µg/kg) in soils collected from each treatment tank at weeks 0 (November 5, 6, and 7, 2001) and 16 (February 25, 26, and 27, 2002). Significant week differences within treatments are also shown (ANOVA, p values: * 0.05 and ** 0.01). Dotted line shows approximate average of method detection level (MDL) for all soils run (9 µg/kg).

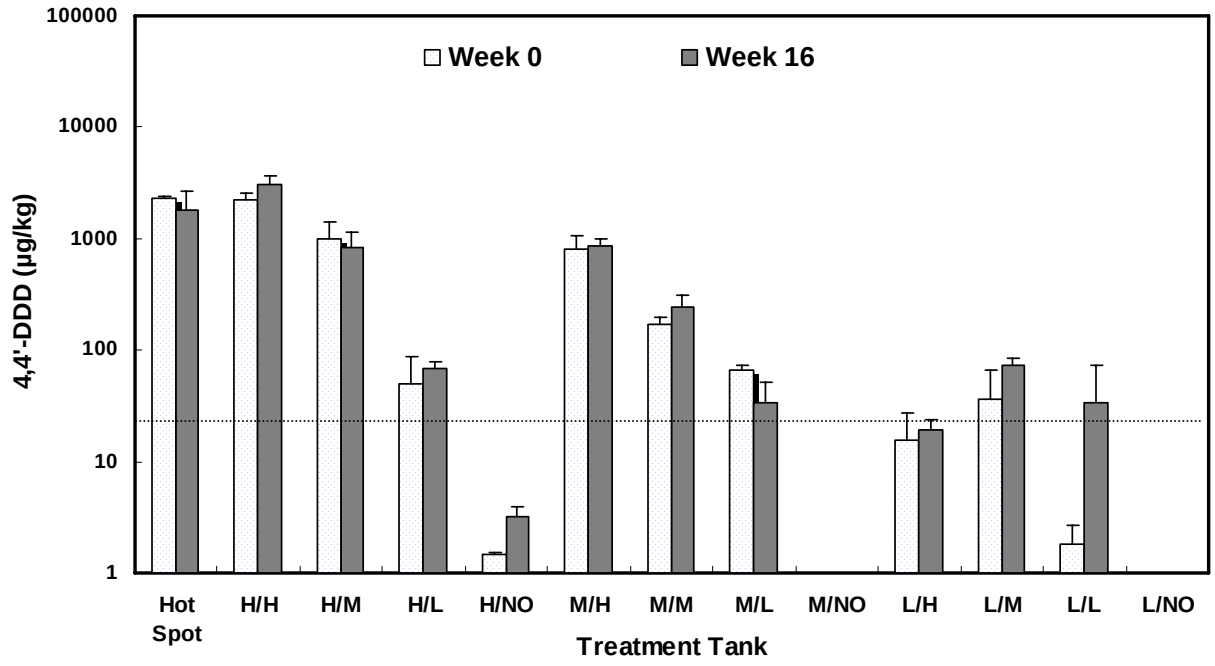


Figure 4-4. Mean ± SD of 4,4'-DDD (µg/kg) in soils collected from each treatment tank at weeks 0 (November 5, 6, and 7, 2001) and 16 (February 25, 26, and 27, 2002). There were no significant week differences within treatments. Dotted line shows approximate average of method detection level (MDL) for all soils run (31 µg/kg).

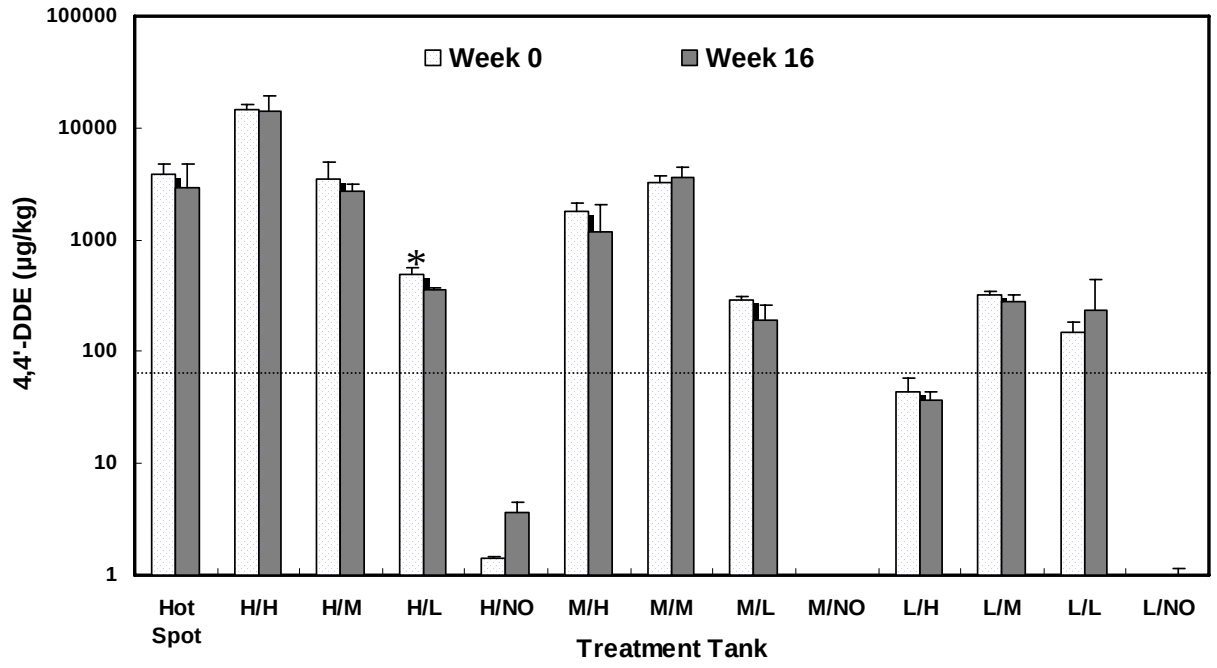


Figure 4-5. Mean ± SD of 4,4'-DDE (µg/kg) in soils collected from each treatment tank at weeks 0 (November 5, 6, and 7, 2001) and 16 (February 25, 26, and 27, 2002). Significant week differences within treatments are also shown (ANOVA, p values: * 0.05 and ** 0.01). Dotted line shows approximate average of method detection level (MDL) for all soils run (80 µg/kg).

*

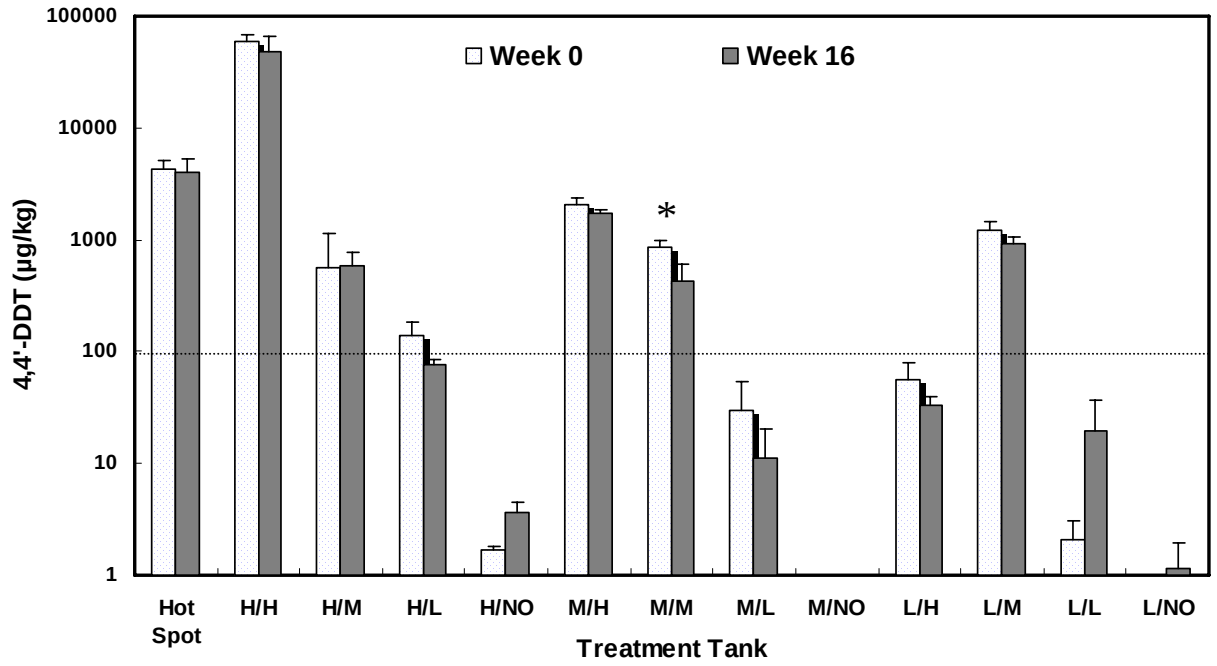


Figure 4-6. Mean ± SD of 4,4'-DDT (µg/kg) in soils collected from each treatment tank at weeks 0 (November 5, 6, and 7, 2001) and 16 (February 25, 26, and 27, 2002). Significant week differences within treatments are also shown (ANOVA, p values: * 0.05 and ** 0.01). Dotted line shows approximate average of method detection level (MDL) for all soils run (95 µg/kg).

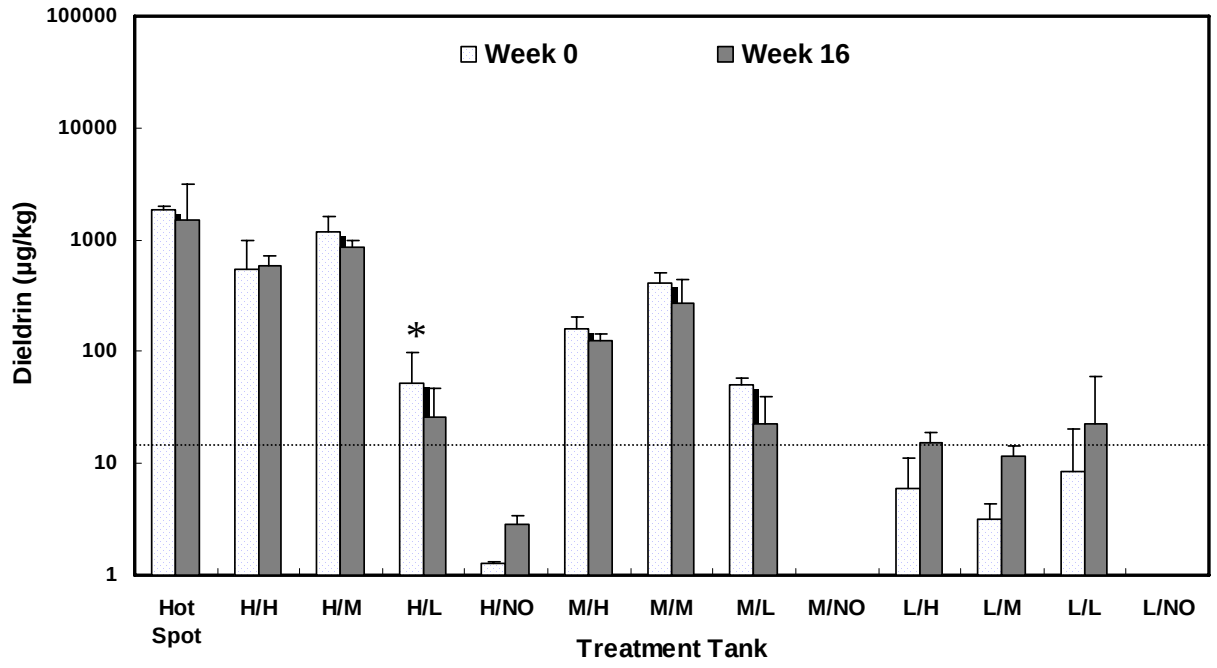


Figure 4-7. Mean ± SD of dieldrin (µg/kg) in soils collected from each treatment tank at weeks 0 (November 5, 6, and 7, 2001) and 16 (February 25, 26, and 27, 2002). Significant week differences within treatments are also shown (ANOVA, p values: * 0.05 and ** 0.01). Dotted line shows approximate average of method detection level (MDL) for all soils run (18 µg/kg).

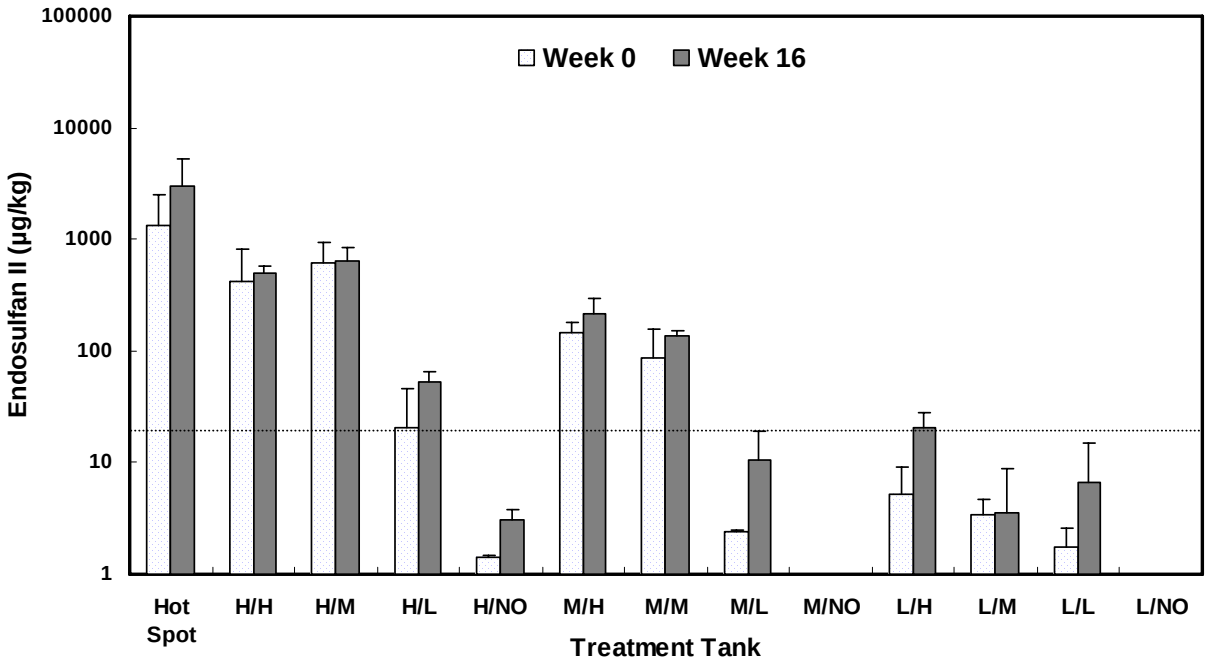


Figure 4-8. Mean ± SD of endosulfan II (µg/kg) in soils collected from each treatment tank at weeks 0 (November 5, 6, and 7, 2001) and 16 (February 25, 26, and 27, 2002). There were no significant week differences within treatments. Dotted line shows approximate average of method detection level (MDL) for all soils run (20 µg/kg).

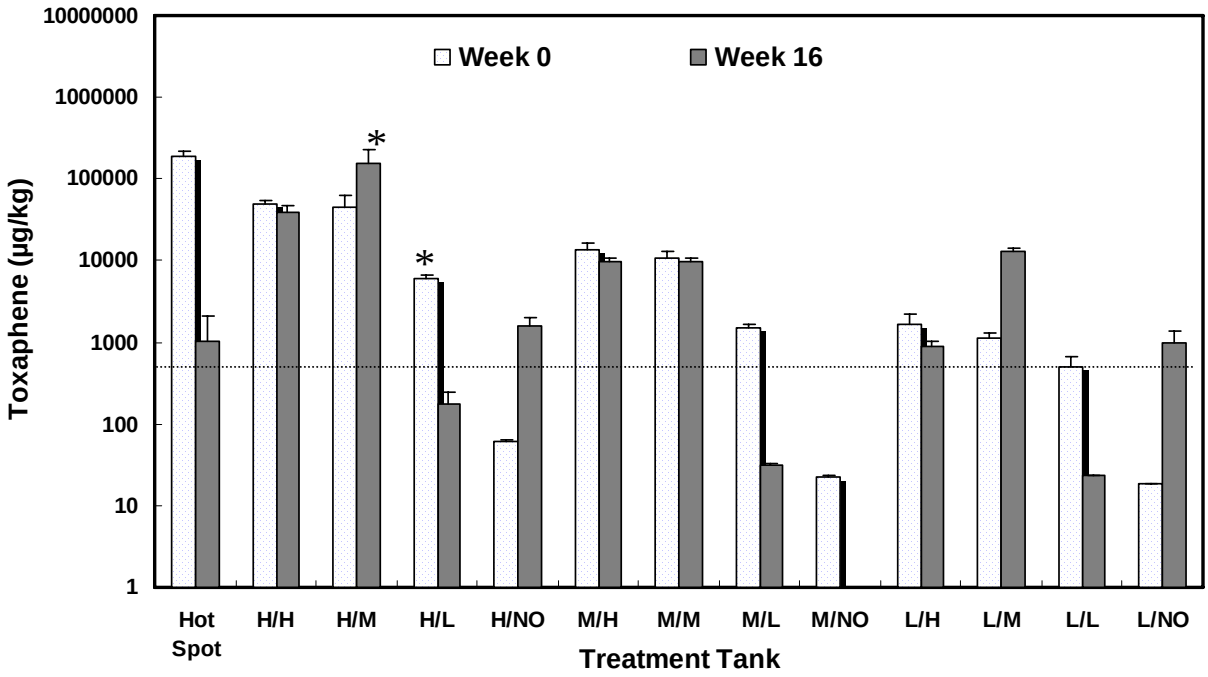


Figure 4-9. Mean ± SD of toxaphene (µg/kg) in soils collected from each treatment tank at weeks 0 (November 5, 6, and 7, 2001) and 16 (February 25, 26, and 27, 2002). Significant week differences within treatments are also shown (ANOVA, p values: * 0.05 and ** 0.01). Dotted line shows approximate average of method detection level (MDL) for all soils run (711 µg/kg).

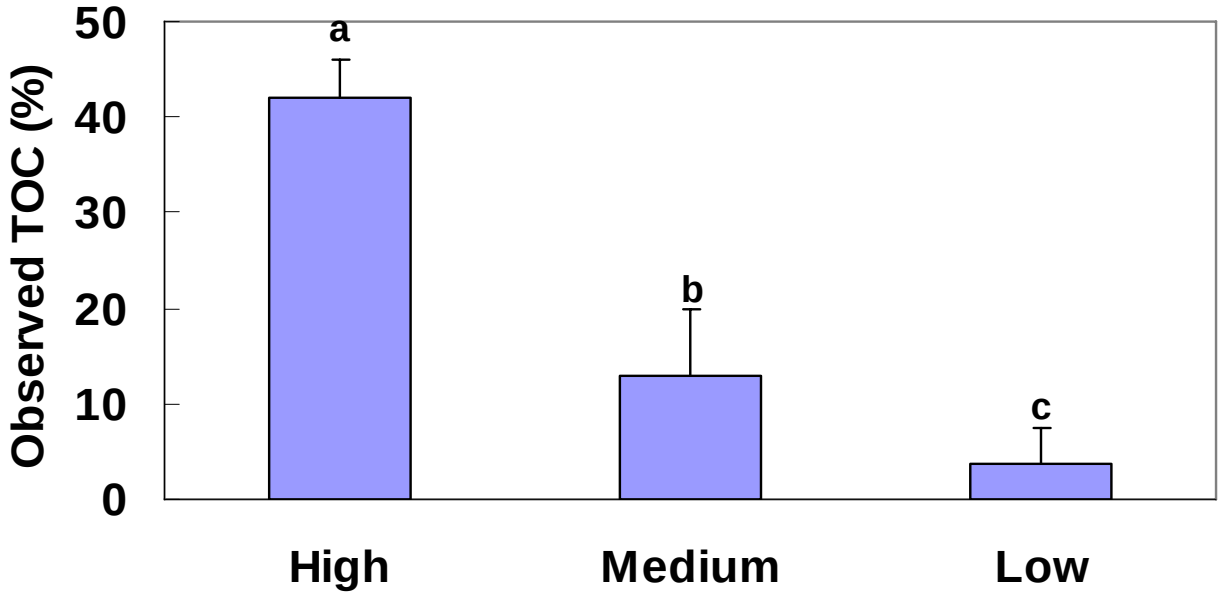


Figure 4-10. Mean ± SD of observed soil total organic carbon (TOC%), by category. There was a significant difference in TOC between categories.

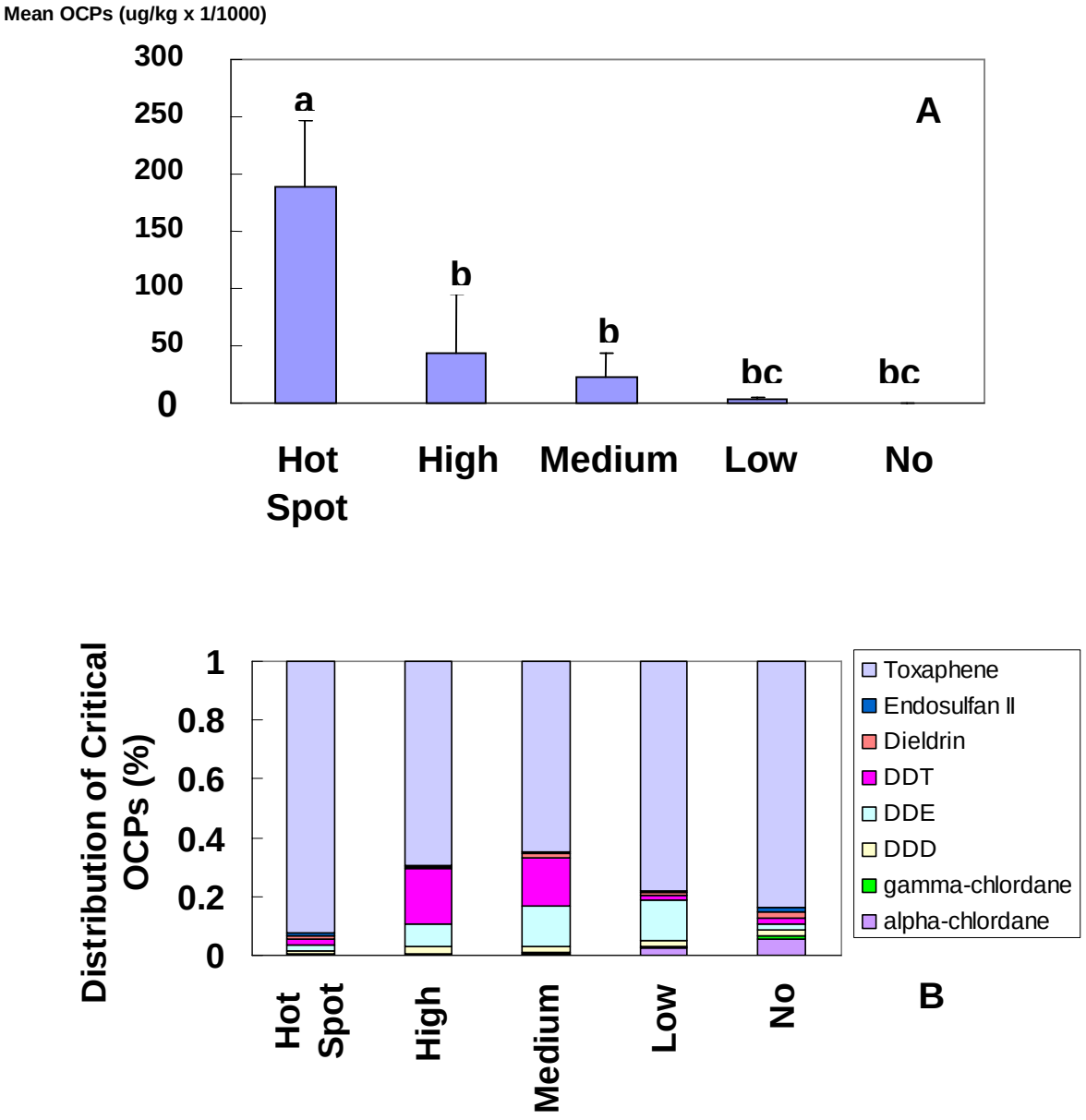


Figure 4-11. Mean ± SD of all soil OCPs of interest (A) in treatments "Hot Spot", "High", "Medium", "Low", and "No" OCPs. The percent distribution of each OCP in each treatment is also shown (B).

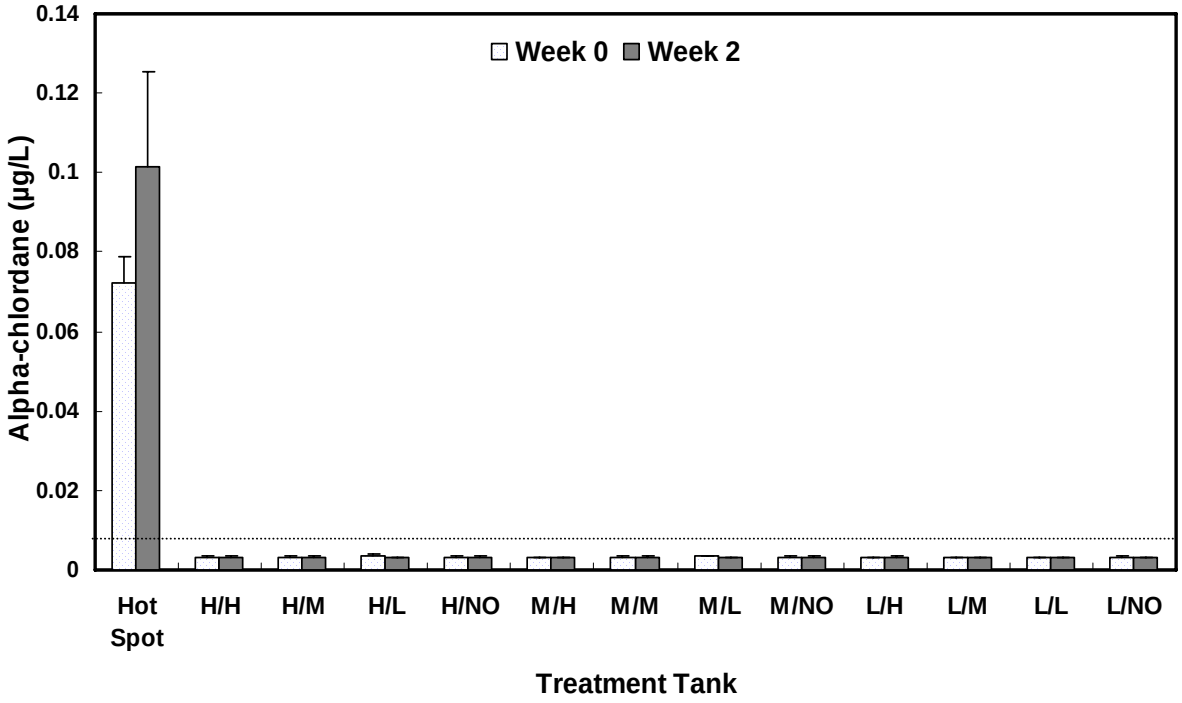


Figure 4-12. Mean ± SD of apha-chlordane (µg/L) in water collected from each treatment tank at weeks 0 (November 5, 6, and 7, 2001) and 2 (November 19, 20, and 21, 2001). Dotted line indicates average of method detection level (MDL) for all water samples run (0.006 µg/L).

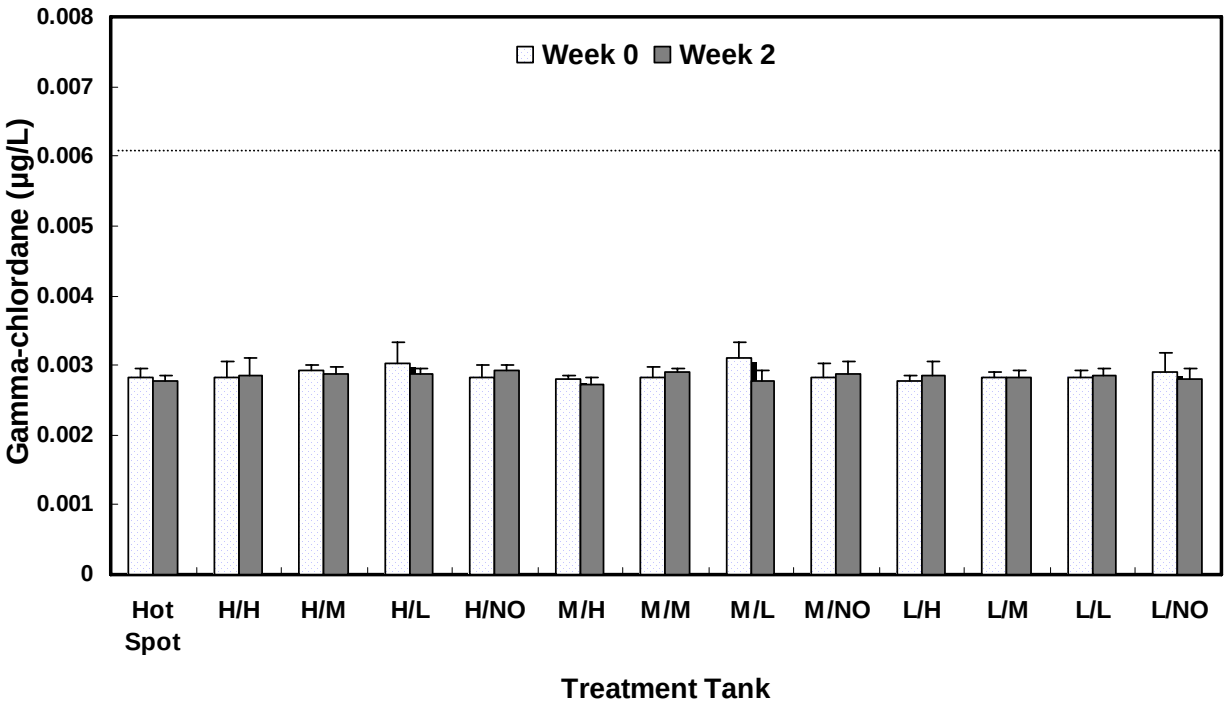


Figure 4-13. Mean ± SD of gamma-chlordane (µg/L) in water collected from each treatment tank at weeks 0 (November 5, 6, and 7, 2001) and 2 (November 19, 20, and 21, 2001). Dotted line indicates average of method detection level (MDL) for all water samples run (0.006 µg/L).

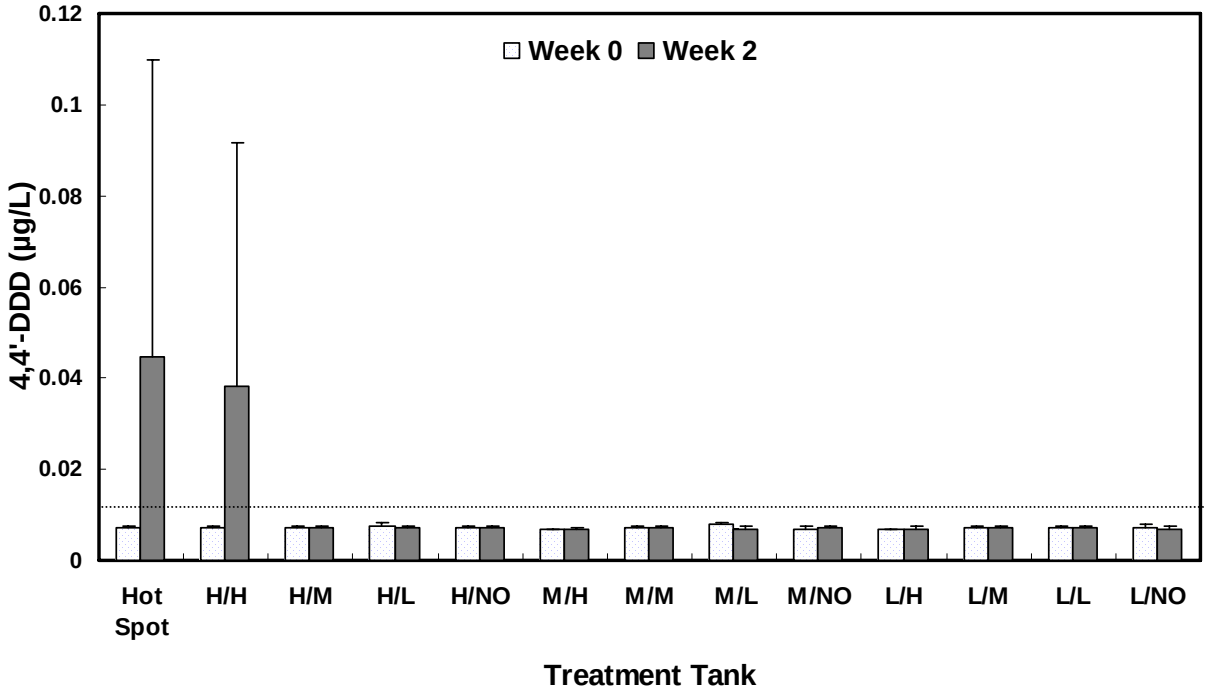


Figure 4-14. Mean ± SD of 4,4'-DDD (µg/L) in water collected from each treatment tank at weeks 0 (November 5, 6, and 7, 2001) and 2 (November 19, 20, and 21, 2001). Dotted line indicates average of method detection level (MDL) for all water samples run (0.014 µg/L).

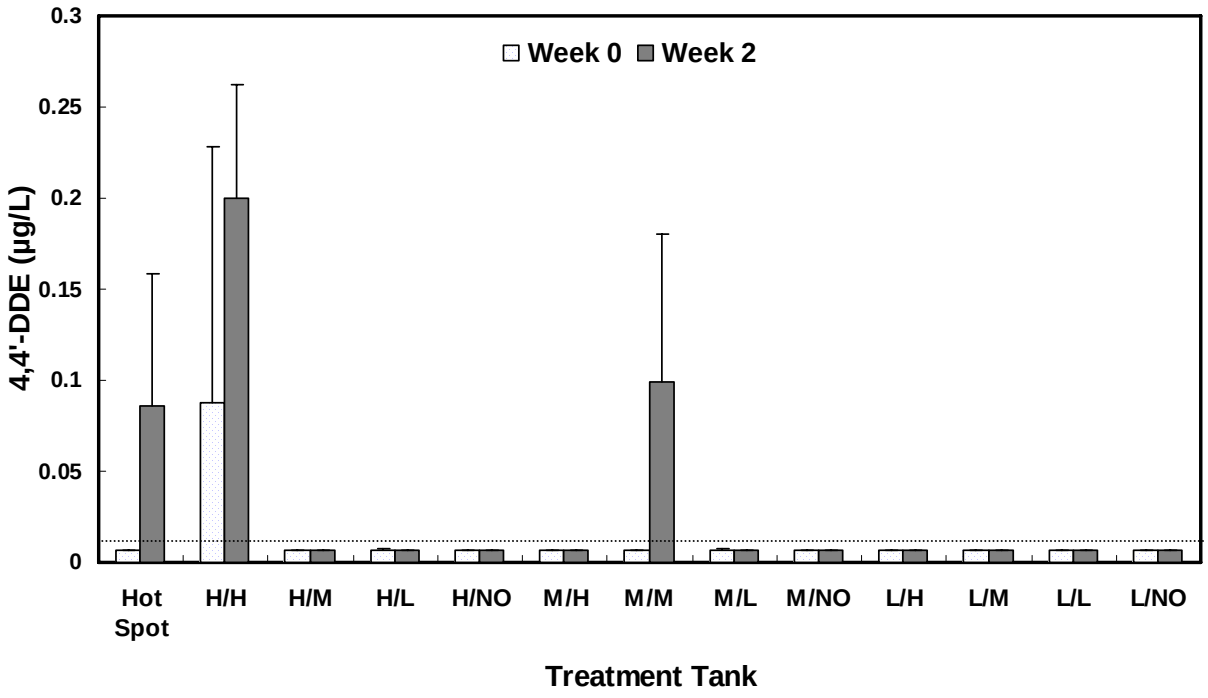


Figure 4-15. Mean ± SD of 4,4'-DDE (µg/L) in water collected from each treatment tank at weeks 0 (November 5, 6, and 7, 2001) and 2 (November 19, 20, and 21, 2001). Dotted line indicates average of method detection level (MDL) for all water samples run (0.012 µg/L).

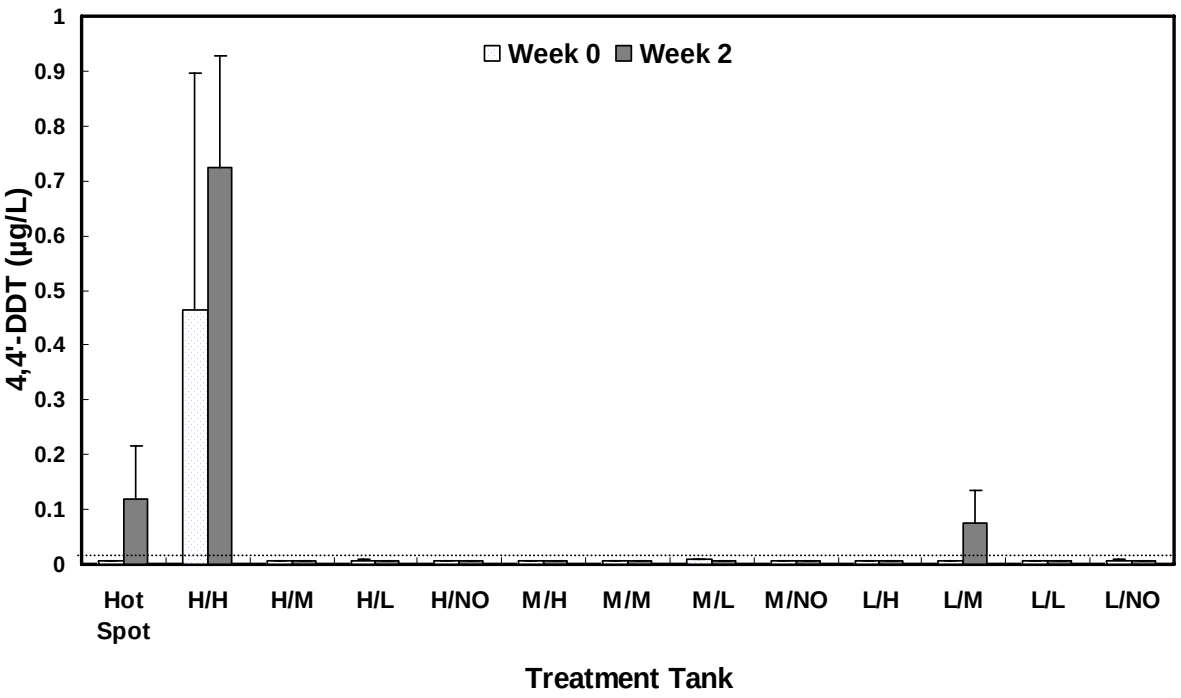


Figure 4-16. Mean ± SD of 4,4'-DDT (µg/L) in water collected from each treatment tank at weeks 0 (November 5, 6, and 7, 2001) and 2 (November 19, 20, and 21, 2001). Dotted line indicates average of method detection level (MDL) for all water samples run (0.014 µg/L).

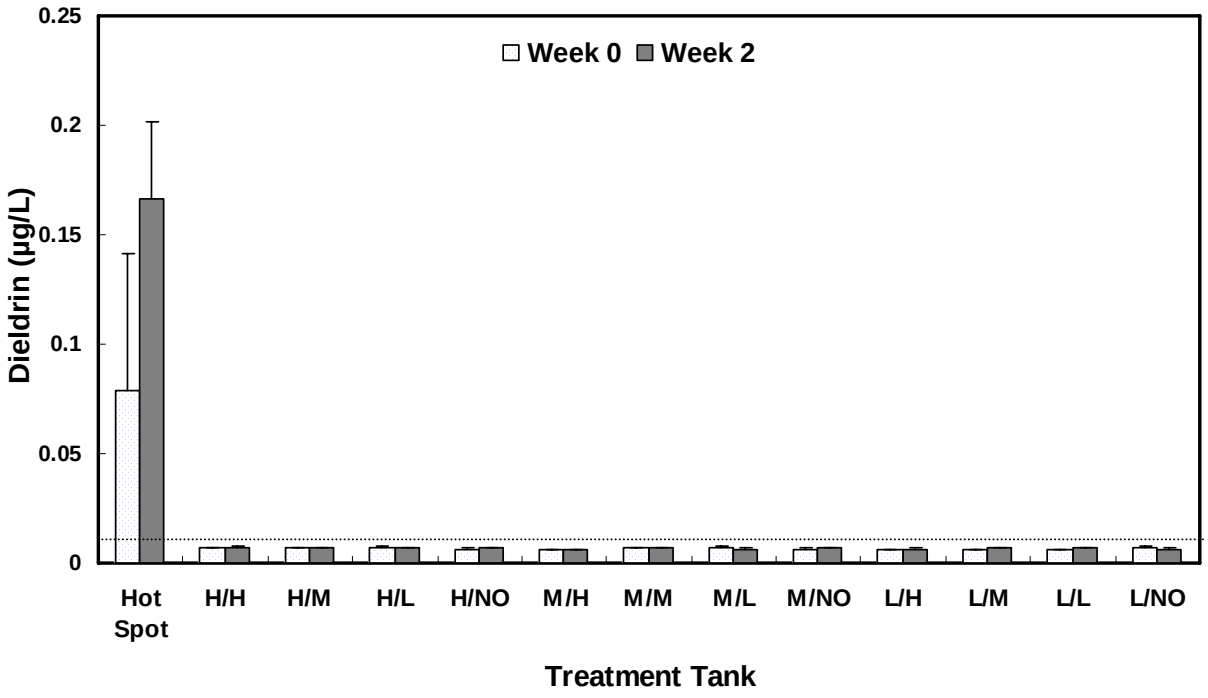


Figure 4-17. Mean ± SD of dieldrin (µg/L) in water collected from each treatment tank at weeks 0 (November 5, 6, and 7, 2001) and 2 (November 19, 20, and 21, 2001). Dotted line indicates average of method detection level (MDL) for all water samples run (0.013 µg/L).

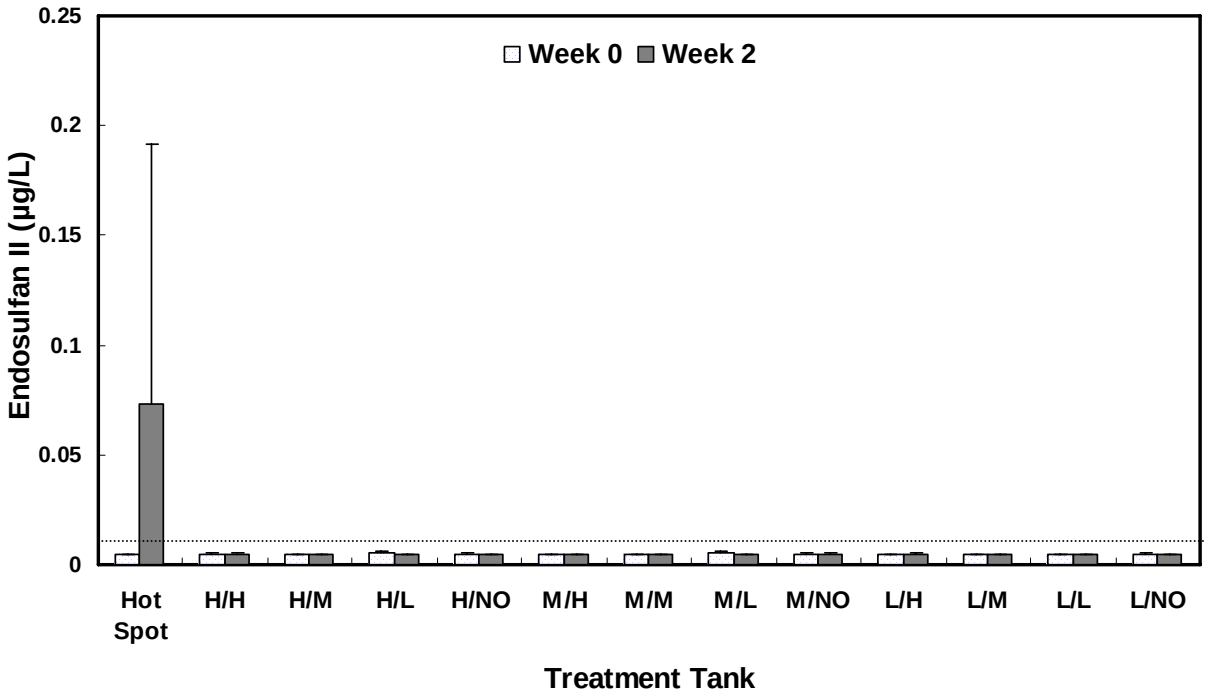


Figure 4-18. Mean ± SD of endosulfan II (µg/L) in water collected from each treatment tank at weeks 0 (November 5, 6, and 7, 2001) and 2 (November 19, 20, and 21, 2001). Dotted line indicates average of method detection level (MDL) for all water samples run (0.01 µg/L).

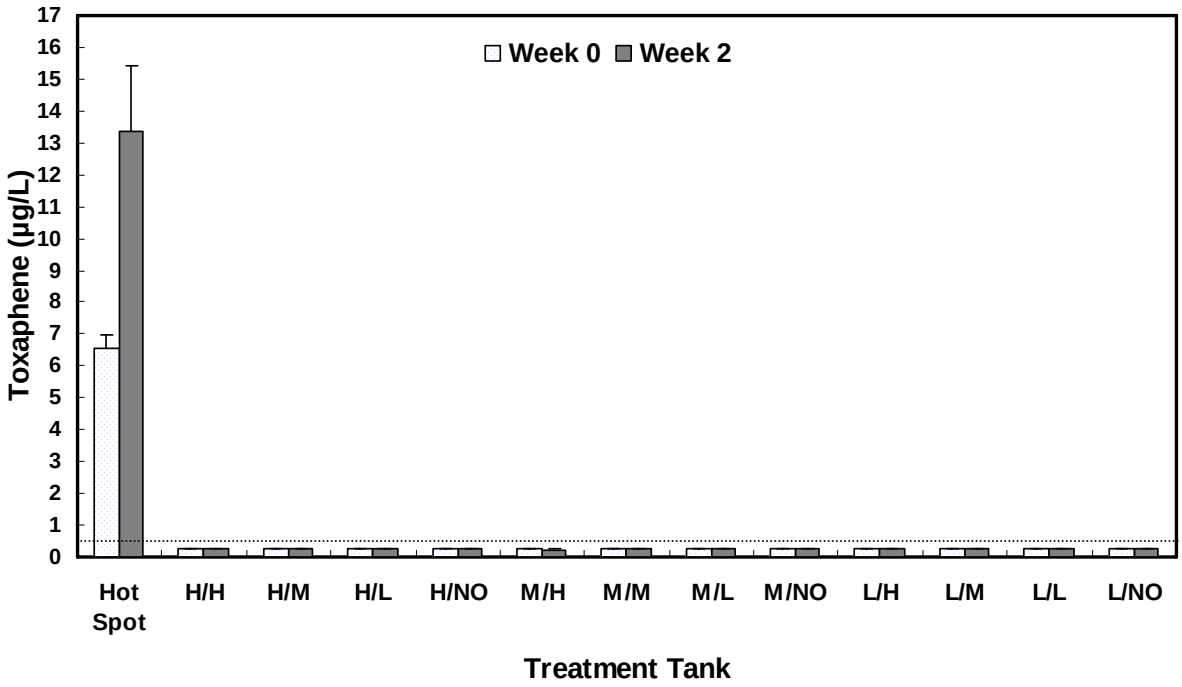


Figure 4-19. Mean ± SD of toxaphene (µg/L) in water collected from each treatment tank at weeks 0 (November 5, 6, and 7, 2001) and 2 (November 19, 20, and 21, 2001). Dotted line indicates average of method detection level (MDL) for all water samples run (0.5 µg/L).

CHAPTER 5
RESULTS AND DISCUSSION: ZOOPLANKTON AND PHYTOPLANKTON ANALYSES

This chapter will summarize zooplankton and phytoplankton data collected from the microcosm tanks throughout the study. As explained in Chapter 2, water samples from each treatment tank (5 L total) were collected for zooplankton and phytoplankton quantification approximately every 15 d. Zooplankton was quantified by counting the total number of organisms present in a I-mL subsample under a light microscope. Individual zooplankton organisms were then keyed out to Family using a freshwater invertebrate textbook (Pennak, 1991). Phytoplankton was quantified by measuring chlorophyll *a* concentrations using a portable fluorometer and turbidometer.

Zooplankton

In the present study, zooplankton was classified in Phylums Arthropoda and Rotifera (Figure 5-1). Within the Arthropoda, two Classes were identified: Crustacea and Insecta. Crustaceans were represented by the Orders Cladocera and Copepoda, and the Families Sididae, Daphnidae, Bosminidae, Chydoridae, and Diaptomidae and Cyclopidae, respectively (Figure 5-1). The Class Insecta was represented by a single Order (Diptera) and Family (Chironomidae).

The Phylum Rotifera was represented by a single Class (Monogononta), and the Orders Ploima (free-swimming) and Flosculariacea (sessile). The former was represented by the Families Lecanidae, Branchionidae, and Trichoceridae, whereas the latter was represented by the Family Filiniidae (Figure 5-1).

The percent distribution of zooplankton categories by treatment is presented in Figure 5-2. Regardless of treatment, numerically over half of the zooplankton identified throughout the course of this study was represented by copepods (81.3 %,), the majority of which were immature and thus could not be identified to Family (Figure 5-2). The second most abundant class of zooplankton was the rotifers (16.7 %), represented mostly by members of the Family Branchionidae. The apparent higher abundance of Families Branchionidae and Filiniidae in the Hot Spot treatment, was due to an unusual high number of individuals recovered from one of the replicates at week 5 (see Figure 5-5B and D). Cladocerans ("water fleas") made only 2% of the total number of zooplankton recovered, and almost half of them were members of the Family Sididae.

The mean ± standard deviation concentrations of each Family of zooplankton identified in this study are presented in Figures 5-3 to 5-5 by treatment and time of collection. In general and regardless of treatment and time of collection, there was a great variation in the numbers of zooplankton counted which probable accounted for the lack of significant trends observed. An interesting pattern, however, was the general tendency for an increase in zooplankton numbers during the course of the experiment (see Figure 5-6B for all zooplankton types combined). This would suggest that zooplankton were capable of reproducing and maintaining viable populations under the microcosm conditions tested. This is supported by the increase in numbers of immature

copepods over time. It is known that these organisms are capable of reproducing year-round, giving life to over four generations per year (Pennak, 1991). However, since the experimental design for this study called for the collection of fish and crayfish over time, this increase in zooplankton numbers could also be a reflection of a decrease in predator pressure over time.

As already mentioned, the overall concentrations of cladocerans, copepods, and rotifers found in the present study were quite variable (0.9 ± 1.4, 37 ± 54, and 2.2 ± 1.6 individuals/L, respectively), but within ranges reported in the literature for natural environments (Pennak, 1991). This is not surprising, since the environmental conditions present in the microcosm tanks (mostly DO, temperature, and pH) were well within tolerable ranges for the types of zooplankton identified (Pennak, 1991).

We had hypothesized that increased concentrations of OCPs in soils would result in increased zooplankton mortality. This hypothesis however was not supported by the data obtained in this study. We regressed the number of each zooplankton type collected in relation to soil TOC and OCP concentrations, and only found significant positive relationships between the total number of Cladocerans, Copepods, and Rotifers and the concentration of 4,4'-DDD, 4,4'-DDE, and dieldrin in soil (Figure 5-7). Since the number of zooplankton was greatly variable in this study, this relationship should be interpreted with caution and viewed as very preliminary.

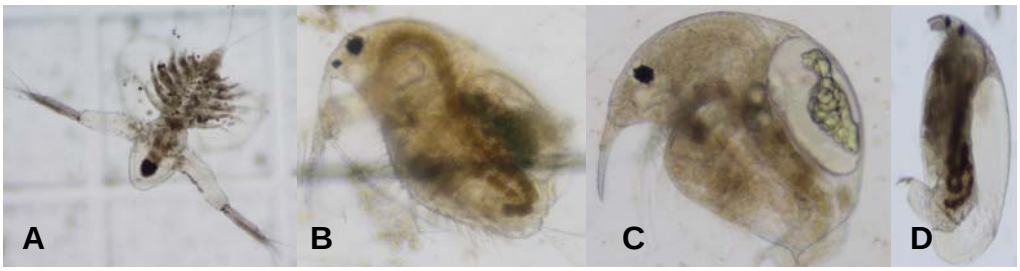
Phytoplankton Measured as Chlorophyll *a*

The mean ± SD of chlorophyll *a* concentrations over the course of the experiment are presented in Figure 5-8, by treatment. The results of the 2-way ANOVA showed a significant effect of both treatment and time on the amount of chlorophyll *a* measured in the water column.

Time changes of chlorophyll *a* were evidenced by an almost monocyclic pattern, with a peak in concentration between weeks 4 and 6 (Figure 5-8). This peak more or less followed the changes in water temperature with chlorophyll *a* concentrations dropping when water temperatures fell below 8ºC (see Chapter 3, Figure 3-2).

Although chlorophyll *a* concentrations were significantly different across treatments, these differences were not clearly explained by differences in soil TOC and OCP concentrations (for the latter, only the main OCPs were examined by regression analyses). For instance, and contrary to predictions, treatments with very low soil TOC (e.g. L/H) and with very high soil OCP (e.g. H/H) were the ones that contained the highest amount of chlorophyll *a* in the water (Figure 5-8).

PHYLUM ARTHROPODA

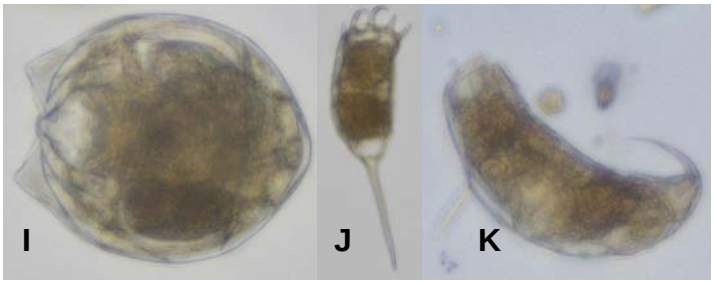


Class: Crustacean, Order: Cladocera. Families: A)Sididae; B)Daphnidae; C)Bosminidae; D)Chydoridae

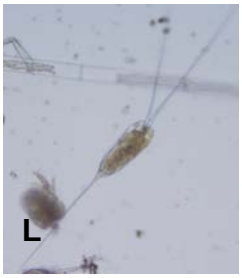
Class: Crustacean, Order: Copepoda. Families: E)Immature; F)Diaptomidae; G)Cyclopidae

Class: Insecta, Order: Diptera. Family: H)Chironomidae

PHYLUM ROTIFERA



Class: Monogononta, Order: Ploima. Families: I)Lecanidae; J)Branchionidae; K)Trichoceridae



Class: Monogononta, Order: Flosculariacea. Family: L)Filiniidae

Figure 5-1. Diagram showing representative individuals of each zooplankton Family identified from the microcosm tanks, by Phylum, Class, and Order.

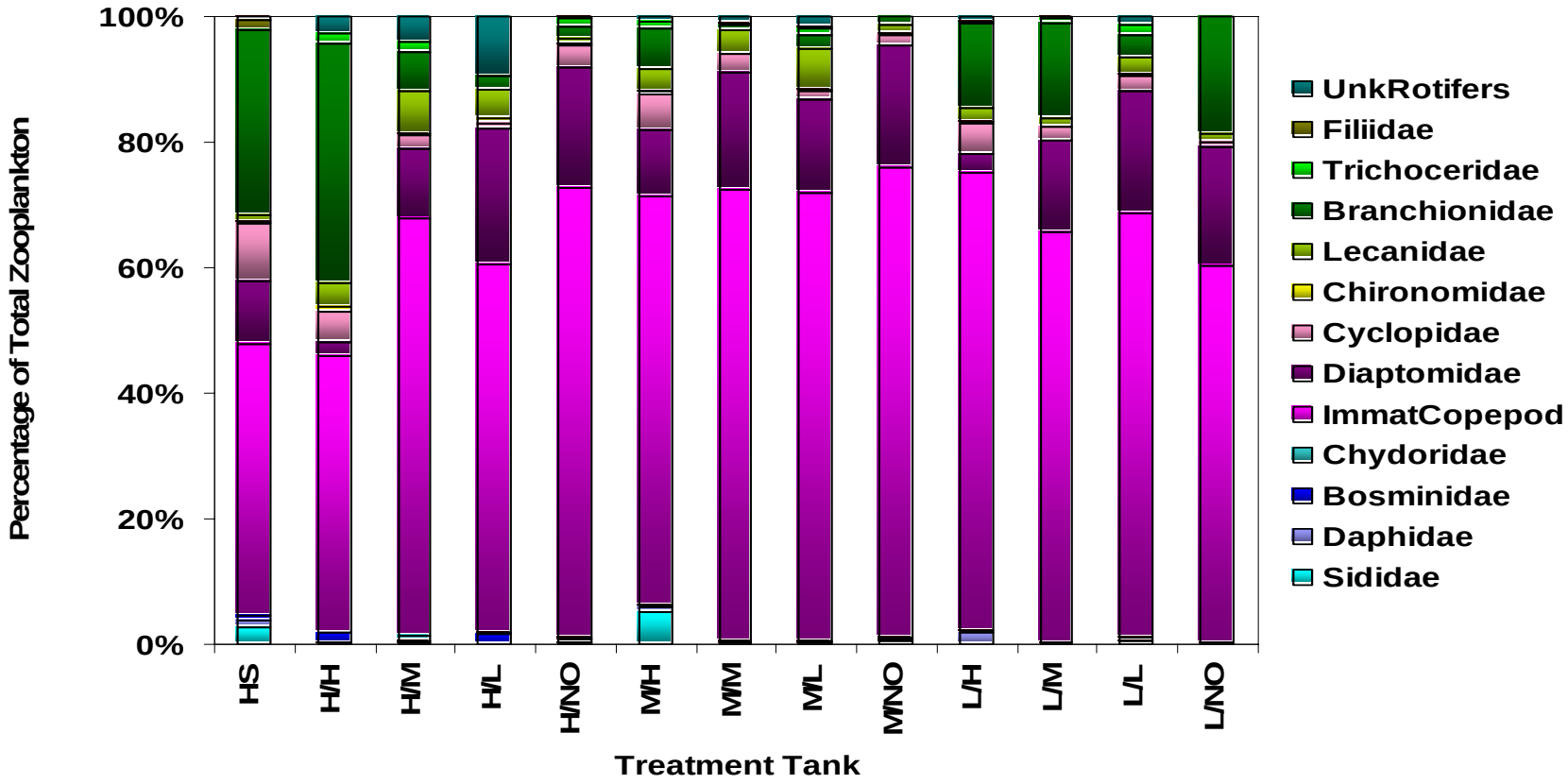


Figure 5-2. Percent distribution of zooplankton categories, by treatment.

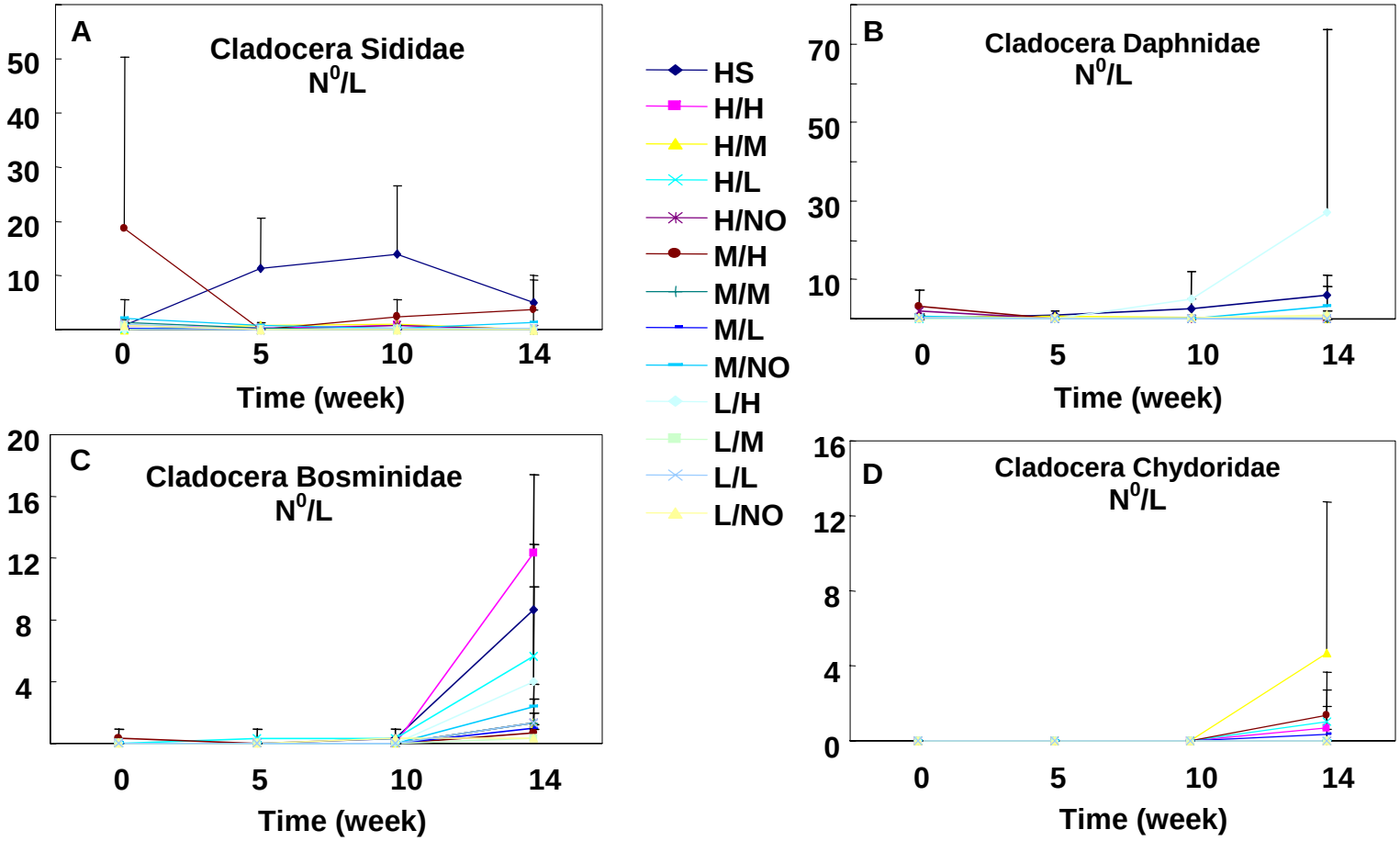


Figure 5-3. Mean number ± SD of Cladocera zooplankton per L of microcosm tank water, by family, date of collection, and treatment.

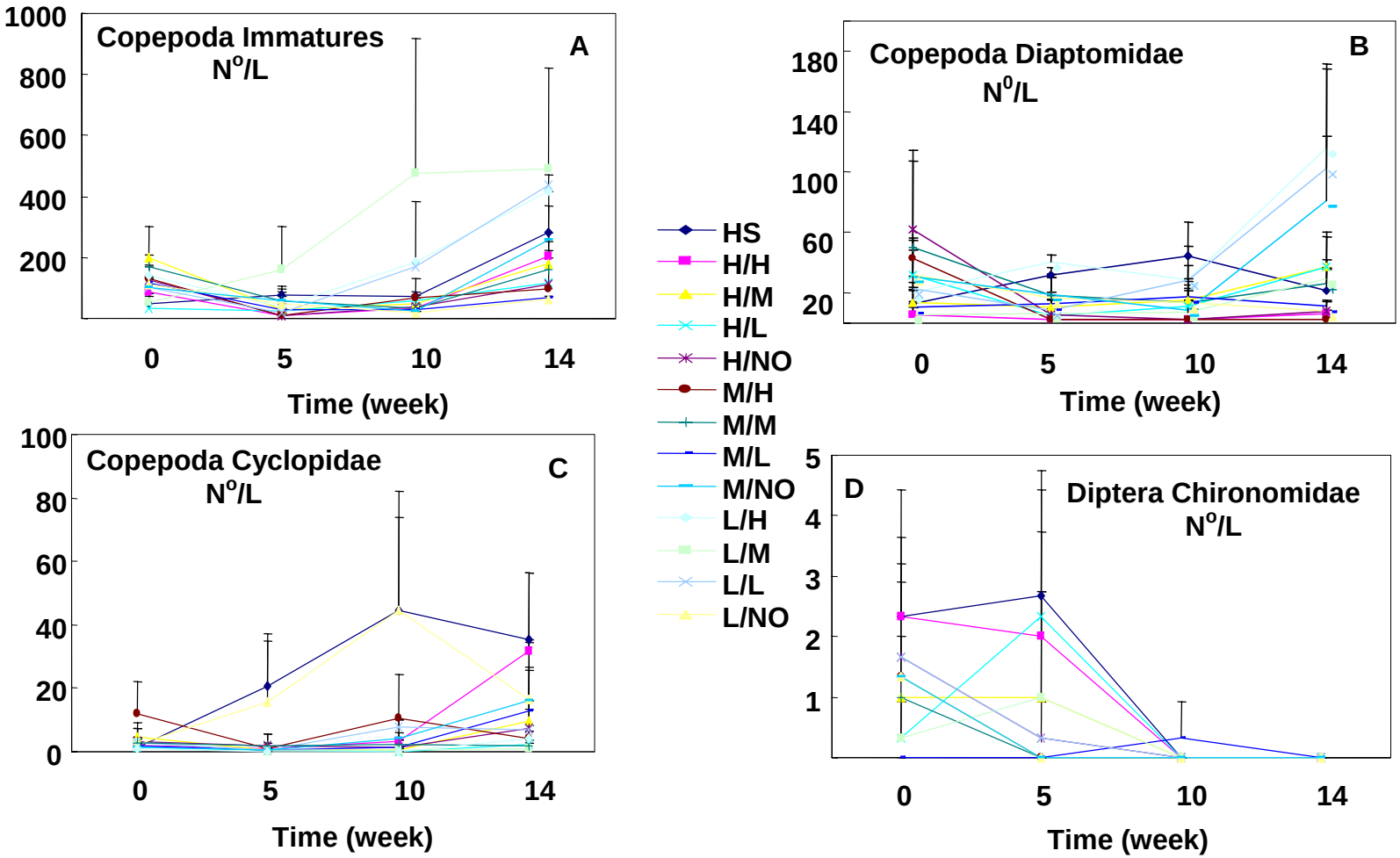


Figure 5-4. Mean number ± SD of Copepoda (A, B, and C) and Diptera (D) zooplankton per L of microcosm tank water, by family, date of collection, and treatment.

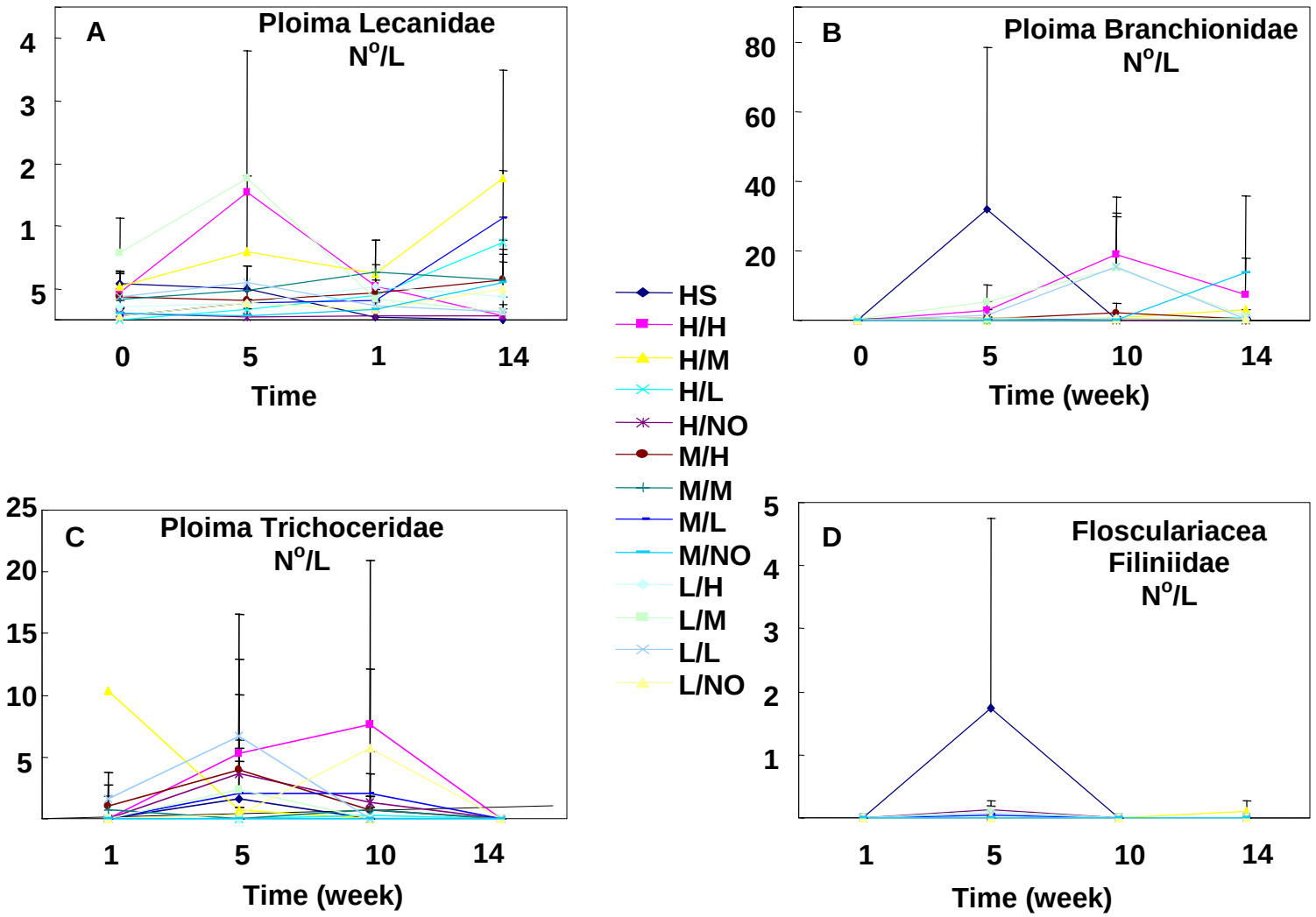


Figure 5-5. Mean number ± SD of Ploima (A, B, and C) and Flosculariacea (D) zooplankton per L of microcosm tank water, by family, date of collection, and treatment.

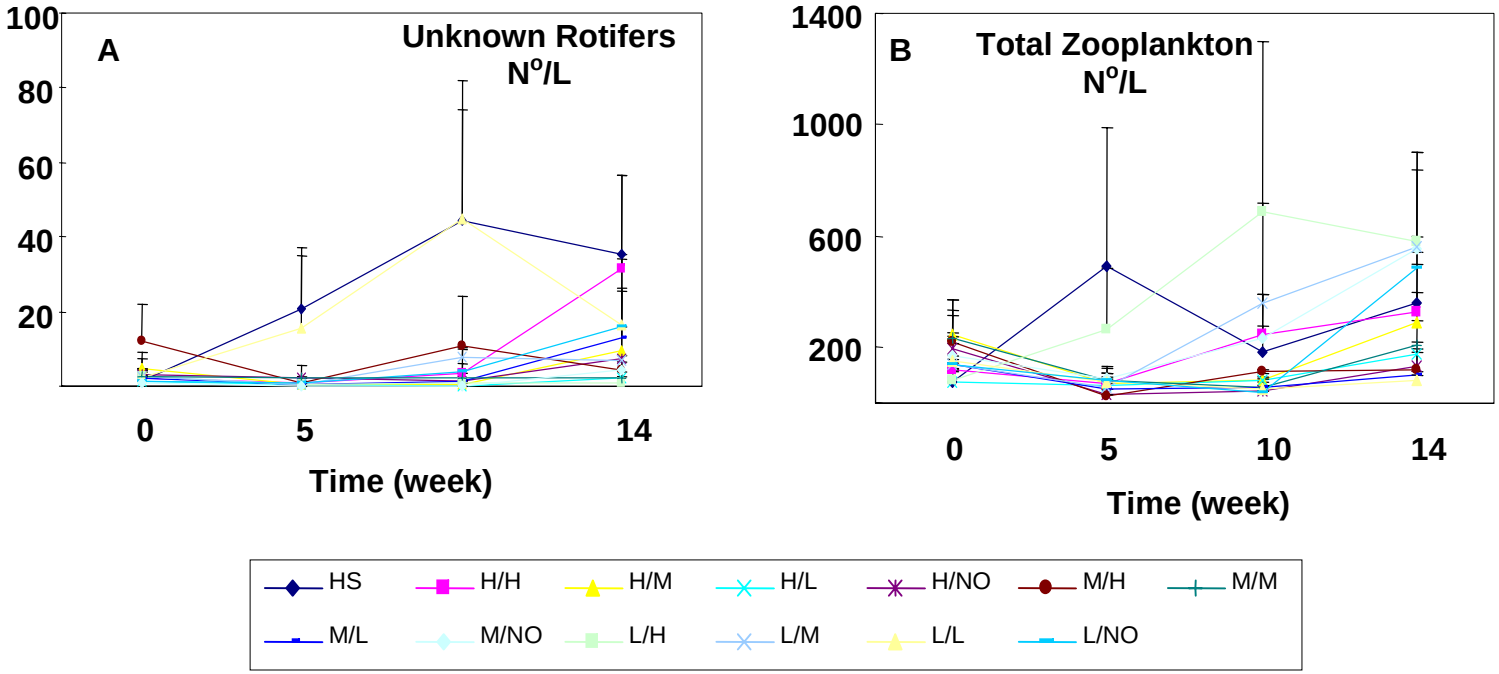


Figure 5-6. Mean number ± SD of unknown rotifers (A) and total numbers (B) of zooplankton per L of microcosm tank water, by date of collection, and treatment.

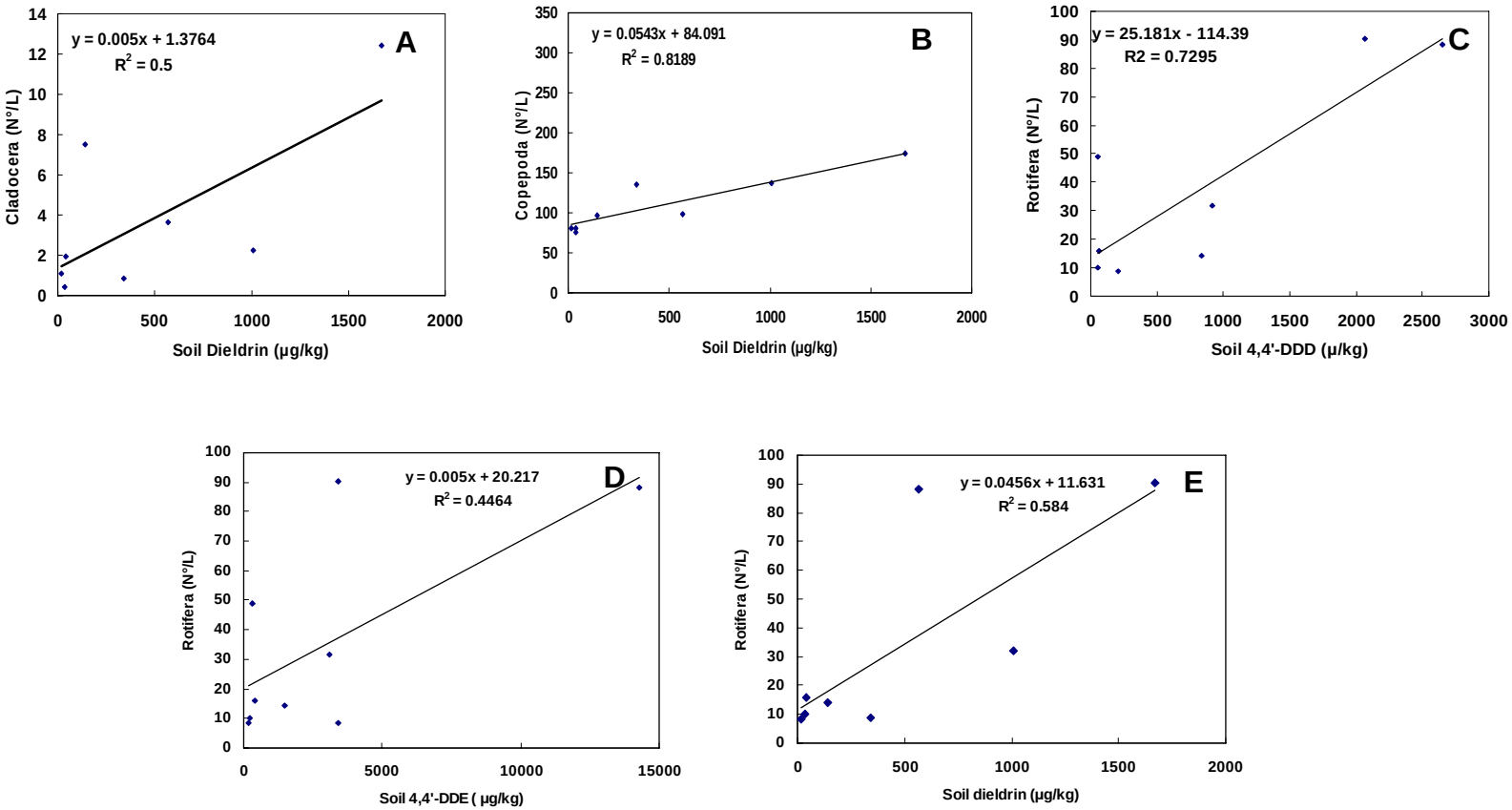


Figure 5-7. Relationship between total number of Cladocera (A), Copepoda (B), and Rotifera (D and E) zooplankton and concentrations of dieldrin, 4,4'-DDD, and 4,4'-DDE in soil. Only soil values above detection limit were included in the regression analyses.

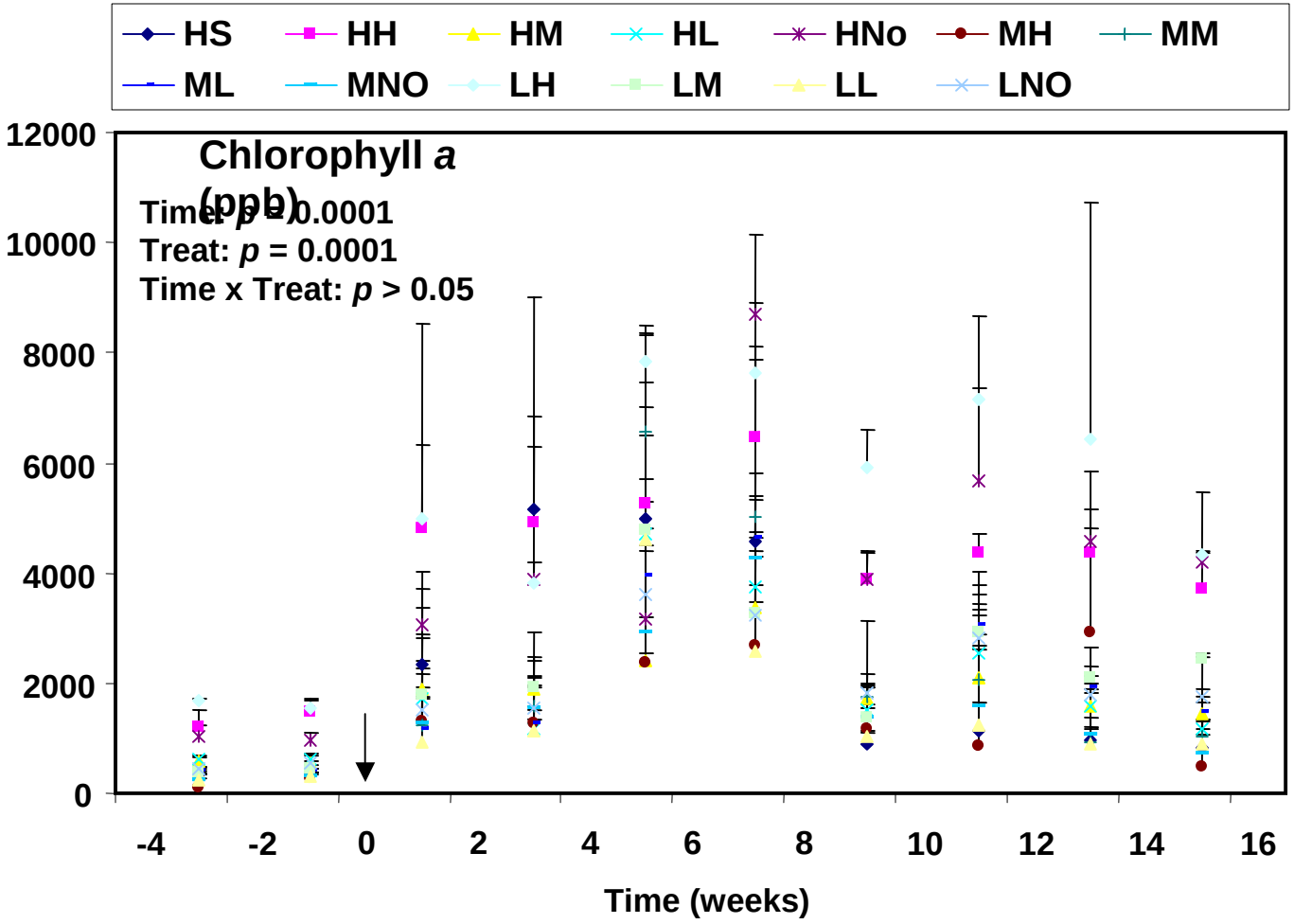


Figure 5-8. Changes in chlorophyll *a* over the course of the microcosm study, by treatment. Plotted are averages ± SD of the three replicates/treatment. The arrow (week 0) indicates the time microcosms were stocked with mosquitofish and crayfish (November, 5 – 7, 2001). Results of the 2-way repeated measures ANOVA are also shown.

◄

CHAPTER 6
RESULTS AND DISCUSSION: BIOACCUMULATION OF ORGANOCHLORINE PESTICIDES IN MOSQUITOFISH AND CRAYFISH

This chapter will summarize crayfish and mosquitofish lipid and OCP data. Data was summarized in relationship to treatment, time of collection, and in some instances, in relation to soil OCP and TOC. A summary of the amount of biota collected by treatment is also presented.

Similar to previous chapters, data analysis was focused only on a subset of OCPs (i.e. 4,4'-DDD, 4,4'-DDE, and 4,4'-DDT; alpha and gamma-chlordane; dieldrin; endosulfan II; and toxaphene) (see Chapter 2 for a justification on why these OCPs were chosen for a more detailed analysis). Nevertheless, summary Tables (6-1 through 6-6) are given with data on all OCPs analyzed in this study.

Amount of Biota Collected

The amount of biota (grams in wet weight) collected during the course of the experiment is summarized in Figures 6-1 and 6-2 for crayfish and mosquitofish, respectively. For crayfish, the overall mass collected averaged 332 ± 108 g, which represents a slight increase (10.6 %) in relation to the amount stocked (Figure 6-1A).

Figure 6-1B summarizes the mean mass of crayfish collected per sampling event. Both sets of data followed the same trend, in that in two of the treatments with highest

soil OCP (Hot Spot and H/H) lower amounts of crayfish (< 8.3 g) were collected (Figure 6-1B).

The overall mass of mosquitofish collected during the course of the study averaged 75 ± 30 g, which represents a 50 % increase in relation to the amount stocked (Figure 6-2A). This large increase in mass is more likely a result of an increase in fish numbers and not fish size. It is unlikely mosquitofish grew significantly over the course of the study, since most of the population of fish stocked were in the adult size class. An increase in fish numbers due to reproductive output, on the other hand, is known to occur under similar captive conditions (Paul Leberg, University of Louisiana, Lafayette, personal communication). Considering that in mosquitofish age to maturity is about 60 d (Jessica Noggle, University of Florida, Gainesville, personal communication) production of more than one generation of fish was possible in this study.

Similarly to what was observed with crayfish, treatment had a significant effect on the amount of mosquitofish recovered (Figure 6-2B). Fish were never retrieved from the Hot Spot tank, and very low amounts were collected from treatments H/H and L/H (< 3.7 g/event). High values were collected from treatments L/L and M/H (> 7.0 g), and the remaining treatments had intermediate values (4.8 – 6.0 g). The extremely low values of mosquitofish collected from the Hot Spot tank would suggest an increased mortality of fish in relation to the other treatment tanks. It is unlikely fish died due to water quality problems, since there were no differences in such parameters across treatments (see Chapter 3). Fish, however, could have died due to acute toxicity. If this was indeed what happened, one could calculate LC_{100} values for mosquitofish based on the scant amount of OCP data measured in water from the Hot Spot tank during the first two

weeks of experiment (see Chapter 4). The OCP with highest concentration reported in water was toxaphene which was detected at an average concentration of 10 ± 4 µg/L, while the remaining OCPs were quantified at a mean of 0.03 ± 0.05 µg/L. See below for a more detailed discussion on OCP toxicity threshold values.

Regression analyses were also run to further evaluate the relationship between certain soil OCPs and amount of biota collected, and results are summarized in Figures 6-3 and 6-4 for crayfish and mosquitofish, respectively. No significant trends were observed for crayfish (Figure 6-3), whereas in mosquitofish, increasing concentrations of 4,4'-DDD, dieldrin, and toxaphene resulted in significant declines in the amount of fish collected (Figure 6-4).

Total Lipid Content in Biota

Percent whole body lipid content was analyzed for all of the biota samples collected during the course of this study. Figure 6-5 summarizes the overall lipid concentrations detected in crayfish and mosquitofish, by treatment. Regardless of treatment, mosquitofish contained almost twice the amount of lipids compared to crayfish (2.0 vs. 0.9 %). Both mosquitofish and crayfish from the H/H tank contained the highest percent body lipid (1.7 and 2.9 % for crayfish and mosquitofish, respectively). Crayfish from the Hot Spot tank also had above average lipid concentrations (1.8 %). These high percent lipid contents could be a reflection of the lower densities of fish and crayfish observed in these tanks. Since animals were not fed during the study, a lower density could have led to a higher amount of natural food/animal, and thus to higher fat levels.

Temporal changes in lipid contents are presented in Figures 6-6 and 6-7 for crayfish and mosquitofish, respectively. For crayfish, body lipid contents decreased

significantly after week 2, regardless of treatment (Figure 6-6A). As can be seen in Figure 6-6B, during the second week of the study, crayfish had body lipid concentrations that were similar to those observed prior to the start of the experiment (1.9 ± 0.6 %). These concentrations, however, fell rapidly and steadily over the course of the experiment, and reached a minimum of 0.5 ± 0.13 % by week 16.

In the case of mosquitofish, treatment effects on lipid content were not as clear. Indeed, a significant interaction effect was observed between treatment and week of collection (Figure 6-7A). Temporal changes in lipid content in this fish species followed a “U” shape-curve, with high values between weeks 0 and 4 (2.5 ± 0.4 %); lowest values between weeks 8 and 12 (1.8 ± 0.4 %); and baseline values again at the end of the experiment (2.6 ± 1.1 %) (Figure 6-7B). This “dip” in lipid values could have been related to a decrease in water temperatures and a decrease in food intake during the winter months. This dip could also have been due to food stress which later decreased as sampling events reduced competition for food. Indeed, between weeks 7 and 10, water temperatures reached their lowest (< 8°C, see Figure 3-2, Chapter 3). This decline coincided with the lowest lipid contents in mosquitofish (between weeks 8 and 12).

In summary, mosquitofish had almost twice the amount of lipid compared to crayfish. In relation to treatment effects on lipid contents, although some significant differences were observed, no clear patterns were discernible. Temporal changes in lipid content were evident for crayfish and mosquitofish. Lipid contents steadily decreased over time in crayfish, and in mosquitofish lowest values were observed approximately in the middle of the study, with values increasing towards the end. This

differential rate of lipid accumulation across species could due to several factors. First, since crayfish were much harder to retrieve from the tanks (they were usually found in direct contact with sediment, sometimes buried in it), the last sampling event (week 16) had a proportionally higher number of individuals (between 3 and 5/tank) than the remaining time points (single animal/tank). This large sample size could have "skewed" the lipid determination towards a more "diluted" concentration. Second, crayfish could have been eating less than their fish counterparts, and thus accumulating less fat. This possibility is supported by the relatively small increase in the mass of crayfish in relationship to amount stocked, compared to mosquitofish. Lower food consumption could have been the result of behavioral differences between species. For instance, it is possible that crayfish did not adapt as well to captive conditions compared to mosquitofish, and thus not only ate less, but used more energy to deal with the increased stress conditions. Second, the type of food present (mostly phyto and zooplankton, see Chapter 5) likely were not adequate for a higher top predator such as the crayfish. Decreased food consumption could also have been a subtle effect induced by the OCPs themselves (see below for threshold toxicity values in other crustaceans). And thirdly, crayfish could have been overstocked compared to mosquitofish. Overcrowding could have led to a decrease in food consumption due to a combination of stress and lack of enough food. This is however an unlikely possibility, because we should have seen an increase in overall body condition as the experiment progressed due to decrease numbers of crayfish due to collections.

Chlorinated Pesticides in Biota

Frequency Distribution of Data Qualifiers

The percent distributions of different laboratory qualifiers reported by EN CHEM for biota are summarized in Figure 6-8. Only the most common qualifiers were summarized. Many more were provided, but were mostly combinations of the ones presented here.

The most common qualifier reported was “U”, which stands for “non-detect” values and as such get assigned the laboratory method detection limit or MDL instead. Pie charts with a dark grey background represent chemicals for which most of the values reported fell into the non-detect category (mean of 82 %; 60 – 99 %). Mostly because of this reason, these chemicals were left out of more detailed analyses in this report. The only exception to this was trans-nonachlor, which had a 35 % of “U” values, similar to what was reported in the remaining chemicals examined with more detail in this study (mean of 42 %; 8 – 70 %). As already explained in Chapter 2, for our analyses we chose to use half the concentration of these non-detect values. Non-detect values were not included in BSAF calculations, however.

The second most common qualifier was “J”, which is attached to a value when there are laboratory Quality Assurance (QA) problems involved in the analyses. These values are only estimates, and thus may not be accurate. Here the opposite to what was seen with “U” values was true, in that the chemicals discussed in more detail throughout this report had a higher percentage of values with a “J” qualifier (24 %; 12 – 41 %) compared to the rest (17 %; 1 – 31 %). For instance, for dieldrin and endosulfan II, a total of 34 and 41 % of the values, respectively, fell into this category.

It is interesting to note that, of all the chemicals analyzed by this laboratory, only one (4,4,'-DDE) was reported with no qualifiers most of the time (78 % of the values, Figure 6-8). For all the remaining chemicals, less than 10 % of the cases included values with no qualifiers attached. With these results in mind, it would be beneficial for future studies to compare the QA/QC procedures and standards of this commercial laboratory with that of others.

It is important to mention that for toxaphene, the large number of "JN" coded values reported is due to a stipulation in the District's contract with En Chem. Specifically, the District requested that a "JN" qualifier be assigned to Toxaphene values when a satisfactory pattern match with the standard was not observed. This means that it was not possible to match a minimum of the 4 peaks based on the retention time and peak height. This is not surprising, since the Toxaphene in the Apopka soil and tissue samples is weathered, so would not likely match the standard. This is especially true for the 5-9 peaks that are typically selected for the pattern match comparison. These peaks tend to represent relatively high concentrations of highly chlorinated congeners. These are the congeners that are either greatly reduced in concentration (lower peak height) or absent in weathered Toxaphene. However, it was shown in independent high resolution GC-MS analyses using multiple single congeners as standards that Toxaphene is indeed present in the Apopka soil and tissue samples in relatively high concentrations. Thus, even though the peak pattern of the Apopka samples does not match the standard, there is high confidence that these peaks do represent Toxaphene.

Bioaccumulation of OCPs in Biota

Pattern of bioaccumulation

The mean relative concentration of the different OCPs analyzed in biota samples is summarized in Figure 6-9. As can be seen from this Figure, there was no difference in the pattern of pesticide bioaccumulation between crayfish and mosquitofish. Ninety eight percent of the chemicals bioaccumulated by crayfish and mosquitofish were composed of: toxaphene (overall mean of 56 %); 4,4'-DDE (22 %); 4,4'-DDT (15 %); 4,4'-DDD (2.5 %); and dieldrin (2.5 %).

Amount of bioaccumulation

The concentrations of the most critical OCPs in crayfish and mosquitofish are summarized in Figures 6-10 through 6-17. An "A" suffix after each Figure number represents OCP non-lipid normalized, wet-weight (ww) values, whereas a "B" suffix indicates values were OCP lipid-normalized. In addition, the remaining OCPs (not lipid-normalized, ww) quantified in crayfish and mosquitofish are summarized in Tables 6-1 through 6-4 for cyclodienes; and Tables 6-5 and 6-6 for hexachlorocyclohexanes (BHCs).

Degree of bioaccumulation differed across chemicals. For both species, highest values were reported for 4,4'-DDE, 4,4'-DDT, and toxaphene. Lowest values were observed for chlordanes, dieldrin, and endosulfan II (Figure 6-18).

Values in relationship to MDLs

MDLs were calculated as an overall average of all the MDLs reported by the laboratory for that specific chemical, by biota. As expected, for both crayfish and mosquitofish, tissue concentrations were below MDL values for treatments with little to no OCPs in soil, i.e. H/L, H/NO, L/L, and L/NO. An exception to this pattern was

observed with treatment M/NO in which non-detect values were observed mostly in crayfish, but not in mosquitofish. Several treatments (M/H, M/M, M/L, L/M, and L/H) induced low levels of accumulation for alpha and gamma-chlordanes. All chemical values were above detection limit in treatments with high soil OCP (i.e. Hot Spot, H/H, and H/M).

Effect of species on bioaccumulation

Overall, mosquitofish tended to bioaccumulate higher concentrations of OCPs compared to crayfish (Figure 6-18). This is not surprising, since mosquitofish contained almost twice as much fat (ww) than crayfish (see above). However, when chemical data was normalized by lipid contents, these differences either disappeared (as was the case for alpha-chlordane and endosulfan II) or were lessened (remaining of chemicals).

Effect of treatment on bioaccumulation

For all the chemicals analyzed, non-lipid normalization (i.e. ww concetration) resulted in complex biota and treatments interactions. However, when values were lipid-normalized, clearer differences emerged. For instance, in both species, treatments Hot Spot, H/M, and L/H induced the highest magnitude of bioaccumulation for both chlordanes (Figures 6-101B and 6-11B), endosulfan II (Figure 6-16B), and toxaphene (Figure 6-17B). On the other hand, dieldrin, 4,4'-DDD, 4,4'-DDE, and 4,4'-DDT followed a more complex pattern. Interactions persisted following lipid-normalization, and were similar among species. Again, treatments with high soil OCP induced higher rates of bioaccumulation (Hot Spot, H/H, H/M, M/H, M/M, and L/H).

Regression analyses between biota OCP lipid-normalized concentrations and soil OCP-TOC normalized concentrations

The relationship between biota OCP lipid-normalized concentrations and soil OCP-TOC normalized concentrations are presented in Tables 6-8 and 6-9 for crayfish and mosquitofish, respectively. For these analyses, datasets were not log-transformed, and only OCP values attained between weeks 8 and 16 were included. There was a significant positive relationship between the TOC-normalized soil OCP concentrations and the OCP lipid-normalized concentrations in crayfish and mosquitofish for most of the chemicals studied. The exceptions were aldrin, endosulfan sulfate, and endrin ketone for both biota species, and heptachlor epoxide and methoxychlor for mosquitofish (Tables 6-8 and 6-9).

Temporal changes in bioaccumulation

Temporal changes in bioaccumulation of OCPs in crayfish and mosquitofish are summarized in Figures 6-19 through 6-26. An “A” suffix after each Figure number represents non-lipid normalized values (ww), whereas a “B” suffix indicates values were lipid-normalized. In general, however, lipid normalization of chemical data resulted in no changes on temporal patterns for the chemicals examined.

Effect of species and treatment on temporal changes

Regardless of species and treatment, chemical concentrations in tissues increased significantly during the first two weeks of experiment. Indeed, for all the chemicals examined, the slope from week 0 to week 2 was the steepest observed. From there on, chemical concentrations remained more or less the same until the end of the study (week 16), although with some variation due to outliers coming from treatments with low or no soil OCPs (i.e. H/NO, M/NO, L/NO, and H/L). An exception to

this pattern was alpha-chlordane, for which an unexplained drop in concentrations was detected on week 12 (Figures 6-19A and B). Concentrations at week 16, however, were comparable to those observed during week 8.

For BSAF calculations, it was assumed that animals attained “steady state” regardless of treatment after 4 weeks of exposure. Thus, only chemical values reported from weeks 8, 12, and 16 were utilized for these calculations (see Chapter 7).

Comparison of OCP Values in Crayfish and Mosquitofish with toxicity values reported in the literature

Organochlorine pesticide toxicity data in mosquitofish is limited to a couple of studies. Mosquitofish (1.1 g body weight/individual) were exposed to toxaphene through water (2 µg/mL) for 9.6 hr and in that short period of time accumulated 0.7 µg/g ww or 700 µg/Kg ww of toxaphene (whole body concentrations) (Schaper and Crowder, 1976). Decreased survival was reported. In another study, mosquitofish (age/size not reported) were exposed to DDT through water (4 µg/L) for 16 days and survival was reduced 50% (Pillai, et al., 1977). Total body burdens of DDT in the latter study were 27,000 µg/kg ww. Although we do not have OCP values for mosquitofish from the Hot Spot tanks, overall ranges from the other treatments in this study (0.3 – 1,500, 0.8 – 4,900, and 0.6 – 7,300 µg/kg for 4,4’-DDD, 4,4’-DDE, and 4,4’-DDT, respectively and 26 – 30,000 µg/kg for toxaphene) fell well within those reported in the previous two mosquitofish studies. Even when the maximum levels of the DDT metabolites in the microcosm fish are totaled (13.7 ppm), the sum does not fall within the Pillai et al, study value of 27 ppm. Only the tissue levels of toxaphene were at or exceeded the literature values presented.

Toxicity data on other freshwater fish species (Table 6-8) would suggest that total body burdens of dieldrin in the range of 500 – 2,100 µg/kg would not be associated with adverse effects, with a reduction in survival at values around 6,000 µg/kg. Data on endosulfan show effects on survival at concentrations between 70 and 1,500 µg/kg (Table 6-8). Mosquitofish in the present study accumulated dieldrin at ranges between 0.3 – 410, below levels associated in the literature with adverse effects. Levels of endosulfans (I, II, and endosulfan sulfate combined) in mosquitofish from this study ranged from 0.1 – 510 µg/kg ww. Whole body residues of 70 µg/kg have been associated with reduced survival in freshwater fish in aerially sprayed African river systems (Table 6.8). Again, these OCP values fall within ranges associated with adverse health effects in other freshwater fish species. This is a significant finding because it would suggest that mosquitofish are less likely to die if exposed to relatively high concentrations of OCPs. Effects on growth and reproduction, however, generally occur at lower doses compared to mortality, and thus should be kept in mind and potentially considered in future studies.

The lack of a similar mortality in crayfish from the Hot Spot tanks would suggest that these invertebrates are less sensitive to the effects of OCPs and/or that their rate of pesticide accumulation was lower than in mosquitofish and that OCPs did not reach lethal levels. There is limited information on threshold toxicity values for these crustaceans. In saltwater shrimp, whole body concentrations of 500 – 3,300 and 80 – 5,000 µg/kg of toxaphene and endosulfan, respectively are associated with decreased survival (Table 6-8). In the present study, concentrations of toxaphene and endosulfans ranged between 12 – 7,600 and 0.1 – 150 µg/kg, again well within threshold toxicity

values previously reported in closely related species. As already mentioned, although exposure of crayfish to OCPs in this study did not appear to affect survival, it could have led to decreased growth and altered nutritional status due to declines in food consumption.

Examination of bioaccumulation of remaining chemicals

As already mentioned, the remaining chemicals quantified in crayfish and mosquitofish are summarized in Tables 6-1 through 6-6. The average concentrations of aldrin and endrins are summarized by week of collection and treatment in Tables 6-1 and 6-2 for crayfish and mosquitofish, respectively. As can be seen from these tables, most values fell below detection limit. An exception was endrin aldehyde. This cyclodiene pesticide was detected at significant concentrations mostly in treatments containing high soil OCPs (Hot Spot, H/H, H/M, M/H, and L/H). Interestingly, most of the high and outlier values were reported from the L/H treatment. It is also worth mentioning the extremely high values reported from mosquitofish collected from tanks containing “NO” OCP soils (H/NO and M/NO, Table 6-2).

Another set of seven cyclodiene OCPs are summarized in Tables 6-3 and 6-4 for crayfish and mosquitofish, respectively. Of the seven cyclodienes, only four (endosulfan I, endosulfan sulfate, heptachlor, and heptachlor epoxide) were analyzed consistently throughout the study. The remaining three (cis-nonachlor, trans-nonachlor, and oxychlordane) were only included in the last two sampling dates (weeks 12 and 16). In addition, methoxychlor was also present consistently in biota samples throughout this study. Again, for both species, most values fell below detection limits. Treatments Hot Spot, H/H, H/M, M/H, M/M, and L/H contained most of the values above detection limit. In the case of mosquitofish, the latter treatment also contained most of the outlier

values, particularly in week 16 (Table 6-6). In general, trans-nonachlor in both species and methoxychlor in mosquitofish, stood out as the chemicals with higher values, including many outliers.

Hexachlorocyclohexanes or BHCs, are summarized in Tables 6-5 and 6-6 for crayfish and mosquitofish, respectively. For both species, few values were above their respective MDLs.

Table 6-1. Additional cyclodiene OCPs (aldrin and endrins) measured in crayfish, by week and treatment. Bold indicates above MDL; red are outlier values (> 10x MDLs). Given are overall means.

Week	Analyte (µg/kg, ww)	Hot Spot	H/H	H/M	H/L	H/NO	M/H	M/M	M/L	M/NO	L/H	L/M	L/L	L/NO	Blank	Grand Average
0	**Aldrin**														0.36	0.36
	Endrin														0.72	0.72
	Endrin aldehyde														0.85	0.85
	Endrin ketone														0.77	0.77
2	**Aldrin**	**1.26**	0.28	0.22	0.22	0.29	0.25	0.21	0.23	0.30	**0.44**	0.27	0.30	0.25		0.35
	Endrin	**4.03**	0.55	0.43	0.44	0.55	0.49	0.40	0.45	0.57	0.88	0.52	0.59	0.49		0.80
	Endrin aldehyde	0.65	0.65	0.53	0.53	0.67	0.58	0.48	0.54	0.68	**1.05**	0.62	0.70	0.58		0.64
	Endrin ketone	0.60	0.60	0.46	0.48	0.60	0.54	0.43	0.50	0.63	0.94	0.57	0.63	0.53		0.58
4	**Aldrin**	**1.89**	0.29	0.29	0.28	0.25	0.26	0.25	0.35	0.28	0.29	0.24	0.26	0.34		0.40
	Endrin	0.50	0.57	0.59	0.53	0.48	0.51	0.48	0.70	0.53	0.57	0.47	0.51	0.67		0.55
	Endrin aldehyde	73	0.67	**5.80**	**1.63**	0.58	**3.37**	**8.77**	**2.87**	0.65	47	3.05	**1.38**	**1.60**		12
	Endrin ketone	**6.13**	0.60	0.63	0.57	0.52	0.56	0.52	0.73	0.58	**2.35**	0.51	0.55	0.72		1.15
8	**Aldrin**	0.34	**0.64**	0.31	0.23	0.26	0.33	0.39	0.29	0.33	0.35	0.36	0.37	**0.47**		0.36
	Endrin	0.65	**1.25**	0.61	0.45	0.51	0.65	0.76	0.59	0.67	**1.55**	0.70	0.72	0.90		0.77
	Endrin aldehyde	0.80	**1.50**	**3.28**	**1.02**	0.61	0.77	**1.95**	0.97	0.79	11	0.83	0.87	**1.08**		1.93
	Endrin ketone	0.70	**1.34**	0.66	0.50	0.55	0.70	0.83	0.63	0.73	0.73	0.75	0.77	0.97		0.76
12	**Aldrin**	0.34	-[a]	0.18	0.18	0.17	0.17	0.18	0.19	0.22	0.16	0.29	0.18	0.17		0.20
	Endrin	0.65	-	0.36	0.35	0.33	0.33	0.35	0.38	0.43	0.32	0.56	0.35	0.34		0.39
	Endrin aldehyde	40	-	**2.36**	0.43	0.40	**8.73**	**3.48**	**1.64**	0.51	**6.25**	0.68	0.42	0.41		5.44
	Endrin ketone	0.70		0.39	0.37	0.36	1.31	0.74	0.41	0.45	0.91	0.61	0.38	0.37		0.58
16	**Aldrin**	-	-	0.11	0.11	0.11	0.18	0.17	0.11	0.11	**0.47**	0.22	0.11	0.11		0.16
	Endrin	-	-	0.21	0.21	0.21	0.34	0.34	0.21	0.21	3.74	0.44	0.21	0.21		0.57
	Endrin aldehyde	-	-	**2.03**	0.60	0.44	0.87	0.40	0.25	0.25	**2.88**	0.53	0.40	0.46		0.83
	Endrin ketone	-	-	0.52	0.23	0.23	0.37	0.57	0.23	0.23	0.94	**1.98**	0.23	0.23		0.52

[a] No data reported by EN CHEM.

Table 6-2. Additional cyclodiene OCPs (aldrin and endrins) measured in mosquitofish, by week and treatment. Bold indicates above MDL; red are outlier values (> 10x MDLs). Given are overall means.

Week	Analyte (µg/kg, ww)	H/H	H/M	H/L	H/NO	M/H	M/M	M/L	M/NO	L/H	L/M	L/L	L/NO	Blank	Grand Average
0	**Aldrin**													0.41	0.41
	Endrin													0.81	0.81
	Endrin aldehyde													0.95	0.95
	Endrin ketone													0.85	0.85
2	**Aldrin**	0.61	0.83	1.15	0.78	**1.87**	0.82	0.82	0.97	0.97	1.03	0.78	0.88		0.96
	Endrin	1.22	1.63	2.27	1.55	**3.57**	1.60	1.63	1.88	1.88	**2.03**	1.55	1.72		1.88
	Endrin aldehyde	1.47	**24**	**8.42**	**13**	**27**	**15**	**4.23**	2.25	**91**	2.43	**7.47**	2.07		**17**
	Endrin ketone	1.33	1.68	2.47	1.67	**3.98**	1.70	1.75	2.02	**6.53**	2.18	1.68	2.57		2.46
4	**Aldrin**	1.22	0.85	1.02	1.12	0.82	0.80	0.92	0.80	**3.10**	1.05	0.88	0.83		1.12
	Endrin	**2.38**	1.67	1.98	**2.22**	1.65	1.60	1.80	1.55	**2.87**	**2.03**	1.77	1.63		1.93
	Endrin aldehyde	2.85	**9.12**	2.37	2.67	**20**	**21**	2.17	**13**	3.42	2.45	2.13	1.93		**6.94**
	Endrin ketone	**8.98**	1.80	2.13	2.40	2.78	**3.83**	1.95	2.58	**24**	2.20	1.92	1.75		**4.72**
8	**Aldrin**	1.20	0.93	0.87	1.02	0.88	0.95	0.80	1.28	-[a]	0.75	0.80	1.10		0.96
	Endrin	**4.43**	1.82	1.70	1.98	1.75	1.85	1.58	**2.52**	-	1.48	1.58	**2.18**		**2.08**
	Endrin aldehyde	2.80	2.18	3.78	3.07	**5.00**	2.22	1.88	3.00	-	**5.08**	4.38	2.60		3.27
	Endrin ketone	2.53	1.97	1.85	2.13	1.88	2.00	1.70	2.70	-	1.60	1.72	2.33		2.04
12	**Aldrin**	0.53	0.53	0.57	0.55	0.53	0.47	0.63	0.52	0.60	0.55	0.80	0.57		0.57
	Endrin	1.05	1.08	1.10	1.08	1.03	0.95	1.23	1.03	1.15	1.08	1.55	1.10		1.12
	Endrin aldehyde	1.28	1.28	1.32	1.28	1.23	1.13	1.47	1.23	1.35	1.30	1.83	1.33		1.34
	Endrin ketone	**6.50**	1.15	1.20	1.17	1.12	**3.70**	1.32	1.10	1.25	**4.57**	1.67	1.20		2.16
16	**Aldrin**	1.20	0.73	0.44	0.75	0.32	0.70	0.41	0.68	2.77	1.07	0.27	0.57		0.82
	Endrin	**2.30**	1.40	0.88	1.47	0.63	1.35	0.79	1.35	**3.88**	**2.12**	0.53	1.13		1.49
	Endrin aldehyde	2.80	**25**	1.05	1.75	0.75	1.62	0.95	1.62	**4.77**	2.55	1.52	1.33		3.78
	Endrin ketone	2.50	**4.80**	0.93	1.58	1.59	1.47	0.87	1.46	**18**	2.48	0.57	1.22		3.11

[a] No data reported by EN CHEM.

Table 6-3. Remaining OCPs measured in crayfish, by week and treatment. Bold indicates above MDL. Given are overall means.

Week	Analyte (µg/kg, ww)	Hot Spot	H/H	H/M	H/L	H/NO	M/H	M/M	M/L	M/NO	L/H	L/M	L/L	L/NO	Blank	Grand Average
0	**Endosulfan I**														0.37	0.37
	Endosulfan sulfate														1.73	1.73
	Heptachlor														0.63	0.63
	Heptachlor epoxide														0.56	0.56
	Methoxychlor														2.40	2.40
2	**Endosulfan I**	0.28	0.29	0.23	0.23	0.29	0.25	0.21	0.24	0.30	**9.81**	0.27	0.31	0.25		**1.00**
	Endosulfan sulfate	**13**	1.33	1.07	1.07	1.35	1.18	0.98	1.12	1.40	**13**	1.28	1.42	1.18		**3.09**
	Heptachlor	0.47	0.49	0.38	0.38	0.49	0.42	0.35	0.40	0.51	0.76	0.46	0.51	0.42		0.46
	Heptachlor epoxide	**2.72**	0.44	**1.29**	0.35	0.44	0.52	0.41	0.37	0.46	**2.46**	0.42	0.46	0.39		0.83
	Methoxychlor	1.82	1.88	1.47	1.48	1.88	1.63	1.37	1.52	1.95	**2.93**	1.78	1.98	1.63		1.79
4	**Endosulfan I**	0.27	0.29	0.30	0.28	0.25	0.27	0.25	0.36	0.28	0.30	0.25	0.27	0.35		0.29
	Endosulfan sulfate	**16**	1.83	1.40	1.30	1.17	1.25	1.17	1.68	1.32	**13**	2.12	1.25	1.62		**3.46**
	Heptachlor	0.44	0.49	0.50	0.46	0.43	0.46	0.42	0.60	0.47	0.50	0.41	0.45	0.58		0.48
	Heptachlor epoxide	0.40	0.44	0.46	0.42	0.39	0.41	0.38	0.55	0.43	0.45	0.37	0.41	0.53		0.43
8	**Endosulfan I**	0.35	0.64	0.32	0.24	0.27	**1.49**	0.40	0.51	0.35	0.36	0.36	0.38	0.47		0.47
	Endosulfan sulfate	1.65	**5.40**	1.45	1.12	1.25	1.55	1.85	1.42	1.60	1.65	1.68	1.75	2.22		1.89
	Heptachlor	0.60	**1.07**	0.53	0.40	0.44	0.55	0.65	0.50	0.58	0.60	0.60	0.63	0.78		0.61
	Heptachlor epoxide	0.55	0.97	**1.30**	0.36	0.61	0.86	0.61	0.70	0.53	2.05	0.55	0.57	0.72		0.80
	Methoxychlor	2.25	**4.25**	2.03	1.55	1.73	2.17	**2.57**	1.97	2.23	2.30	2.37	2.42	3.07		2.38
12	**cis-nonachlor**	14.00	-[a]	7.10	0.43	0.40	4.64	2.40	0.45	0.51	6.89	0.68	0.42	0.42		3.19
	Endosulfan I	0.34	-	0.19	0.18	0.17	1.00	0.18	0.20	0.22	0.17	0.29	0.18	0.18		0.28
	Endosulfan sulfate	1.60	-	0.87	0.85	0.80	0.80	0.85	0.92	1.03	2.13	1.37	0.85	0.83		1.08
	Heptachlor	0.55	-	0.31	0.31	0.29	0.28	0.31	0.33	0.37	0.28	0.49	0.30	0.30		0.34
	Heptachlor epoxide	**4.60**	-	**1.30**	0.28	0.26	**1.01**	0.47	0.68	0.34	**3.30**	**2.92**	0.27	0.27		**1.31**
	Methoxychlor	**20**	-	2.12	1.18	1.12	**5.05**	2.10	1.27	1.43	**8.92**	1.90	1.17	1.15		**3.95**

Table 6-3. Continued.

Week	Analyte (µg/kg, ww)	Hot Spot	H/H	H/M	H/L	H/NO	M/H	M/M	M/L	M/NO	L/H	L/M	L/L	L/NO	Blank	Grand Average
16	**Endosulfan I**	-	-	0.77	0.11	0.11	0.18	0.18	0.11	0.11	0.26	0.23	0.11	0.11		0.21
	Endosulfan sulfate	-	-	0.50	0.50	0.50	0.83	0.82	0.50	0.50	1.42	1.07	0.50	0.50		0.69
	Heptachlor	-	-	0.18	0.18	0.18	0.30	0.29	0.18	0.18	0.58	0.38	0.18	0.18		0.26
	Heptachlor epoxide	-	-	0.17	0.17	0.17	0.28	**1.42**	0.17	0.17	0.38	0.35	0.17	0.17		0.32
	Methoxychlor	-	-	1.70	0.70	0.70	1.17	1.47	0.70	0.70	**4.38**	**4.08**	0.70	0.70		1.55
	Oxychlordane	-	-	**3.20**	0.18	0.18	1.08	0.96	0.42	0.18	**2.25**	0.68	0.28	0.18		0.87
	trans-nonachlor	-	-	**15**	0.44	0.20	**4.83**	**5.50**	**1.73**	0.20	**7.97**	0.82	1.19	0.20		**3.49**

[a] No data reported by EN CHEM.

Table 6-4. Remaining OCPs measured in mosquitofish, by week and treatment. Bold indicates above MDL; red are outlier values (> 10x MDLs). Given are overall means.

Week	Analyte (µg/kg, ww)	H/H	H/M	H/L	H/NO	M/H	M/M	M/L	M/NO	L/H	L/M	L/L	L/NO	Blank	Grand Average
0	**Endosulfan I**													0.41	0.41
	Endosulfan sulfate													1.94	1.94
	Heptachlor													0.69	0.69
	Heptachlor epoxide													0.63	0.63
	Methoxychlor													2.70	2.70
2	**Endosulfan I**	0.63	**14**	1.18	0.82	1.90	0.83	0.83	0.97	**7.32**	1.07	0.82	0.90		**2.63**
	Endosulfan sulfate	**32**	4.02	5.48	3.77	8.90	3.78	3.83	4.50	**13**	5.97	3.77	4.12		7.76
	Heptachlor	1.07	1.43	1.97	1.35	3.10	1.38	1.40	1.65	1.63	1.77	1.35	1.48		1.63
	Heptachlor epoxide	0.97	**2.93**	1.82	1.22	**2.83**	1.25	1.28	1.50	1.48	1.60	1.22	2.30		1.70
	Methoxychlor	4.12	5.33	7.67	5.20	**12**	5.33	5.38	6.40	6.45	6.93	5.12	5.85		6.33
4	**Endosulfan I**	1.23	**2.47**	1.02	**2.02**	0.85	0.85	0.93	0.80	**18**	1.07	0.93	0.85		**2.46**
	Endosulfan sulfate	**60**	4.02	4.83	5.37	5.93	3.90	4.38	3.77	**72**	7.67	4.28	3.95		**15**
	Heptachlor	2.08	1.45	1.72	1.93	1.42	1.38	1.57	1.35	2.48	1.77	1.55	1.40		1.68
	Heptachlor epoxide	**3.68**	1.32	1.55	1.75	1.30	**3.83**	1.43	1.23	**8.40**	2.13	1.38	1.30		2.44
	Methoxychlor	**157**	**13**	6.50	7.43	**23**	**24**	6.17	5.27	**180**	**36**	6.00	5.33		**39**
8	**Endosulfan I**	1.23	0.95	0.90	1.02	0.92	0.97	0.82	1.30	-[a]	0.78	0.83	1.12		0.98
	Endosulfan sulfate	**59**	4.30	4.15	4.78	5.92	4.50	3.83	5.97	-	**10**	3.83	5.20		**10**
	Heptachlor	2.05	1.58	1.48	1.73	1.52	1.62	1.37	2.17	-	1.28	1.38	1.88		1.64
	Heptachlor epoxide	**3.03**	**5.97**	1.37	1.58	**2.87**	1.47	1.25	1.98	-	1.18	1.25	1.72		2.15
	Methoxychlor	**115**	6.17	5.77	6.67	5.83	6.17	5.33	8.50	-	**13**	5.32	7.33		**17**
12	**cis-nonachlor**	1.28	**57**	1.32	1.28	2.82	**9.07**	1.47	1.23	1.40	1.32	1.87	1.35		**6.78**
	Endosulfan I	0.56	0.57	0.58	0.55	0.55	0.49	0.63	0.53	0.60	0.57	0.80	0.58		0.58
	Endosulfan sulfate	**38**	2.62	2.68	2.60	2.53	2.30	2.98	2.48	2.80	**16**	3.73	2.72		6.77
	Heptachlor	0.93	0.93	0.97	0.93	0.90	0.82	1.08	0.90	1.00	0.93	1.33	0.97		0.97
	Heptachlor epoxide	**6.98**	**10**	0.88	0.85	0.82	0.75	0.98	0.80	0.90	0.85	1.22	0.87		2.17

[a] No data reported by EN CHEM.

Table 6-4. Continued.

Week	Analyte (µg/kg, ww)	H/H	H/M	H/L	H/NO	M/H	M/M	M/L	M/NO	L/H	L/M	L/L	L/NO	Blank	Grand Average
12	**Methoxychlor**	**38**	**27**	3.73	3.62	8.23	**18**	4.13	3.45	3.85	**12**	5.17	3.77		11
	Oxychlordane	**6.45**	**39**	1.85	0.92	1.52	3.15	1.03	0.90	1.00	0.93	1.32	0.95		**4.89**
	trans-nonachlor	**28**	**217**	**6.90**	1.02	**18**	**34**	**5.03**	0.98	1.10	**9.07**	**6.77**	1.05		**27**
16	**cis-nonachlor**	2.80	**62**	3.35	1.77	**11**	**18**	0.95	1.63	**103**	3.30	1.47	1.37		**17**
	Endosulfan I	1.20	0.73	0.46	0.77	0.33	**2.83**	0.42	0.71	**22**	1.10	0.28	0.60		**2.60**
	Endosulfan sulfate	5.50	5.00	2.13	3.55	1.53	4.67	1.95	3.20	**50**	6.03	1.30	2.73		**7.26**
	Heptachlor	2.00	1.23	0.76	1.27	0.55	1.17	0.70	1.18	**3.47**	1.84	0.46	0.98		1.30
	Heptachlor epoxide	**22**	1.13	0.88	1.17	**3.76**	2.05	0.63	1.07	**32**	1.67	0.42	0.90		**5.60**
	Methoxychlor	8.00	4.80	2.95	5.02	10.03	4.63	2.70	4.55	**174**	**20**	1.80	3.82		**20**
	Oxychlordane	**12**	**37**	2.80	1.25	**6.87**	**6.97**	1.92	1.16	**31**	2.58	2.43	0.97		**8.88**
	trans-nonachlor	**83**	**104**	**8.60**	1.38	**20**	**30**	3.57	1.30	**149**	**10**	**6.97**	1.07		**35**

Table 6-5. Summary of hexachlorocyclohexane OCPs measured in crayfish, by week and treatment. Bold indicates above MDL. Given are overall means.

Week	Analyte (µg/kg, ww)	Hot Spot	H/H	H/M	H/L	H/NO	M/H	M/M	M/L	M/NO	L/H	L/M	L/L	L/NO	Blank	Grand Average
0	**alpha-BHC**														0.73	0.73
	beta-BHC														0.92	0.92
	delta-BHC														0.58	0.58
	gamma-BHC														0.42	0.42
2	**alpha-BHC**	0.55	0.55	0.45	0.45	0.57	0.50	0.42	0.47	0.60	0.90	0.53	0.61	0.50		0.54
	beta-BHC	0.70	0.72	0.56	0.57	0.72	0.63	0.51	0.58	0.73	**1.13**	0.67	0.77	0.63		0.69
	delta-BHC	0.44	0.45	0.35	0.36	0.45	0.39	0.33	0.37	0.47	0.71	0.43	0.49	0.39		0.43
	gamma-BHC	0.32	0.32	0.25	0.26	0.33	0.28	0.24	0.26	0.34	0.51	0.31	0.34	0.28		0.31
4	**alpha-BHC**	0.52	0.58	0.59	0.56	0.50	0.53	0.49	0.70	0.55	0.58	0.49	0.53	0.68		0.56
	beta-BHC	0.65	0.72	0.75	0.70	0.63	0.68	0.62	0.90	0.70	0.75	0.62	0.67	0.87		0.71
	delta-BHC	0.41	0.45	0.46	0.43	0.40	0.42	0.39	0.57	0.44	0.46	0.38	0.42	0.53		0.44
	gamma-BHC	0.30	0.32	0.34	0.31	0.28	0.30	0.28	0.41	0.32	0.33	0.28	0.30	0.39		0.32
8	**alpha-BHC**	0.70	**1.28**	0.62	0.47	0.53	0.65	0.79	0.59	0.69	0.70	0.70	0.73	0.93		0.72
	beta-BHC	0.85	**1.58**	0.77	0.59	0.66	**1.28**	0.98	0.73	0.85	0.88	**2.73**	0.93	**1.18**		1.08
	delta-BHC	0.55	**1.00**	0.49	0.37	0.41	**2.48**	0.61	0.46	0.53	0.55	0.55	0.59	0.73		0.72
	gamma-BHC	0.39	0.73	0.35	0.27	0.30	0.38	0.44	0.34	0.39	0.40	0.66	0.42	0.54		0.43
12	**alpha-BHC**	0.65	-[a]	0.36	0.36	0.34	0.33	0.36	0.39	0.44	0.33	0.58	0.36	0.35		0.40
	beta-BHC	0.85	-	0.47	0.46	0.43	0.42	0.46	0.49	0.54	0.41	0.72	0.45	0.43		0.51
	delta-BHC	0.55	-	0.29	0.28	0.27	0.26	0.29	0.31	0.35	0.26	0.46	0.28	0.28		0.32
	gamma-BHC	0.38	-	0.21	0.20	0.19	0.19	0.21	0.22	0.25	0.19	0.33	0.20	0.20		0.23
16	**alpha-BHC**	-	-	0.22	0.22	0.22	0.36	0.34	0.22	0.22	0.49	0.46	0.22	0.22		0.29
	beta-BHC	-	-	0.27	0.27	0.27	0.45	0.43	0.27	0.27	0.61	0.57	0.27	0.27		0.36
	delta-BHC	-	-	0.17	0.17	0.17	0.40	0.37	0.17	0.32	0.50	0.35	0.17	0.27		0.28
	gamma-BHC	-	-	0.12	0.12	0.12	0.20	0.20	0.12	0.20	0.37	0.25	0.12	0.12		0.18

[a] No data reported by EN CHEM.

Table 6-6. Summary of hexachlorocyclohexane OCPs measured in mosquitofish, by week and treatment. Bold indicates above MDL. Given are overall means.

Week	Analyte (µg/kg, ww)	H/H	H/M	H/L	H/NO	M/H	M/M	M/L	M/NO	L/H	L/M	L/L	L/NO	Blank	Grand Average
0	**alpha-BHC**													0.81	0.81
	beta-BHC													1.04	01.04
	delta-BHC													0.65	0.65
	gamma-BHC													0.47	0.47
2	**alpha-BHC**	1.25	1.70	2.32	1.60	**3.75**	1.63	1.65	1.92	1.93	2.08	1.58	1.77		1.93
	beta-BHC	1.58	2.13	2.98	2.00	**4.72**	2.05	2.08	2.42	2.43	2.63	2.00	2.23		2.44
	delta-BHC	0.98	1.35	1.85	1.27	**2.87**	1.30	1.32	1.52	1.53	1.65	1.27	1.39		1.52
	gamma-BHC	0.70	0.97	1.32	0.90	**2.12**	0.93	0.95	1.08	1.10	1.18	0.90	1.00		1.10
4	**alpha-BHC**	2.43	1.70	2.03	2.28	1.67	1.65	1.85	1.60	2.92	2.08	1.82	1.67		1.98
	beta-BHC	3.08	2.15	2.57	2.88	2.12	2.08	2.33	2.02	3.70	2.63	2.28	2.10		2.50
	delta-BHC	1.93	1.33	1.60	1.80	1.32	1.30	1.47	1.25	**2.32**	1.65	1.43	1.32		1.56
	gamma-BHC	1.37	0.97	1.13	1.28	0.95	0.93	1.05	0.90	1.65	1.20	1.03	0.95		1.12
8	**alpha-BHC**	2.40	1.85	1.75	2.03	1.78	1.90	1.60	2.58	-[a]	1.53	1.63	2.22		1.93
	beta-BHC	3.03	2.35	2.22	2.57	2.25	2.40	2.05	3.23	-	1.92	2.07	2.80		2.44
	delta-BHC	1.88	1.47	1.38	1.62	1.42	1.50	1.27	2.03	-	1.20	1.28	1.77		1.53
	gamma-BHC	1.35	1.05	1.00	1.15	1.02	1.07	0.92	1.45	-	0.85	0.92	1.27		1.09
12	**alpha-BHC**	1.10	1.10	1.15	1.10	1.07	0.98	1.27	1.05	1.15	1.12	1.58	1.15		1.15
	beta-BHC	1.38	1.38	1.42	1.38	1.33	1.22	1.58	1.33	1.50	1.40	1.98	1.43		1.44
	delta-BHC	0.88	0.87	0.88	0.87	0.85	0.75	0.98	0.83	0.95	0.88	1.23	0.92		0.91
	gamma-BHC	0.63	0.62	0.65	0.63	0.60	0.56	0.73	0.60	0.65	0.63	0.88	0.65		0.65
16	**alpha-BHC**	2.40	1.45	0.88	1.52	0.65	1.38	0.83	1.39	4.10	2.17	0.55	1.15		1.54
	beta-BHC	3.00	1.83	1.13	1.90	0.82	1.75	1.03	2.31	**5.15**	2.77	0.69	1.45		1.99
	delta-BHC	1.90	1.13	0.70	1.18	0.50	1.08	0.66	1.09	**3.18**	1.72	0.43	0.90		1.21
	gamma-BHC	1.35	0.80	0.66	1.28	0.37	0.78	0.46	1.16	**2.27**	1.22	0.31	0.67		0.94

[a] No data reported by EN CHEM.

Table 6-7. Summary of whole body residues (µg/g, wet weight) and effects in several species of shrimp and fish. Data is presented for the most critical OCPs discussed in this report. For detailed information on each reference, see Jarvinen and Ankley (1999).

Analyte	Whole Body Residue (µg/g, ww)	Life Stage	Test Species Common Name	Test Species Scientific Name	Effect	Reference	Habitat
DDT	4.67	Juvenile	Rainbow Trout	*Onconrhynchus mykiss*	survival, growth - no effect	269	freshwater
DDT	1.92	Juvenile	Brook Trout	*Salvelinus fontinalis*	survival - no effect	267	freshwater
DDT	25.6	Juvenile	Brook Trout	*Salvelinus fontinalis*	survival - no effect	267	freshwater
DDT	400	Adult	Goldfish	*Carassius auratus*	survival reduced > 80%	370	freshwater
DDT	200	Adult	Goldfish	*Carassius auratus*	survival reduced >20%	370	freshwater
DDT	130	Adult	Goldfish	*Carassius auratus*	survival no effect	370	freshwater
DDT	3.6	1.9 g	Golden Shiner	*Notemigonus crysoleucas*	survival no effect	94	freshwater
DDT	209	Juvenile - Adult	Fathead Minnow	*Pimephales promelas*	survival reduced 79%	218 - 219	freshwater
DDT	160	Juvenile - Adult	Fathead Minnow	*Pimephales promelas*	survival reduced 50%	218 - 219	freshwater
DDT	86	Juvenile - Adult	Fathead Minnow	*Pimephales promelas*	survival reduced 26%	218 - 219	freshwater
DDT	57	Juvenile - Adult	Fathead Minnow	*Pimephales promelas*	survival reduced 25%	218 - 219	freshwater
DDT	40	Juvenile - Adult	Fathead Minnow	*Pimephales promelas*	survival no effect	218 - 219	freshwater
DDT	26.5	NA[a]	Mosquitofish	*Gambusia affinis*	survival reduced 50%	363	freshwater
DDT	24	Juvenile	Green Sunfish and Pumpkinseed	*Lepomis cyanellus and L. gibossus*	survival reduced	161	freshwater
DDT	1.73	Adult	Mummichog	*Fundulus heteroclitus*	survival reduced 94%	96	saltwater
4,4'-DDT	0.15 - 0.21	Adult	Pink shrimp	*Penaeus duorarum*	survival reduced	330	saltwater
4,4'-DDT	0.06	Adult	Pink shrimp	*Penaeus duorarum*	survival no effect	331	saltwater
4,4'-DDT	1 - 5	Juvenile	Brook Trout	*Salvelinus fontinalis*	survival, growth - no effect	5	freshwater
4,4'-DDT	2.8 - 7.6	Yearling - Adult	Brook Trout	*Salvelinus fontinalis*	survival, growth - no effect	265	freshwater
4,4'-DDD	1 - 5	Juvenile	Brook Trout	*Salvelinus fontinalis*	survival, growth - no effect	5	freshwater
4,4'-DDE	1 - 5	Juvenile	Brook Trout	*Salvelinus fontinalis*	survival, growth - no effect	5	freshwater
Dieldrin	5.65	Juvenile	Rainbow Trout	*Onconrhynchus mykiss*	survival reduced >50%	406	freshwater
Dieldrin	0.548	Juvenile	Rainbow Trout	*Onconrhynchus mykiss*	survival no effect	406	freshwater
Dieldrin	2.13	Juvenile	Rainbow Trout	*Onconrhynchus mykiss*	survival, growth - no effect	269	freshwater
Dieldrin	0.36 - 1.4	Juvenile	Rainbow Trout	*Onconrhynchus mykiss*	growth - no effect	406	freshwater

[a] No data available.

Table 6-7. Continued.

Analyte	Whole Body Residue (µg/g, ww)	Life Stage	Test Species Common Name	Test Species Scientific Name	Effect	Reference	Habitat
Endosulfan (Technical grade)	0.48	Juvenile - Adult	Pink shrimp	*Penaeus duorarum*	survival reduced 65%	396	saltwater
Endosulfan (Technical grade)	0.21	Juvenile - Adult	Pink shrimp	*Penaeus duorarum*	survival reduced 35%	396	saltwater
Endosulfan (Technical grade)	0.08	Juvenile - Adult	Pink shrimp	*Penaeus duorarum*	survival reduced 10%	396	saltwater
Endosulfan (Technical grade)	0.07	Juvenile - Adult	Pink shrimp	*Penaeus duorarum*	survival no effect	396	saltwater
Endosulfan (Technical grade)	0.27	Juvenile	Pinfish	*Lagodon rhomboides*	survival reduced 35%	396	saltwater
Endosulfan (Technical grade)	0.2	Juvenile	Pinfish	*Lagodon rhomboides*	survival no effect	396	saltwater
Endosulfan (Technical grade)	0.26	Juvenile	Spot	*Leiostomus xanthurus*	survival reduced 90%	396	saltwater
Endosulfan (Technical grade)	0.07	Juvenile	Spot	*Leiostomus xanthurus*	survival reduced 45%	396	saltwater
Endosulfan (Technical grade)	0.03	Juvenile	Spot	*Leiostomus xanthurus*	survival reduced 35%	396	saltwater
Endosulfan (Technical grade)	0.43 - 0.49	Juvenile	Mullet	*Mugil cephalus*	survival reduced 90%	396	saltwater
Endosulfan (Technical grade)	0.36	Juvenile	Mullet	*Mugil cephalus*	survival reduced 40%		saltwater
Endosulfan (35 % EC)	1.15	Juvenile	Fish	*Serranochromis spp.*	survival reduced	276	freshwater
Endosulfan (35 % EC)	0.07	Juvenile	Fish	*Clarias spp.*	survival reduced	276	freshwater
Endosulfan (35 % EC)	1.08	Juvenile	Fish	*Haplochromis spp.*	survival reduced	276	freshwater
Endosulfan (35 % EC)	1.46	Adult	Fish	*Psuedocrenilabrus philander*	survival reduced	276	freshwater
Endosulfan (35 % EC)	1.1	Juvenile	Tilapia	*Tilapia spp. and Sarotherodon spp.*	survival reduced	276	freshwater

Table 6-7. Continued.

Analyte	Whole Body Residue (µg/g, ww)	Life Stage	Test Species Common Name	Test Species Scientific Name	Effect	Reference	Habitat
Toxaphene	0.83	NA[a]	Pink shrimp	*Penaeus duorarum*	survival reduced > 50%	395	saltwater
Toxaphene	0.54	NA	Pink shrimp	*Penaeus duorarum*	survival reduced 20%	395	saltwater
Toxaphene	3.3	NA	Grass shrimp	*Palaemonetes pugio*	survival reduced > 50%	395	saltwater
Toxaphene	2.7	NA	Grass shrimp	*Palaemonetes pugio*	survival reduced 25%	395	saltwater
Toxaphene	8	Adult	Brook Trout	*Salvelinus fontinalis*	survival reduced	279, 281	freshwater
Toxaphene	2.4	Adult	Brook Trout	*Salvelinus fontinalis*	survival reduced 50%	279, 281	freshwater
Toxaphene	0.4	Adult	Brook Trout	*Salvelinus fontinalis*	survival, growth - no effect	279, 281	freshwater
Toxaphene	0.4	Adult	Brook Trout	*Salvelinus fontinalis*	egg viability reduced	279, 281	freshwater
Toxaphene	0.2	Adult	Brook Trout	*Salvelinus fontinalis*	egg viability - no effect	279, 281	freshwater
Toxaphene	6.0 - 9.0	Adult	Fathead Minnow	*Pimephales promelas*	survival, reproduction - no effect	279, 282	freshwater
Toxaphene	3.3	Adult	Fathead Minnow	*Pimephales promelas*	growth reduced	279, 282	freshwater
Toxaphene	1.0 - 2.7	Adult	Fathead Minnow	*Pimephales promelas*	growth - no effect	279, 282	freshwater
Toxaphene	11	Adult	Channel Catfish	*Ictalurus punctatus*	survival, growth, reproduction - no effect	279, 282	freshwater
Toxaphene	35	NA	Sheepshead Minnow	*Cyprinodon variegates*	survival reduced 85%	395	saltwater
Toxaphene	4.1	NA	Sheepshead Minnow	*Cyprinodon variegates*	survival reduced 25%	395	saltwater
Toxaphene	2.4	NA	Sheepshead Minnow	*Cyprinodon variegates*	survival no effect	395	saltwater
Toxaphene	102	Juvenile	Longnose Killifish	*Fundulus similis*	survival reduced 95%	395	saltwater
Toxaphene	24.7	Juvenile	Longnose Killifish	*Fundulus similis*	survival reduced 35%	395	saltwater
Toxaphene	16	Juvenile	Longnose Killifish	*Fundulus similis*	survival no effect	395	saltwater
Toxaphene	6.1	Adult	Longnose Killifish	*Fundulus similis*	survival reduced 25%	395	saltwater
Toxaphene	2.1	Adult	Longnose Killifish	*Fundulus similis*	survival no effect	395	saltwater
Toxaphene	0.68	1.1 g	Mosquitofish	*Gambusia affinis*	survival reduced -death	390	freshwater
Toxaphene	1.9	NA	Pinfish	*Lagodon rhomboides*	survival reduced 25%	395	saltwater
Toxaphene	1.6	NA	Pinfish	*Lagodon rhomboides*	survival no effect	395	saltwater

[a] No data available.

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Table 6-8. Summary of ANOVAs used to determine the relationship between crayfish OCP lipid-normalized concentrations (dependent variable) and soil total organic carbon (TOC) OCP-normalized concentrations (independent variable). Data sets were not log-transformed for these analyses. Only OCP values attained between weeks 8 and 16 included in these analyses. Regression equation was as follows**: log crayfish OCP lipid-normalized concentration = a (intercept) + X (log soil TOC/OCP-normalized concentration).**

CRAYFISH					
Analyte	**Sum of Squares**	**F**	**P**	**a**	**X**
4,4'-DDD	35.897	55.326	0.0000	1.0968	0.8667
4,4'-DDE	97.828	189.154	0.0000	0.9868	1.0340
4,4'-DDT	154.298	250.658	0.0000	1.1477	0.8873
Aldrin	0.011	0.072	0.8052	4.7902	-0.0752
Dieldrin	47.327	63.048	0.0000	1.5171	0.9425
Endosulfan II	35.565	41.946	0.0000	0.4218	0.9551
Endosulfan sulfate	0.637	2.775	0.1711	4.9044	0.2055
Endrin aldehyde	3.063	17.598	0.0524	1.6884	0.8269
Endrin ketone	0.091	0.511	0.4872	4.6733	0.0973
Heptachlor epoxide	7.185	14.965	0.0006	2.8219	0.5236
Methoxychlor	1.439	6.313	0.0457	-0.6542	1.2395
Toxaphene	37.038	66.404	0.0000	2.9167	0.7158
alpha-Chlordane	14.160	9.978	0.0030	2.4772	0.5065
gamma-Chlordane	5.868	14.082	0.0007	3.6262	0.4123

Table 6-9. Summary of ANOVAs used to determine the relationship between mosquitofish OCP lipid-normalized concentrations (dependent variable) and soil total organic carbon (TOC) OCP-normalized concentrations (independent variable). Data sets were not log-transformed for these analyses. Only OCP values attained between weeks 8 and 16 included in these analyses. Regression equation was as follows: **log mosquitofish OCP lipid-normalized concentration = a (intercept) + X (log soil TOC/OCP-normalized concentration).**

MOSQUITOFISH

Analyte	Sum of Squares	F	P	a	X
4,4'-DDD	70.8153	127.7437	0.0000	0.3481	1.1028
4,4'-DDE	140.7930	290.1841	0.0000	1.4259	1.0337
4,4'-DDT	210.2399	261.4604	0.0000	0.6869	0.9902
Aldrin	0.3802	7.4769	0.1118	2.3185	0.6402
Dieldrin	39.1459	71.6790	0.0000	1.3479	1.0179
Endosulfan II	15.0884	40.2218	0.0000	1.6260	0.8907
Endosulfan sulfate	2.8536	4.8294	0.0703	3.5116	0.5351
Endrin ketone	1.1715	3.0709	0.1178	3.4905	0.3548
Heptachlor epoxide	5.4266	3.1379	0.0982	1.6522	0.7002
Methoxychlor	0.7431	0.9380	0.3519	4.8237	0.4041
Toxaphene	53.6576	188.9106	0.0000	-0.2412	1.0811
alpha-Chlordane	23.4921	30.2424	0.0000	0.9826	0.8984
gamma-Chlordane	13.8689	52.7711	0.0000	2.3799	0.6853

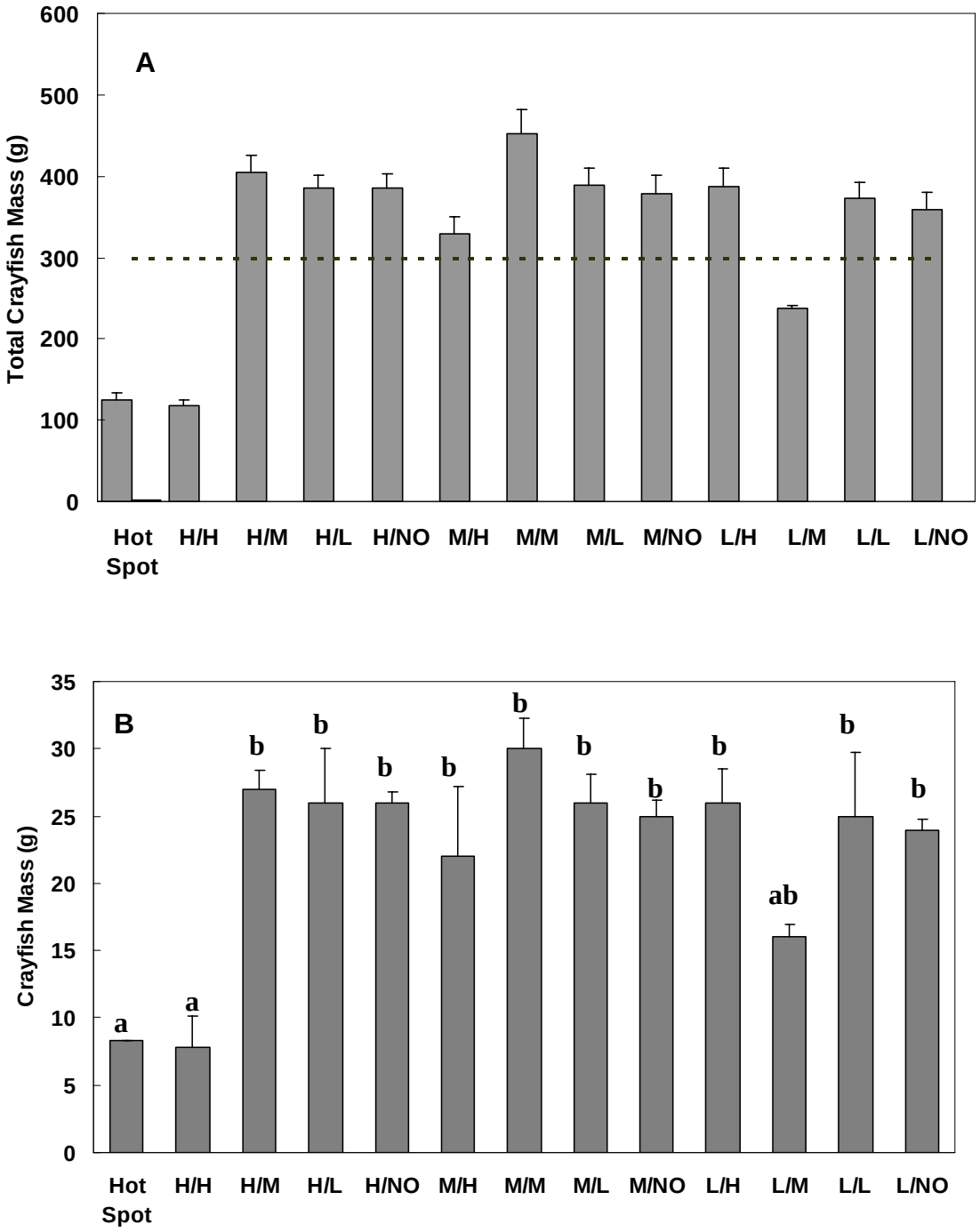


Figure 6-1. Mean ± SD of the sum of total crayfish mass (wet weight) collected for the whole study (A) and per sampling event (B), by treatment. Study consisted of five sampling events over the course of four months. Dotted line shows approximate total mass stocked (~300 g). Different letters denote significant differences in amount of crayfish collected across treatments (ANOVA, p = 0.02, F = 2.1).

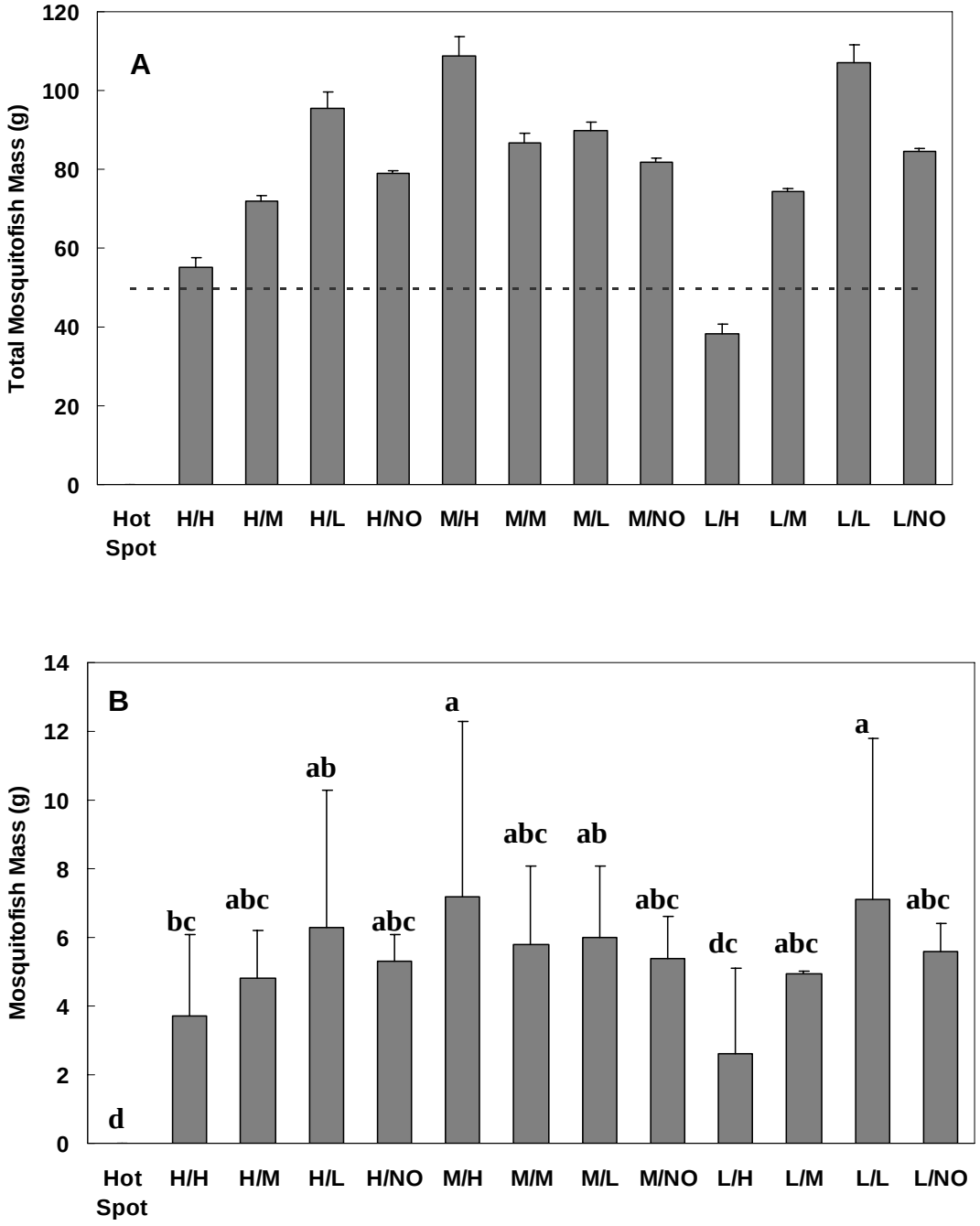


Figure 6-2. Mean ± SD of the sum of total mosquitofish mass (wet weight) collected for the whole study (A) and per sampling event (B), by treatment. Study consisted of five sampling events over the course of four months. Dotted line shows approximate total mass stocked (~50 g). Different letters denote significant differences in mosquitofish collected across treatments (ANOVA, $p < 0.0001$, $F = 8.2$). Note that no mosquitofish were collected from the "Hot Spot" treatment.

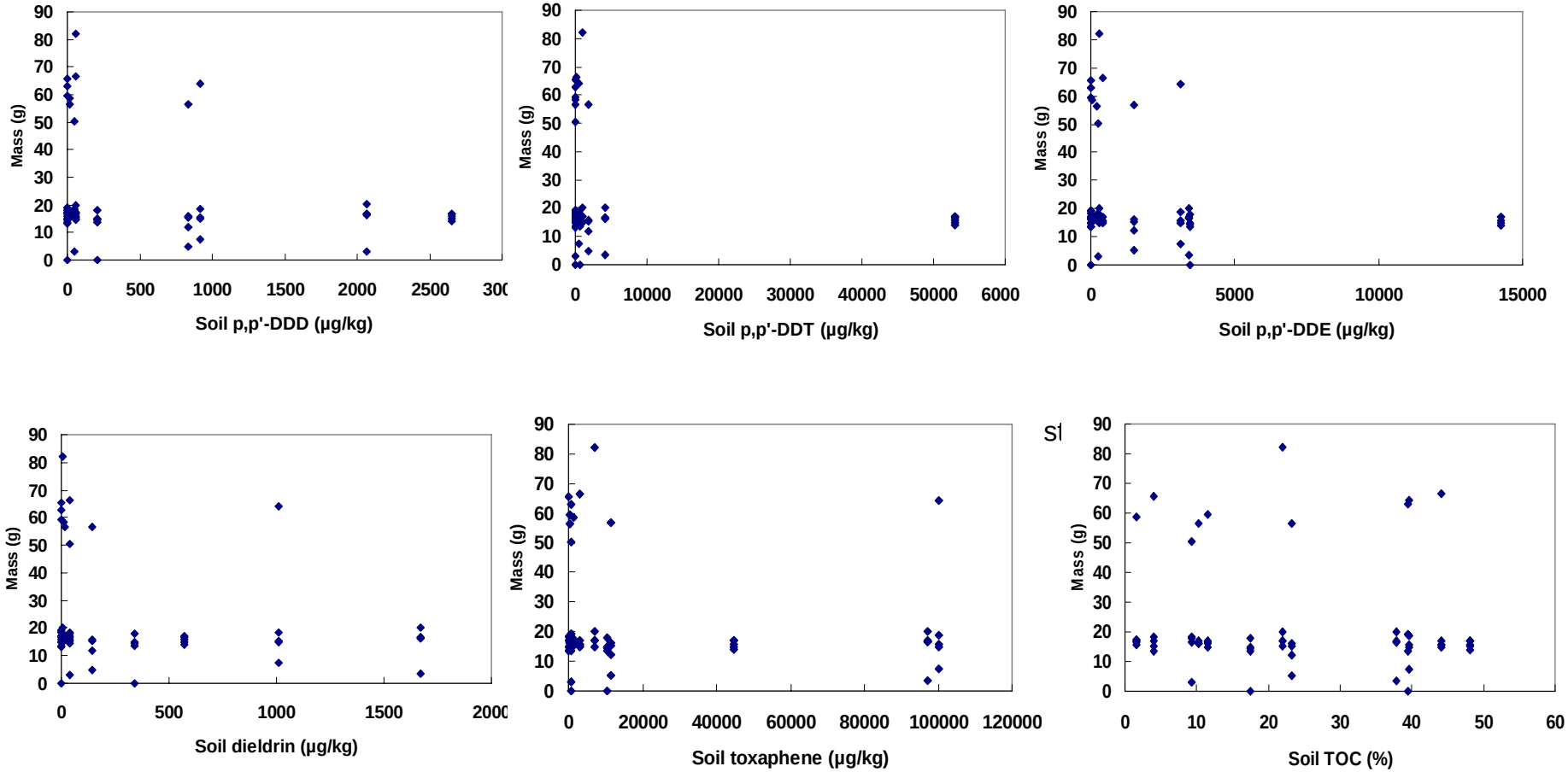


Figure 6-3. Relationship between total crayfish mass collected during the microcosm study and soil OCP and TOC concentrations. No significant relationships were found.

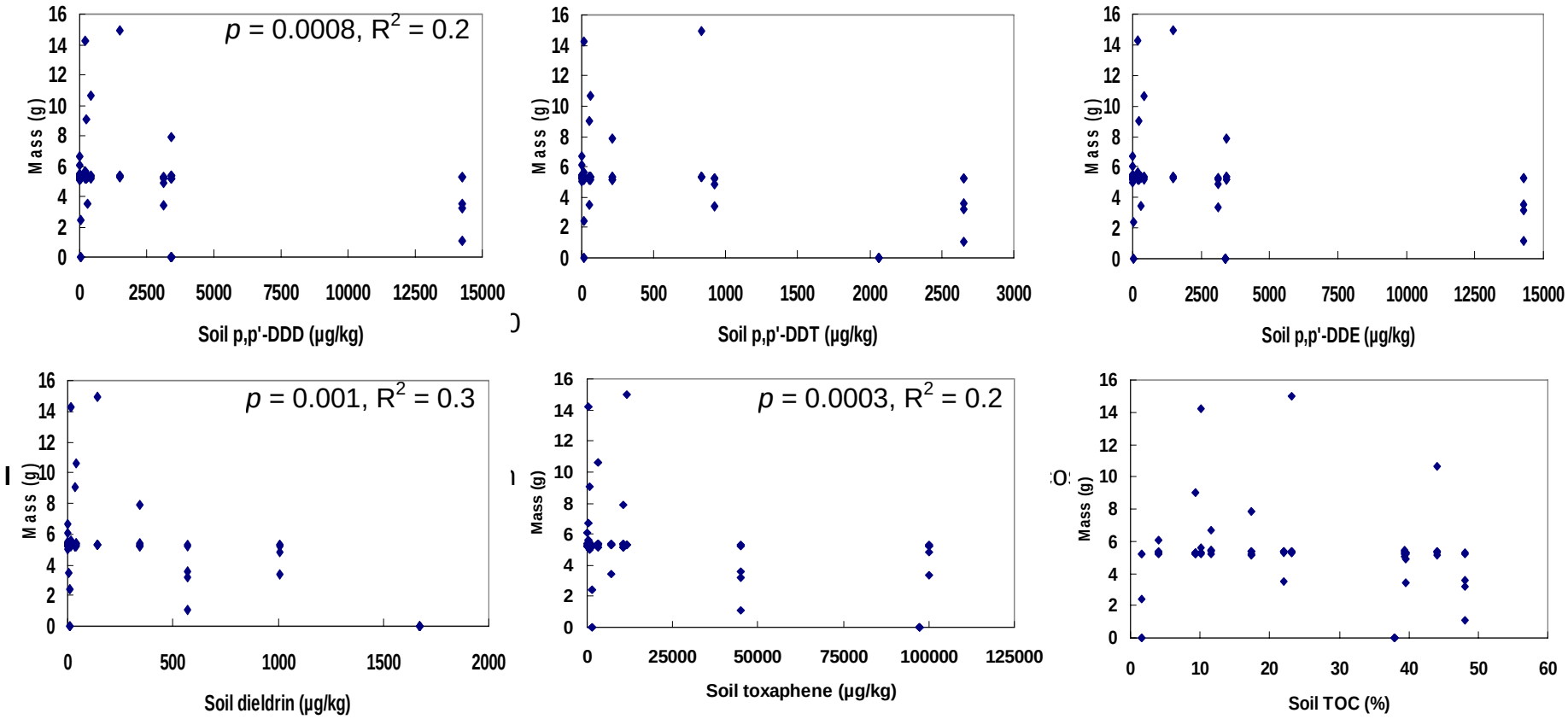


Figure 6-4. Relationship between total mosquitofish mass collected during the microcosm study and soil OCP and TOC concentrations. P and R^2 values are given for those relationships that were significant.

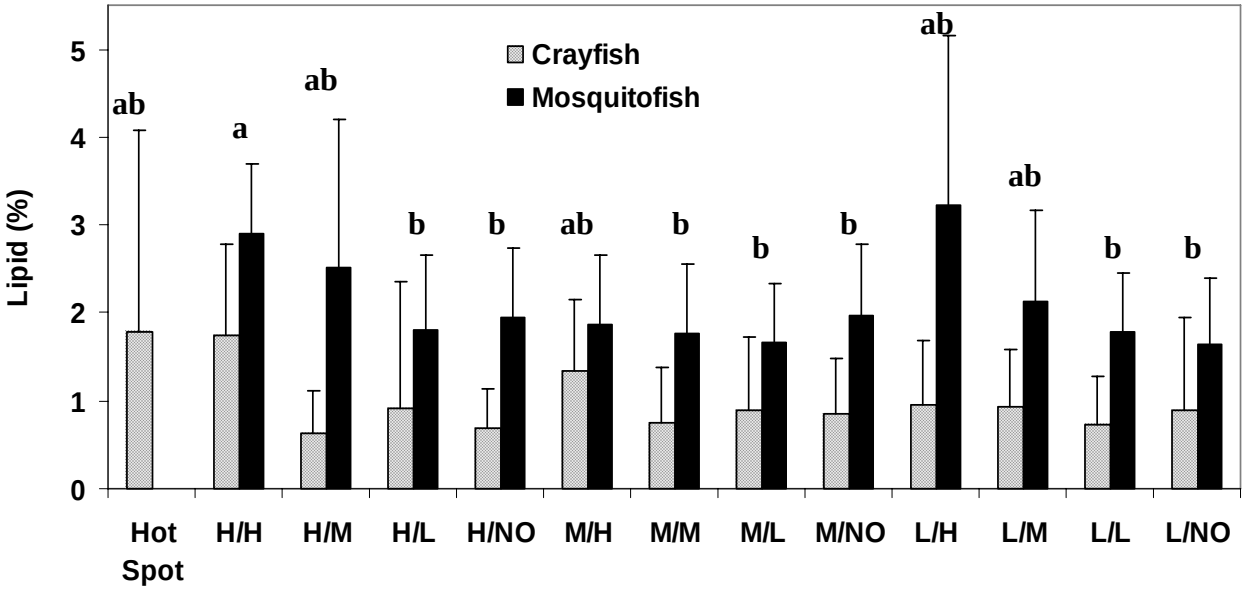


Figure 6-5. Overall mean ± SD of total lipid content (%) by treatment and biota type. Regardless of treatment, mosquitofish contained significantly more lipid than crayfish (2-way ANOVA, $p < 0.0001$, $F = 121$; overall means of 2.0 and 0.9% for mosquitofish and crayfish, respectively). For both species, different small letters indicate significant differences in fat content across treatments (2-way ANOVA, $p = 0.0002$, $F = 3.2$). Sample size was 15, with the following exceptions: For crayfish, Hot Spot and H/H, and M/H with 8 and 14 samples; and for mosquitofish, Hot Spot, H/H, L/H, and H/M and M/NO with 0, 11, 10, and 14 samples, respectively.

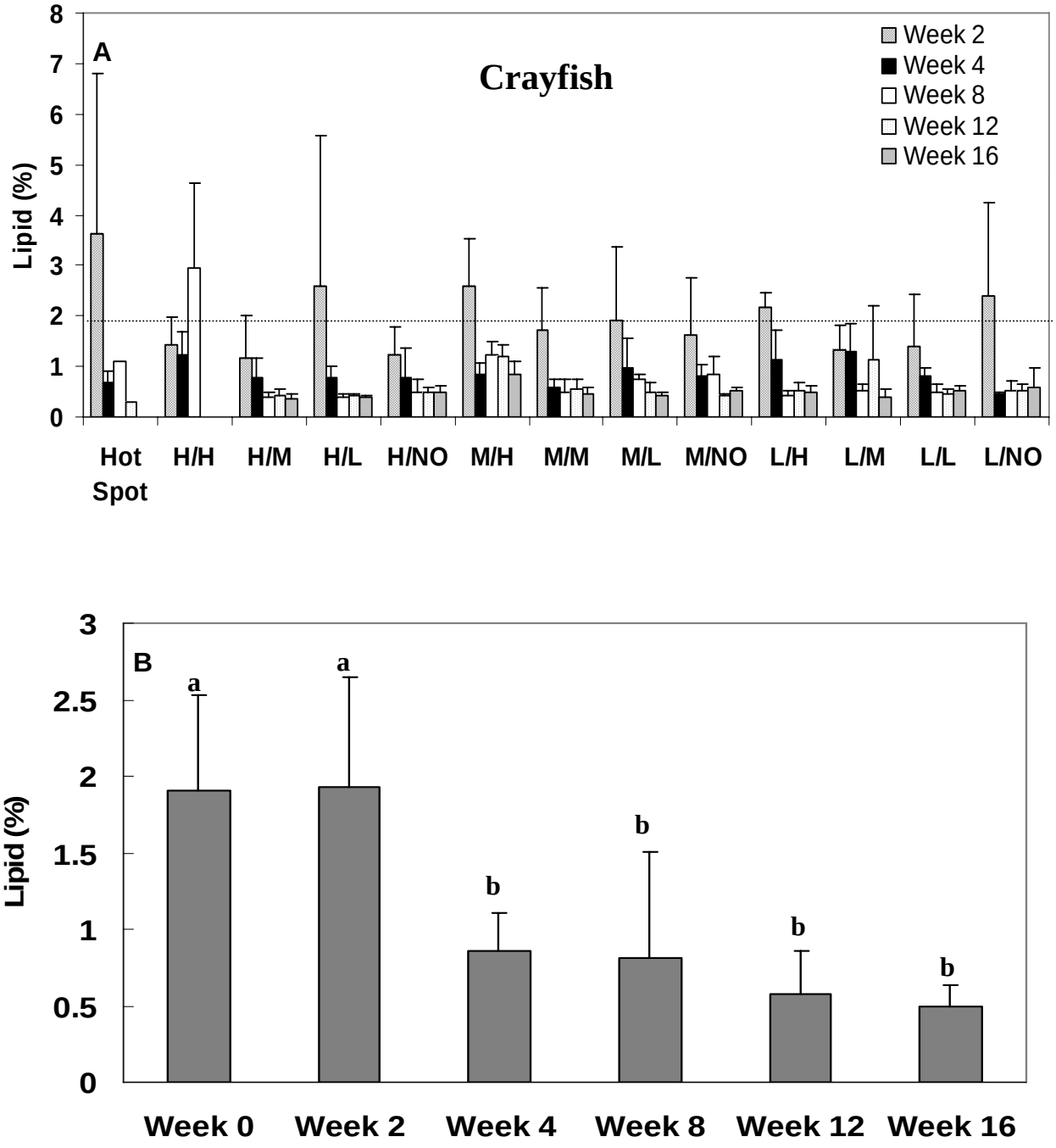


Figure 6-6. Mean ± SD of total lipid content (%) in crayfish, by week of study and treatment. Regardless of treatment, there was a significant week difference in total fat content, with highest values observed on week 2 (2-way ANOVA, $p = 0.001$, $F = 2.3$). Dotted line shows the mean lipid content in crayfish at time 0 (1.9 %, $n = 6$) (A). Overall lipid content decreased significantly and steadily over time (ANOVA, $p < 0.0001$, $F = 18.1$) (B).

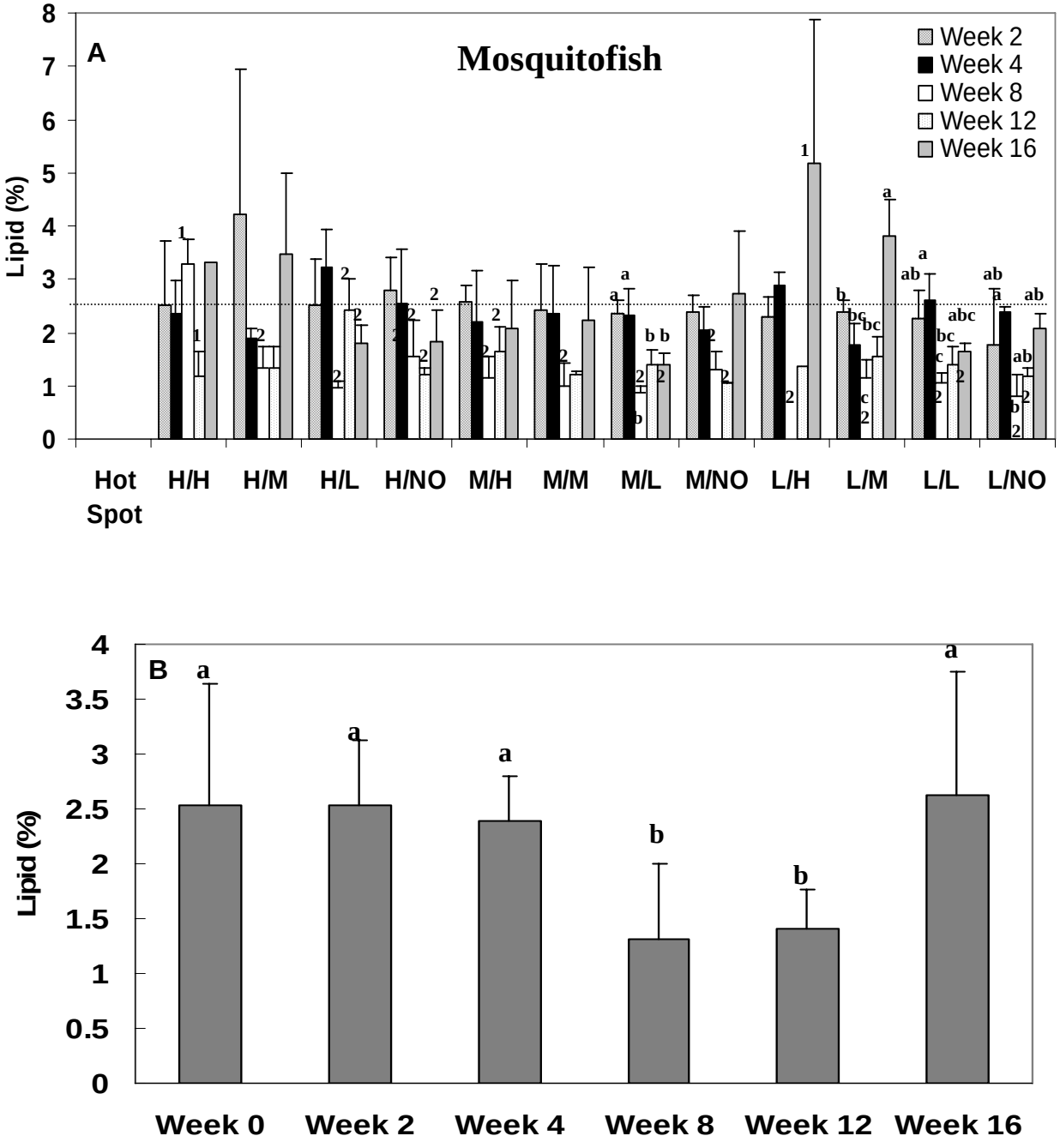
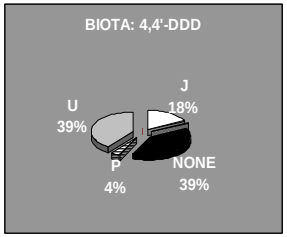
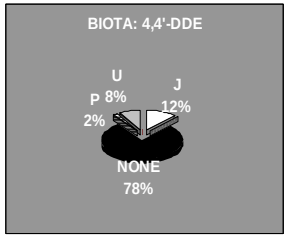
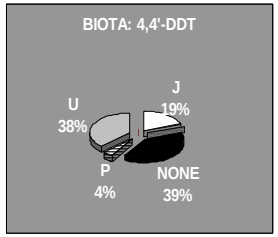


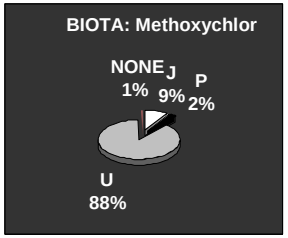
Figure 6-7. Mean ± SD of total lipid content (%) in mosquitofish, by week of study and treatment. Different small letters indicate significant differences across time within a treatment (2-way ANOVA, $p < 0.02$, $F > 5$) and different numbers indicate significant differences in total fat content over time across treatments (2-way ANOVA, $p < 0.01$, $F > 2.5$). Dotted line shows the mean lipid content in mosquitofish at time 0 (2.5 %, n = 5) (A). Overall lipid content followed a "U" shaped curve, with lowest values between weeks 8 and 12 (ANOVA, $p < 0.0001$, $F = 13.7$) (B).

Dichlorodiphenylethanes

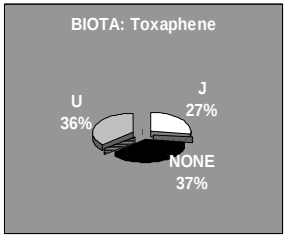




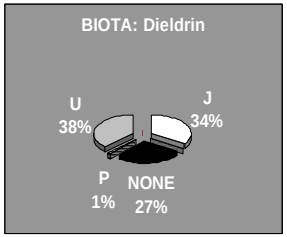


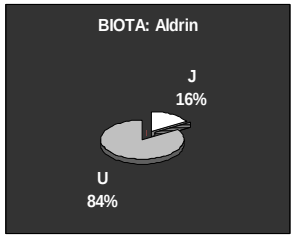


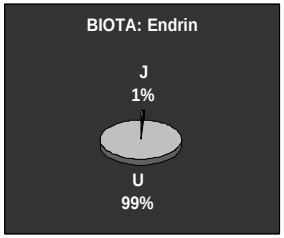
Toxaphene: Chlorinated Camphenes

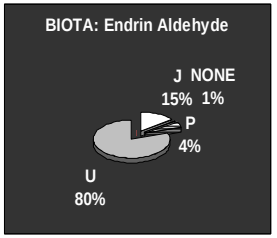


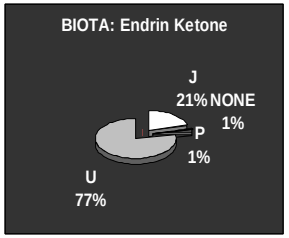
Cyclodienes











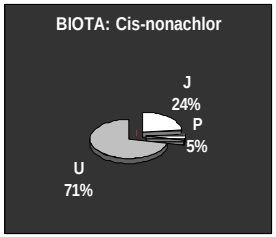
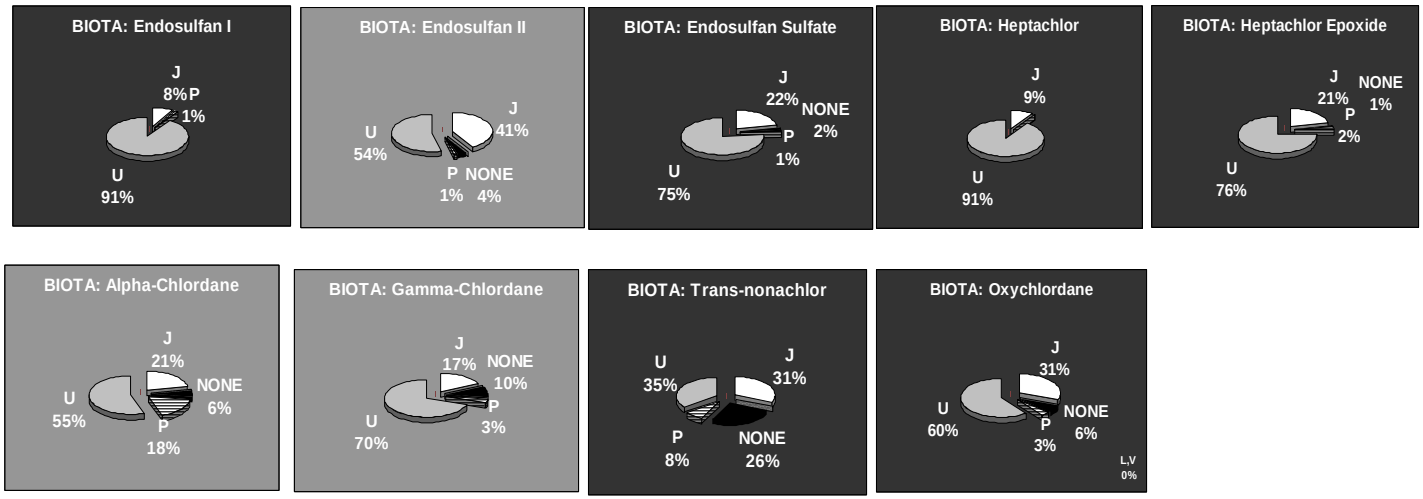


Figure 6-8. Frequency distribution of data qualifiers for biota (crayfish and mosquitofish combined) provided by EN CHEM. Shown are pie charts for each chemical analyzed, by chemical family. All data summarized. Light gray background denotes chemicals that were analyzed in more detail in this report. Definition of qualifiers: U = Not detected; value reported is the laboratory method detection limit (MDL); J = Estimated value; concentration detected is greater than the MDL but less than the reporting limit; P = Relative percent difference for detected concentrations between the two columns was > 40 %; NONE = No qualifiers reported. It is important to note that many others qualifiers were reported, however the ones summarized here were the most common. In addition, toxaphene values with a "JN" qualifier were included in this analysis as "J" values (see text for more discussion on this).

Cyclodienes (cont.)



Hexachlorocyclohexanes

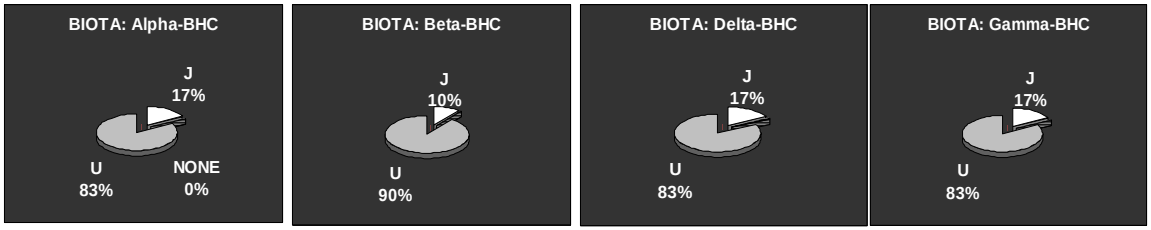


Figure 6-8. Continued.

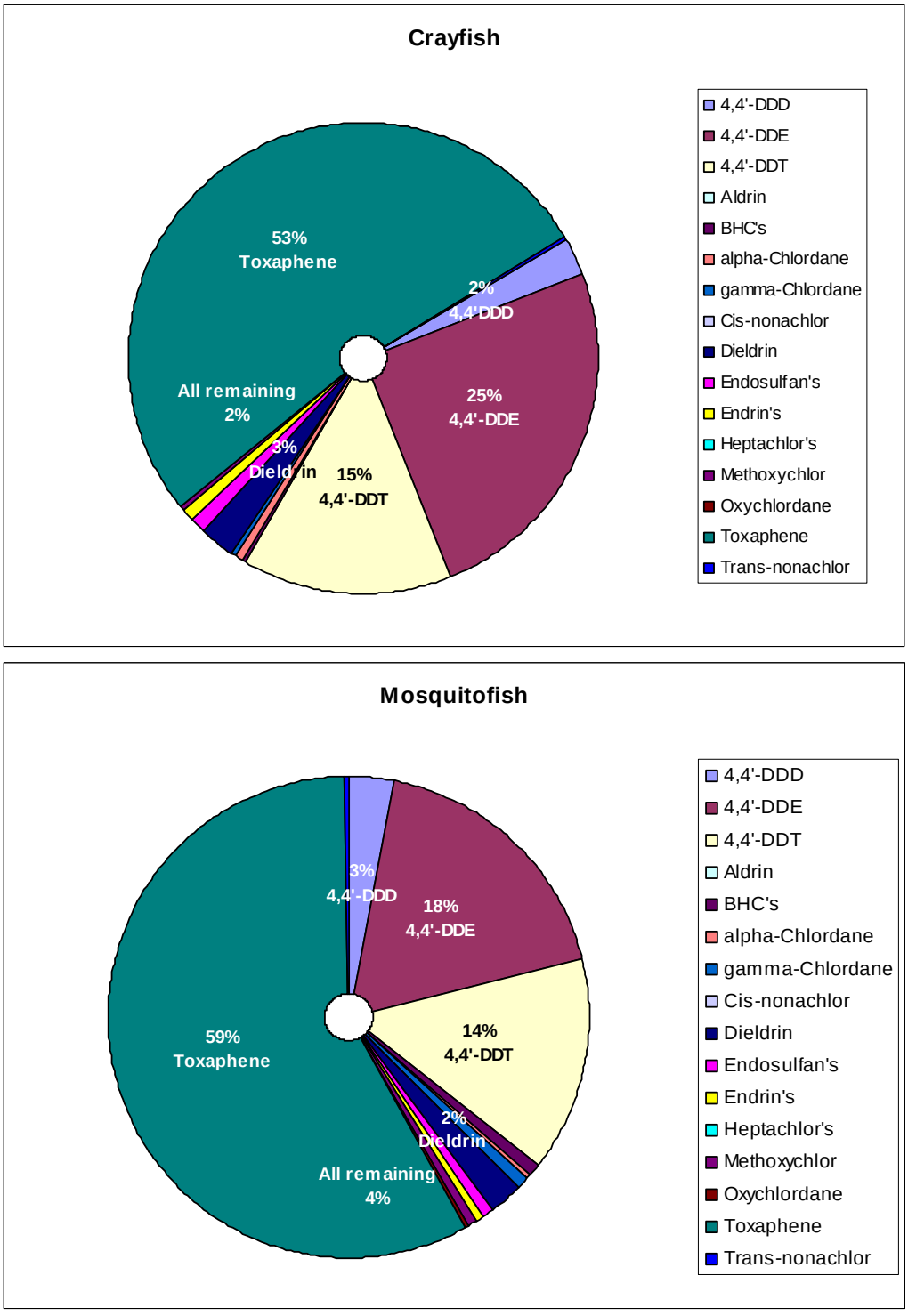


Figure 6-9. Mean relative concentration of each of the 16 OCPs detected in crayfish and mosquitofish throughout the total length of the microcosm study, all treatments combined. Percentages for individual chemicals were calculated after summing up all the OCP values.

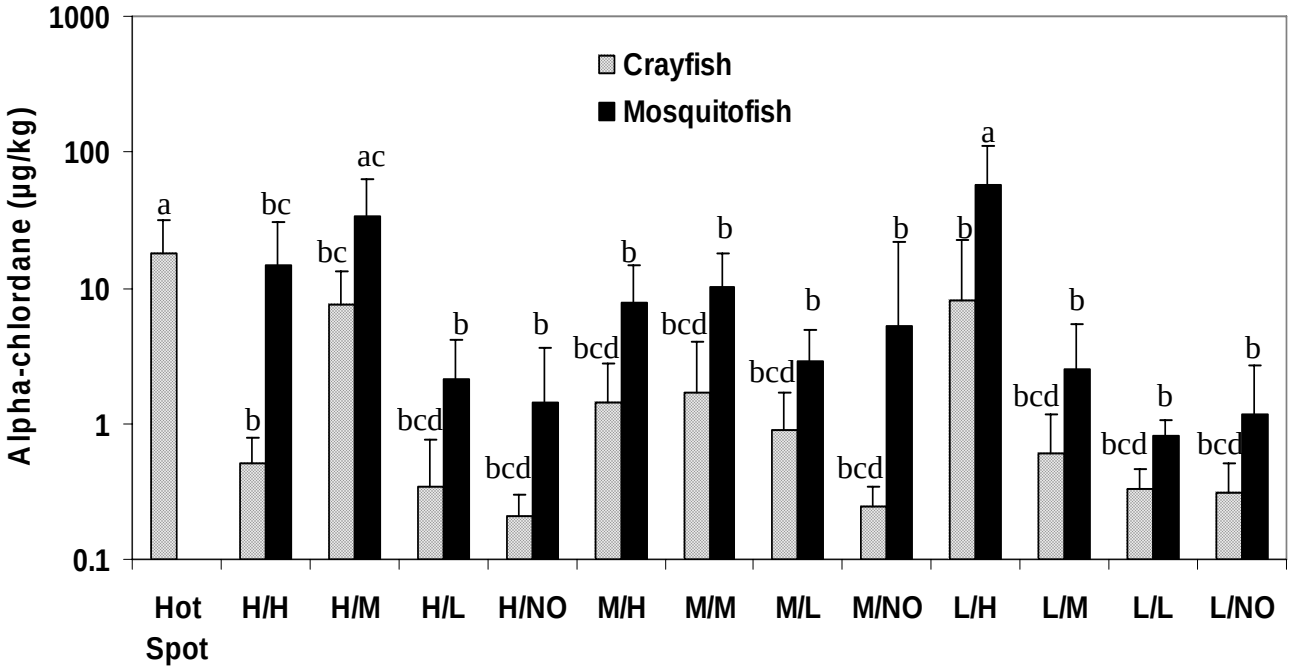


Figure 6-10A. Mean ± SD of alpha-chlordane non-lipid-normalized concentrations (µg/kg, ww) in crayfish and mosquitofish, by treatment. All time periods averaged. There was a significant interaction effect between biota and treatment. Different small letters indicate significant differences across treatments within each biota type (2-way ANOVA, $p < 0.0001$, $F > 9$). With the exception of the Hot Spot treatment in which no mosquitofish samples were analyzed, mosquitofish contained significantly higher concentrations of alpha-chlordane compared to crayfish (2-way ANOVA, $p < 0.04$, $F > 4.0$; overall means of 436 and 338 µg/kg, for mosquitofish and crayfish, respectively).

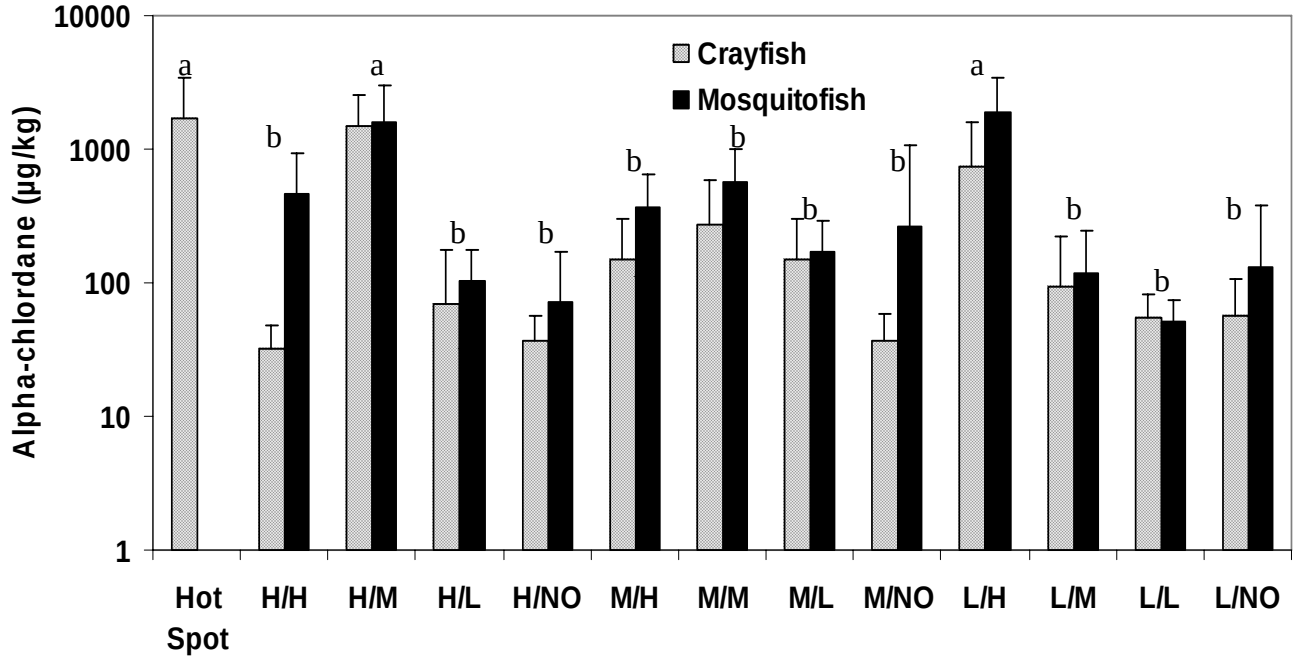


Figure 6-10B. Mean ± SD of alpha-chlordane lipid-normalized concentrations (µg/kg lipid) in crayfish and mosquitofish, by treatment. All time periods averaged. There was no significant interaction effect between biota and treatment. For all treatments, lipid-normalized concentrations did not differ among biota types. For both biota types, treatments Hot Spot, H/M, and L/H contained significantly higher concentrations of alpha-chlordane (2-way ANOVA $p < 0.0001$, $F = 22$).

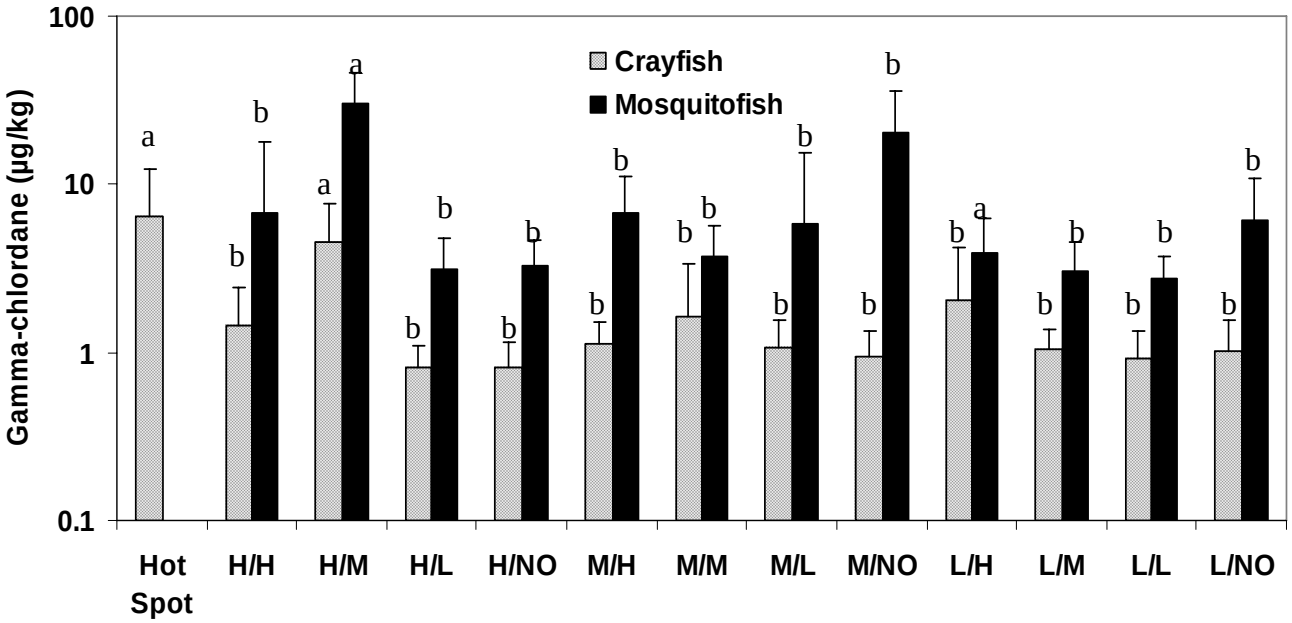


Figure 6-11A. Mean ± SD of gamma-chlordane non-lipid-normalized concentrations (µg/kg, ww) in crayfish and mosquitofish, by treatment. All time periods averaged. There was a significant interaction effect between biota and treatment. Different small letters indicate significant differences across treatments within each biota type (2-way ANOVA, $p < 0.0001$, $F > 10$). With the exception of Hot Spot and H/M treatments, mosquitofish contained significantly higher concentrations of gamma-chlordane compared to crayfish (2-way ANOVA, $p < 0.04$, $F > 4$; overall means of 384 and 272 µg/kg for mosquitofish and crayfish, respectively.

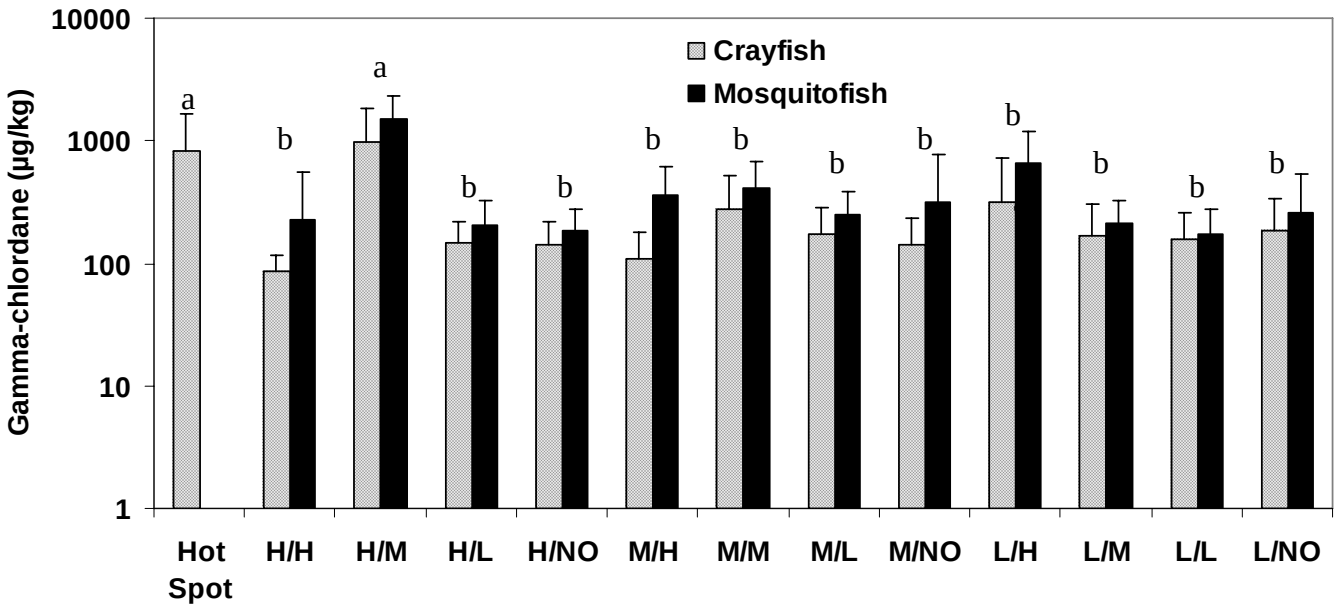


Figure 6-11B. Mean ± SD of gamma-chlordane lipid-normalized concentrations (µg/kg lipid) in crayfish and mosquitofish, by treatment. All time periods averaged. There was no significant interaction effect between biota and treatment. For all treatments, lipid-normalized concentrations were significantly higher in mosquitofish (2-way ANOVA, $p < 0.0001$, $F = 16$). For both biota types, treatments Hot Spot and H/M contained significantly higher concentrations of gamma-chlordane (2-way ANOVA, $p < 0.0001$, $F = 21$).

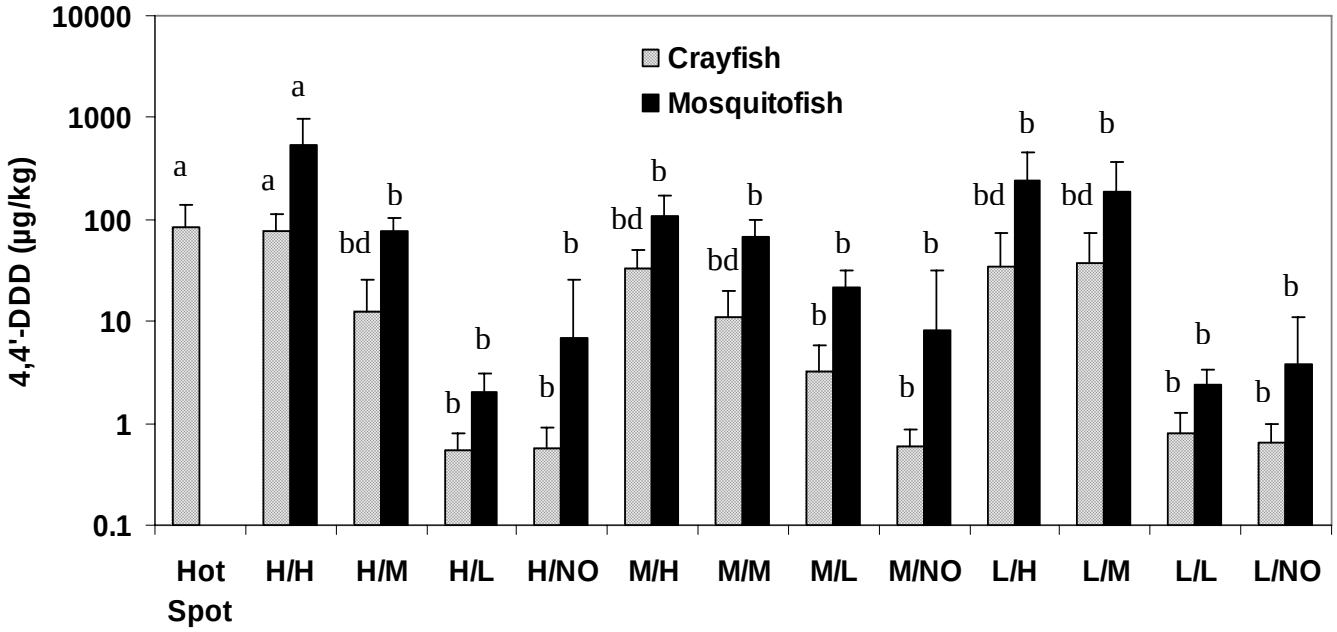


Figure 6-12A. Mean ± SD of 4,4'-DDD non-lipid-normalized concentrations (µg/kg, ww) in crayfish and mosquitofish, by treatment. All time periods averaged. There was a significant interaction effect between biota and treatment. Different small letters indicate significant differences across treatments within each biota type (2-way ANOVA, $p < 0.0001$, $F > 16$). With the exception of Hot Spot, H/NO, and L/NO treatments, mosquitofish contained significantly higher concentrations of 4,4'-DDD compared to crayfish (2-way ANOVA, $p < 0.009$, $F > 8$).

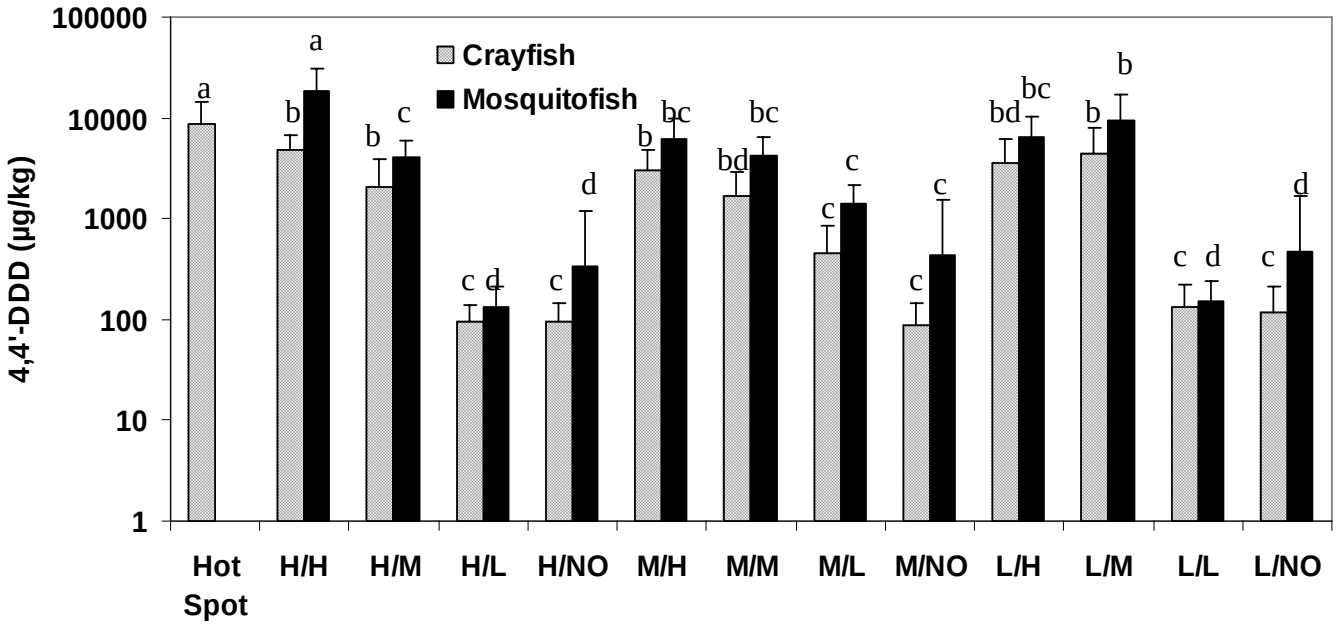


Figure 6-12B. Mean ± SD of 4,4'-DDD lipid-normalized concentrations (µg/kg lipid) in crayfish and mosquitofish, by treatment. All time periods averaged. There was a significant interaction effect between biota and treatment. Different small letters indicate significant differences across treatments within each biota type (2-way ANOVA, $p < 0.0001$, $F > 18$). With the exception of Hot Spot, H/L, H/NO, M/NO, L/L, and L/NO treatments, mosquitofish contained significantly higher concentrations of 4,4'-DDD compared to crayfish (2-way ANOVA, $p < 0.03$, $F > 5$).

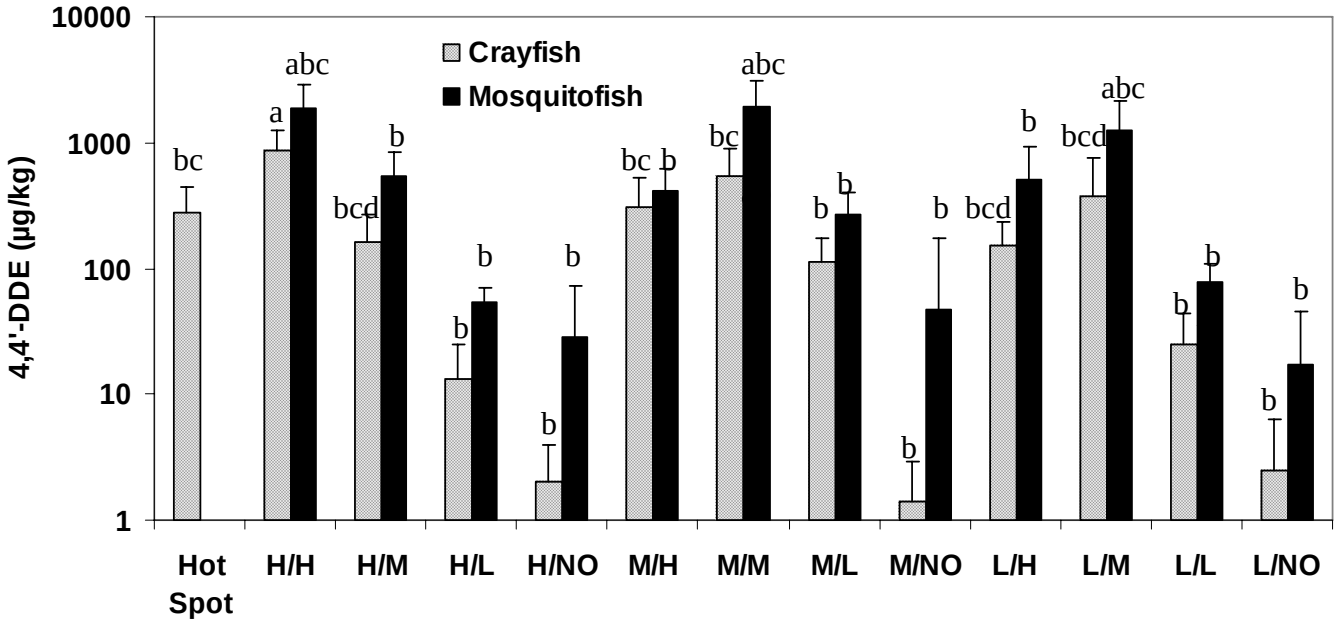


Figure 6-13A. Mean ± SD of 4,4'-DDE non-lipid-normalized concentrations (µg/kg, ww) in crayfish and mosquitofish, by treatment. All time periods averaged. There was a significant interaction effect between biota and treatment. Different small letters indicate significant differences across treatments within each biota type (2-way ANOVA, $p < 0.0001$, $F > 19$). With the exception of "Hot Spot", M/H, and M/NO treatments, mosquitofish contained significantly higher concentrations of 4,4'-DDE compared to crayfish (2-way ANOVA, $p < 0.02$, $F > 5$).

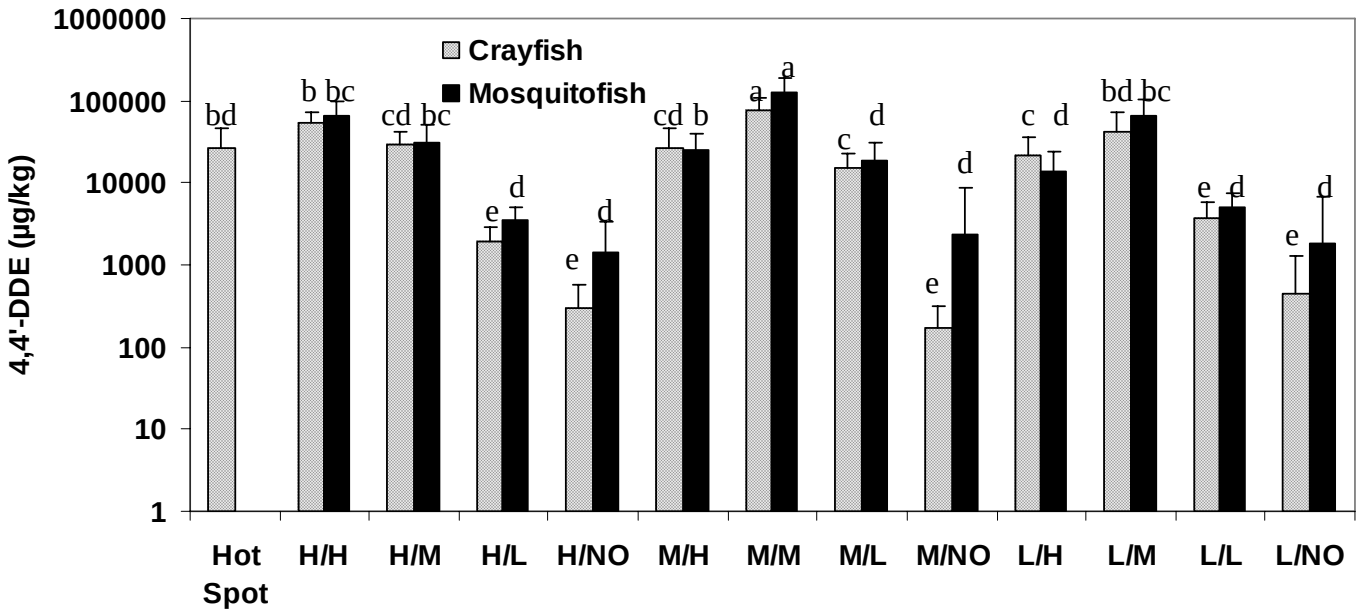


Figure 6-13B. Mean ± SD of 4,4'-DDE lipid-normalized concentrations (µg/kg lipid) in crayfish and mosquitofish, by treatment. All time periods averaged. There was a significant interaction effect between biota and treatment. Different small letters indicate significant differences across treatments within each biota type (2-way ANOVA, $p < 0.0001$, $F > 30$). Mosquitofish contained significantly higher concentrations of 4,4'-DDE only in treatments H/L, H/NO, and M/M (2-way ANOVA, $p < 0.03$, $F > 6$).

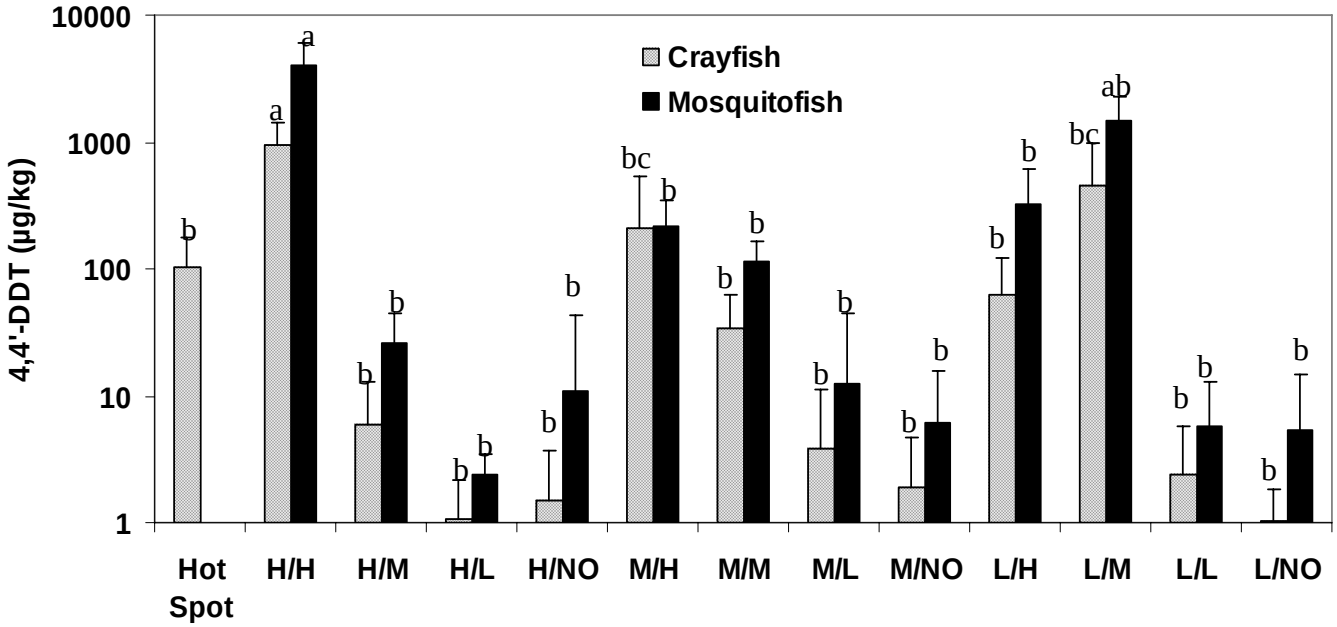


Figure 6-14A. Mean ± SD of 4,4'-DDT non-lipid-normalized concentrations (µg/kg, ww) in crayfish and mosquitofish, by treatment. All time periods averaged. There was a significant interaction effect between biota and treatment. Different small letters indicate significant differences across treatments within each biota type (2-way ANOVA, $p < 0.0001$, $F > 16$). With the exception of Hot Spot, H/NO, M/NO, L/NO, M/H, M/L, and L/L treatments, mosquitofish contained significantly higher concentrations of 4,4'-DDT compared to crayfish (2-way ANOVA, $p < 0.001$, $F > 12$).

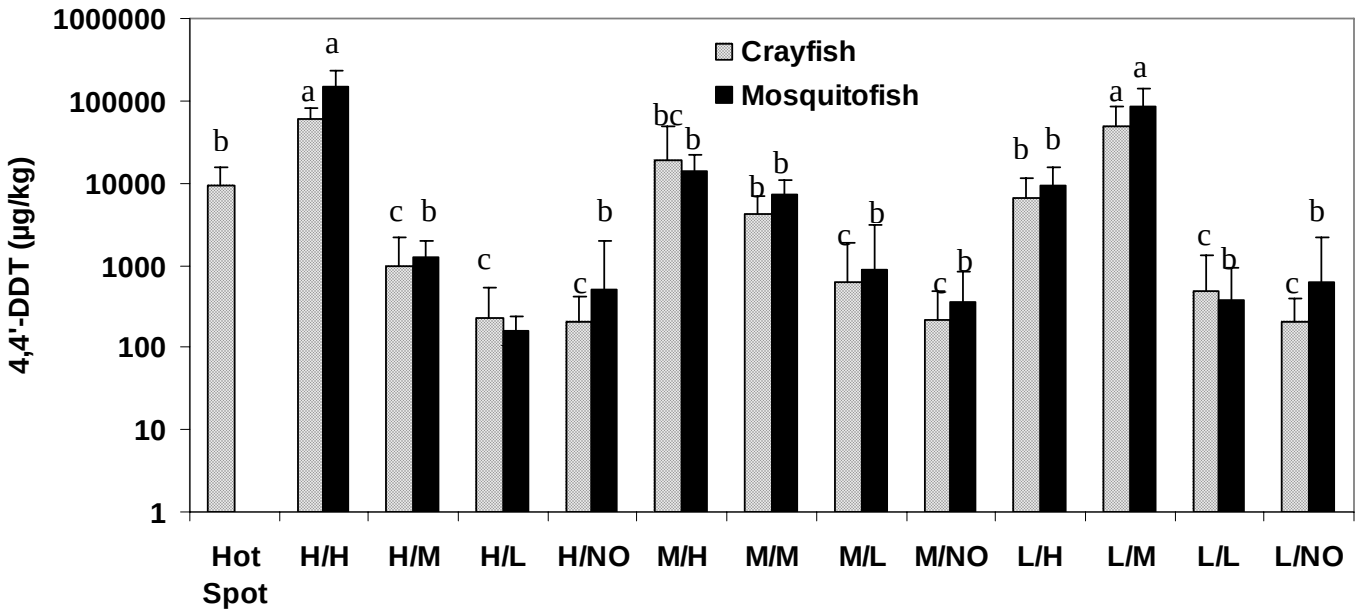


Figure 6-14B. Mean ± SD of 4,4'-DDT lipid-normalized concentrations (µg/kg lipid) in crayfish and mosquitofish, by treatment. All time periods averaged. There was a significant interaction effect between biota and treatment. Different small letters indicate significant differences across treatments within each biota type (2-way ANOVA, $p < 0.0001$, $F > 22$). Mosquitofish contained significantly higher concentrations of 4,4'-DDT only in treatments H/H and M/M (2-way ANOVA, $p < 0.01$, $F > 6$).

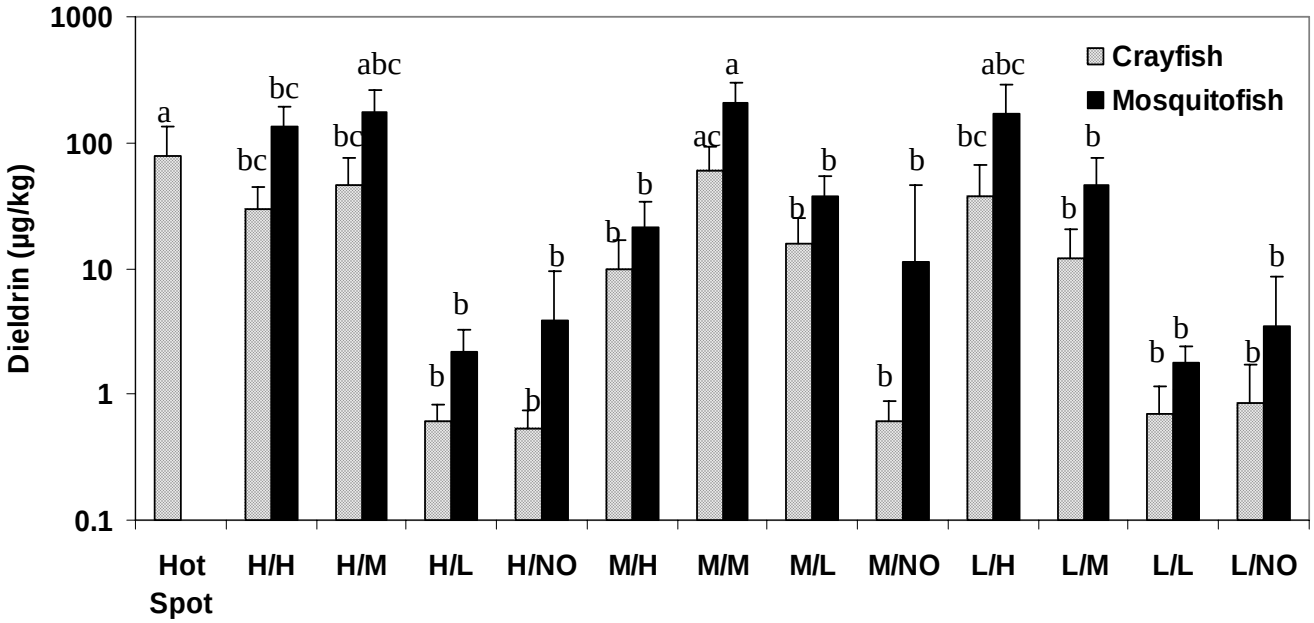


Figure 6-15A. Mean ± SD of dieldrin non-lipid-normalized concentrations (µg/kg, ww) in crayfish and mosquitofish, by treatment. All time periods averaged. There was a significant interaction effect between biota and treatment. Different small letters indicate significant differences across treatments within each biota type (2-way ANOVA, $p < 0.0001$, $F > 16$). With the exception of Hot Spot, mosquitofish contained significantly higher concentrations of dieldrin compared to crayfish (2-way ANOVA, $p < 0.03$, $F > 5$).

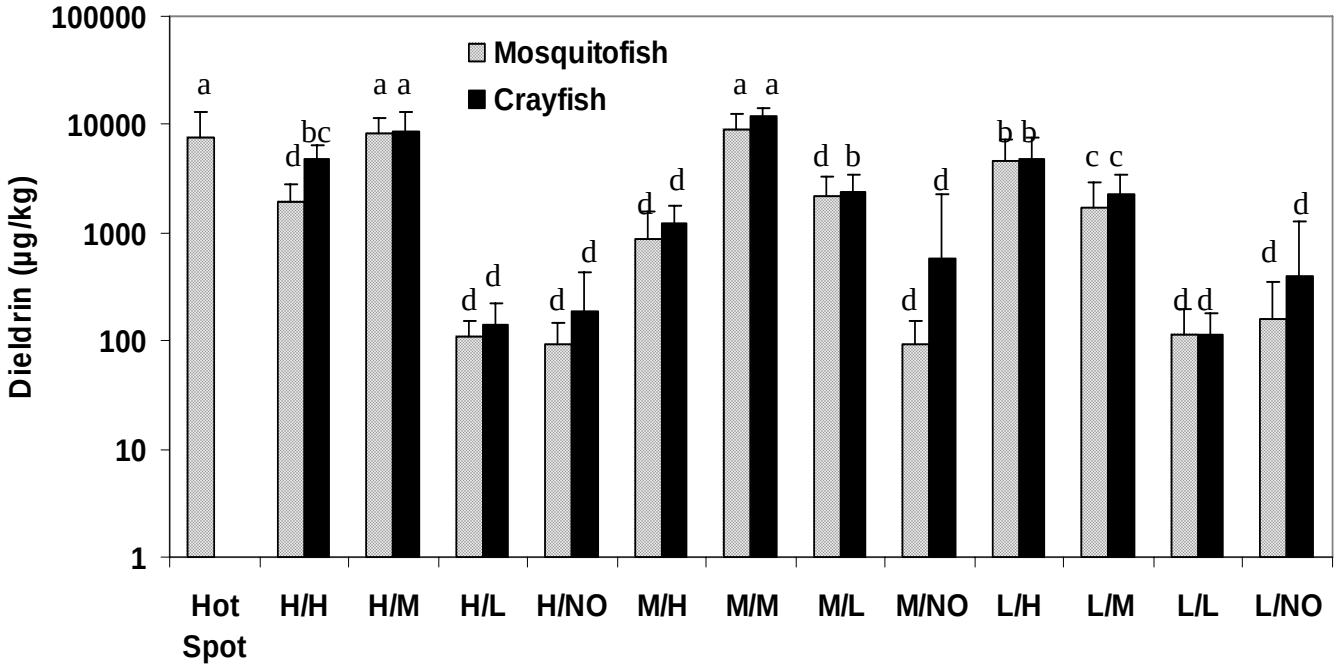


Figure 6-15B. Mean ± SD of dieldrin lipid-normalized concentrations (µg/kg lipid) in crayfish and mosquitofish, by treatment. All time periods averaged. There was a significant interaction effect between biota and treatment. Different small letters indicate significant differences across treatments within each biota type (2-way ANOVA, $p < 0.0001$, $F > 41$). Mosquitofish contained significantly higher concentrations of dieldrin only in treatments H/H and M/M (2-way ANOVA, $p < 0.009$, $F > 8$).

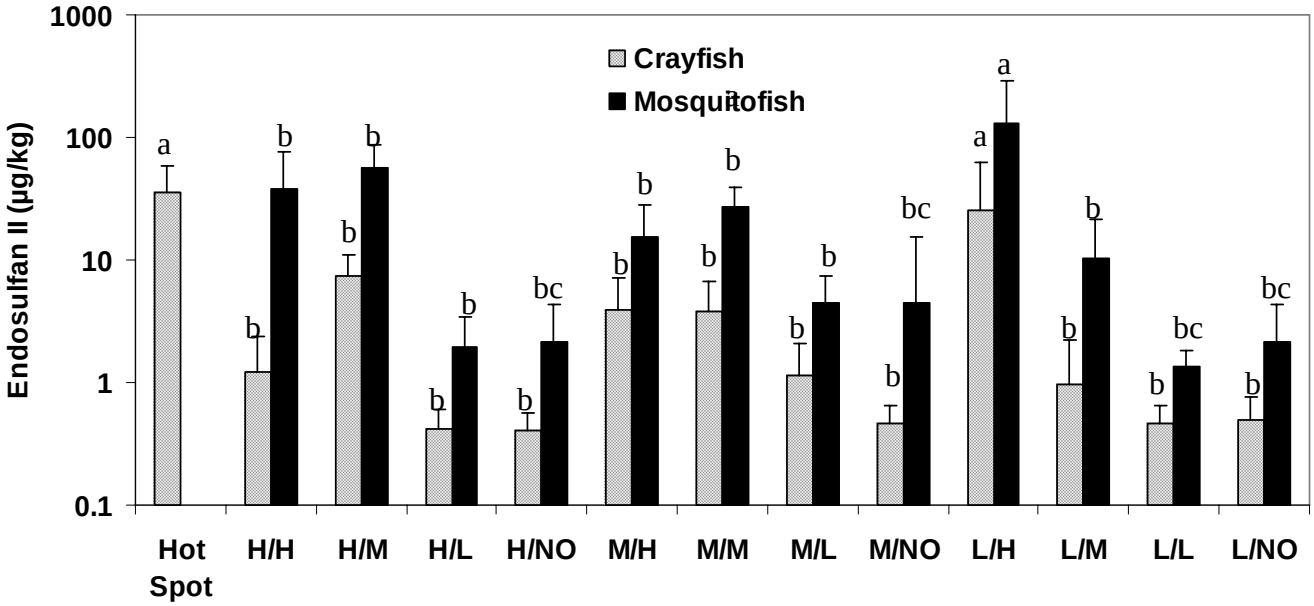


Figure 6-16A. Mean ± SD of endosulfan II non-lipid-normalized concentrations (µg/kg, ww) in crayfish and mosquitofish, by treatment. All time periods averaged. There was a significant interaction effect between biota and treatment. Different small letters indicate significant differences across treatments within each biota type (2-way ANOVA, $p < 0.0001$, $F > 16$). With the exception of Hot Spot, and M/NO treatments, mosquitofish contained significantly higher concentrations of endosulfan II compared to crayfish (2-way ANOVA, $p < 0.02$, $F > 6$).

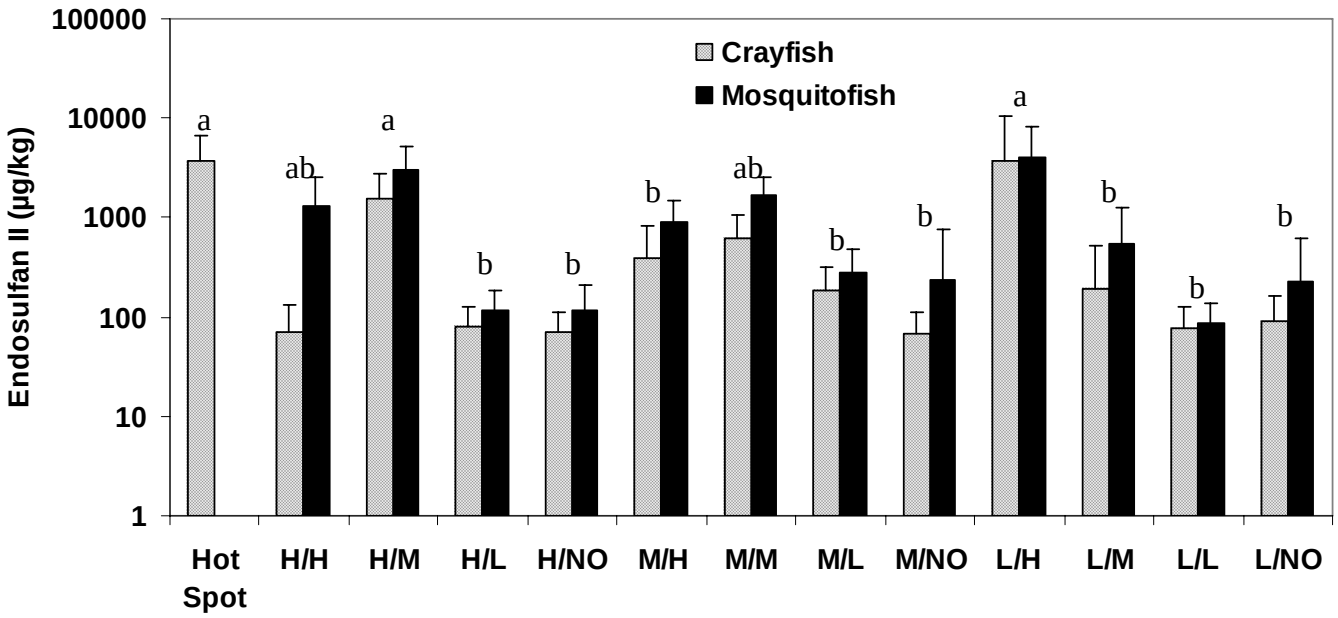


Figure 6-16B. Mean ± SD of endosulfan II lipid-normalized concentrations (µg/kg lipid) in crayfish and mosquitofish, by treatment. All time periods averaged. There was no significant interaction effect between biota and treatment. For all treatments, lipid-normalized concentrations did not differ among biota types (942 and 751 µg/kg, for mosquitofish and crayfish, respectively; 2-way ANOVA, $p = 0.02$, $F = 6$). Different small letters indicate significant differences across treatments for both types (2-way ANOVA, $p < 0.0001$, $F = 12$).

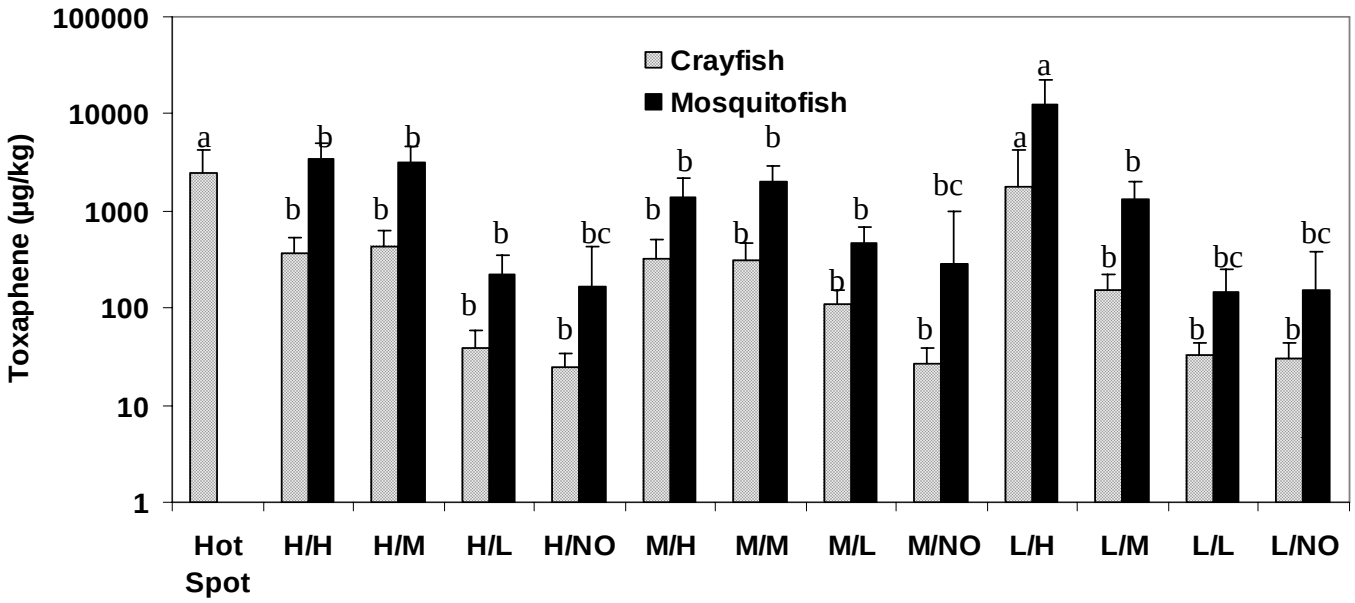


Figure 6-17A. Mean ± SD of toxaphene non-lipid-normalized concentrations (µg/kg, ww) in crayfish and mosquitofish, by treatment. All time periods averaged. There was a significant interaction effect between biota and treatment. Different small letters indicate significant differences across treatments within each biota type (2-way ANOVA, $p < 0.0001$, $F > 16$). With the exception of Hot Spot, and M/NO treatments, mosquitofish contained significantly higher concentrations of toxaphene compared to crayfish (2-way ANOVA, $p < 0.04$, $F > 4$).

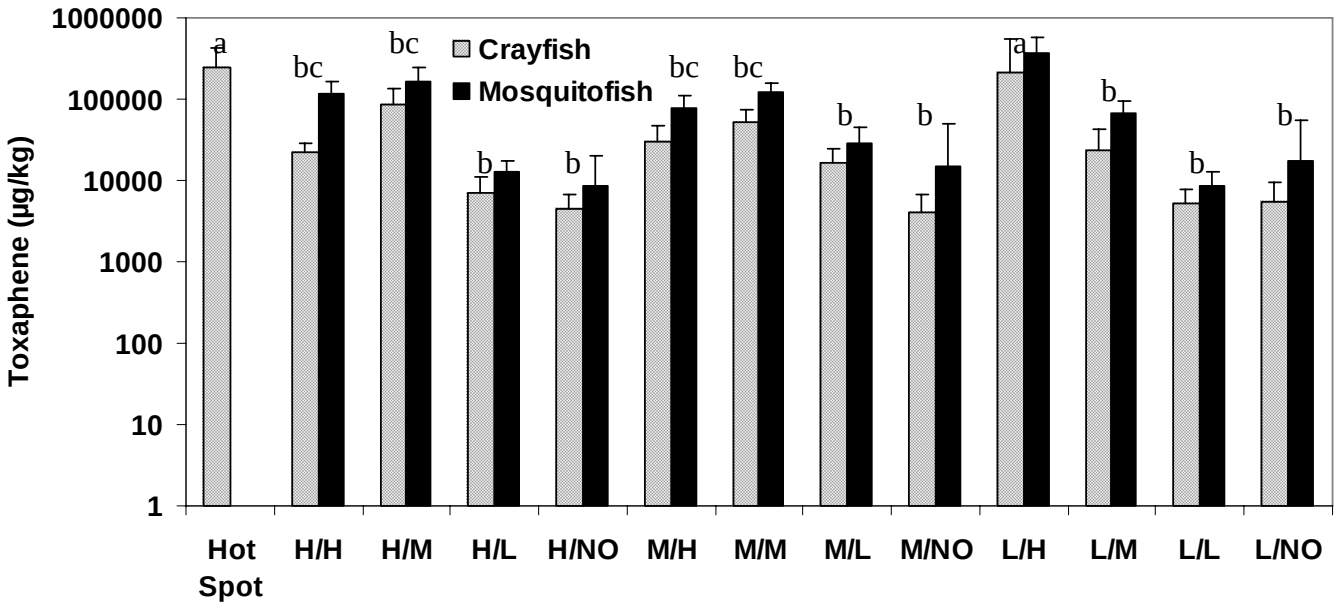


Figure 6-17B. Mean ± SD of toxaphene lipid-normalized concentrations (µg/kg lipid) in crayfish and mosquitofish, by treatment. All time periods averaged. There was no significant interaction effect between biota and treatment. For all treatments, lipid-normalized concentrations were higher in mosquitofish compared to crayfish (74,177 and 49,215 µg/kg, for mosquitofish and crayfish, respectively; 2-way ANOVA, $p < 0.00001$, $F = 24$). Different small letters indicate significant differences across treatments for both types (2-way ANOVA, $p < 0.0001$, $F = 24$).

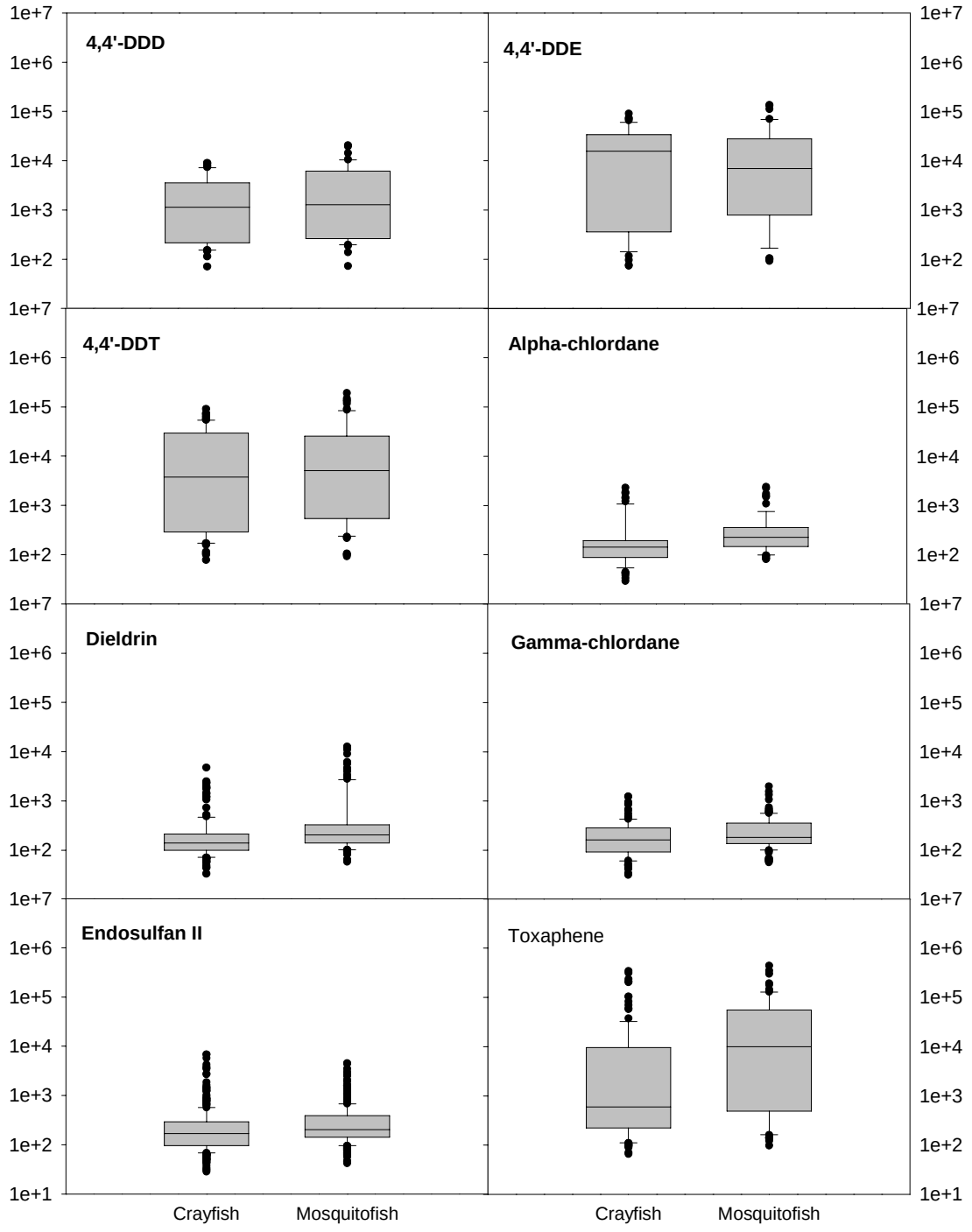


Figure 6-18. Box plots of OCP lipid-normalized concentrations (µg/kg lipid) in crayfish and mosquitofish. Summarized are values collected at steady state conditions (i.e. weeks 8, 12, and 16).

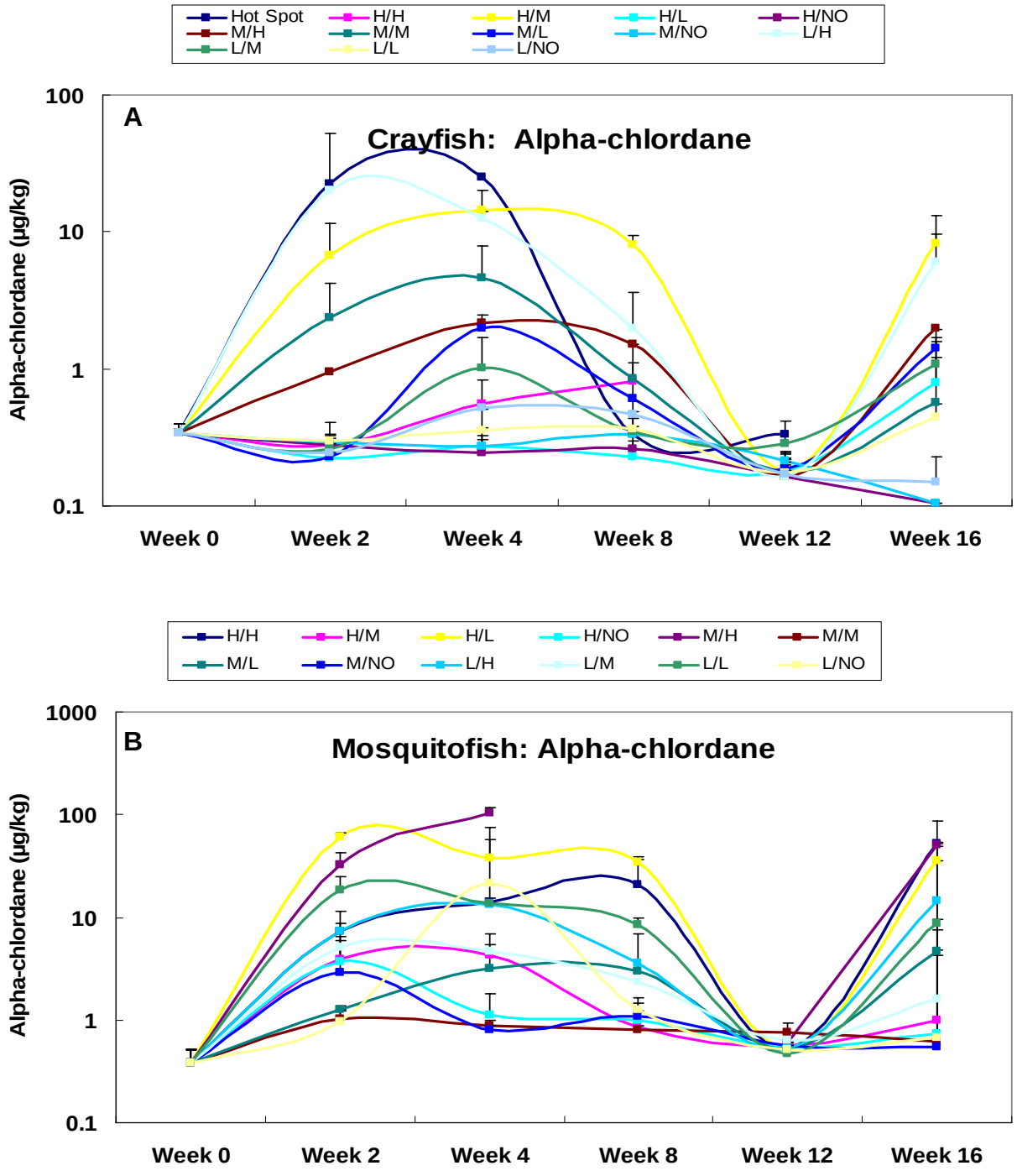


Figure 6-19A. Mean ± SD of alpha-chlordane non-lipid-normalized concentrations (µg/kg, ww) in crayfish (A) and mosquitofish (B) over the length of the experiment (week 0 = Nov. 5 – 7, 2001 through week 16 = Feb. 25 – 27, 2002) by treatment.

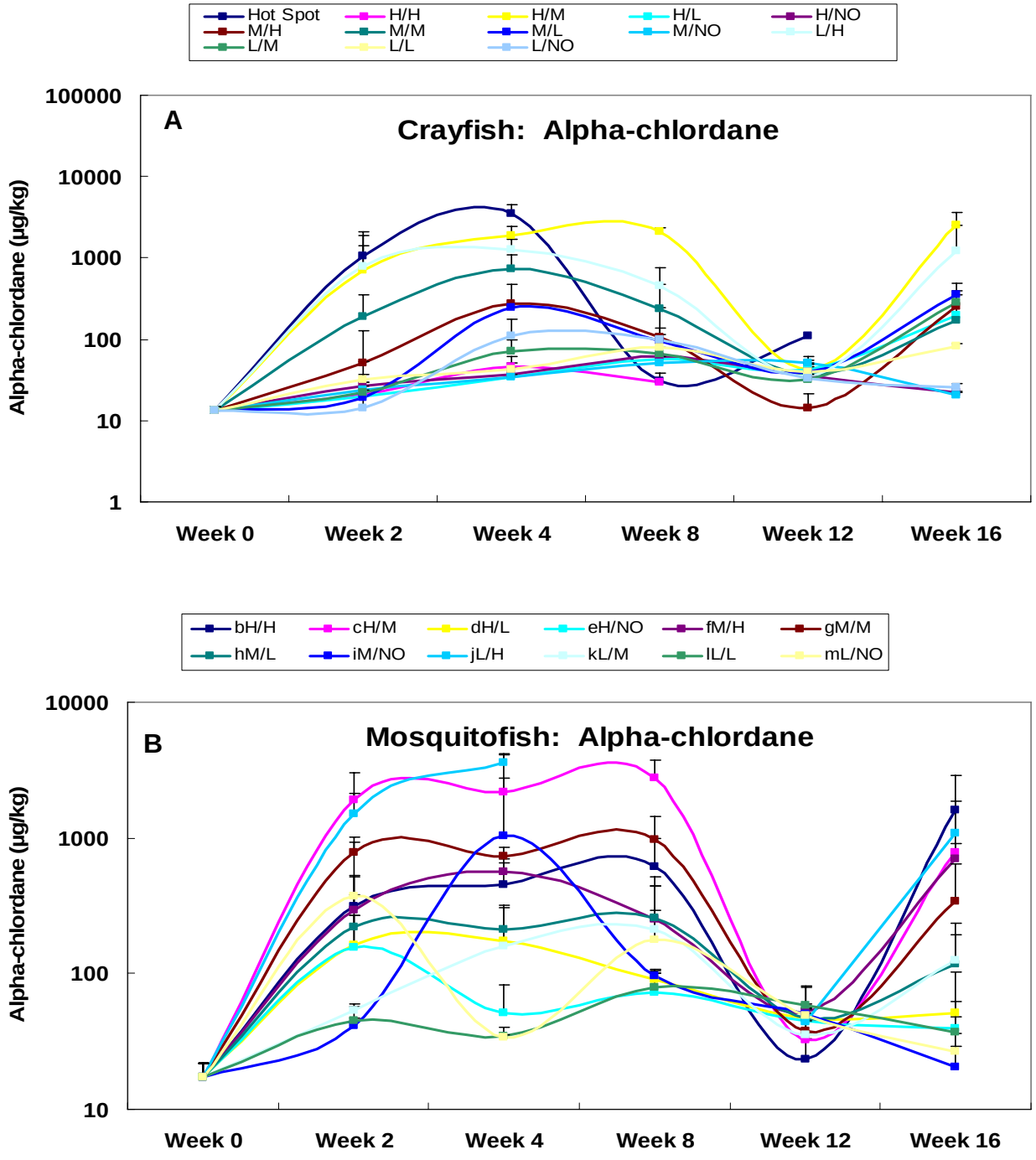


Figure 6-19B. Mean ± SD of alpha-chlordane lipid-normalized concentrations (µg/kg lipid) in crayfish (A) and mosquitofish (B) over the length of the experiment (week 0 = Nov. 5 – 7, 2001 through week 16 = Feb. 25 – 27, 2002) by treatment.

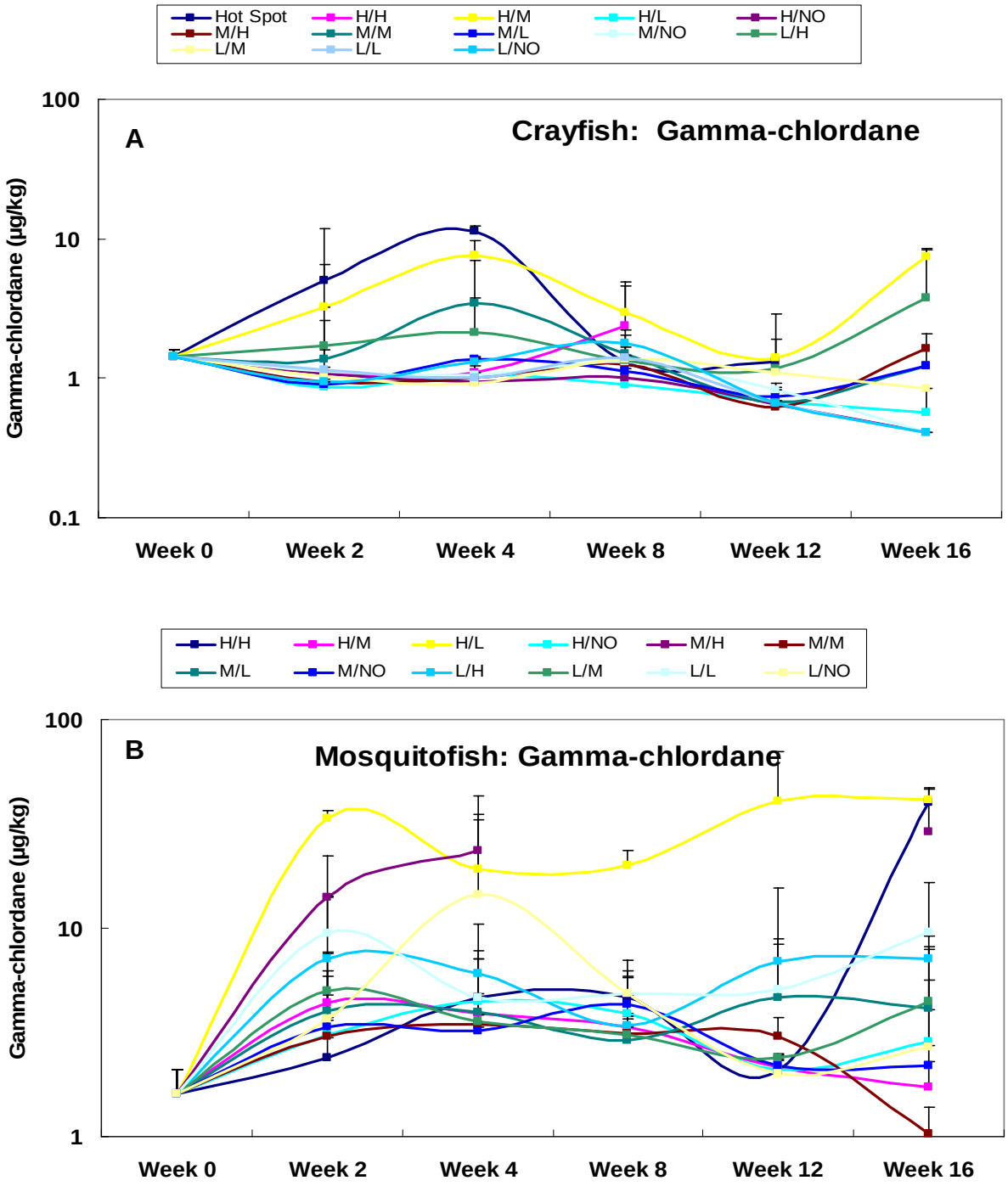


Figure 6-20A. Mean ± SD of gamma-chlordane non-lipid-normalized concentrations (µg/kg, ww) in crayfish (A) and mosquitofish (B) over the length of the experiment (week 0 = Nov. 5 – 7, 2001 through week 16 = Feb. 25 – 27, 2002) by treatment.

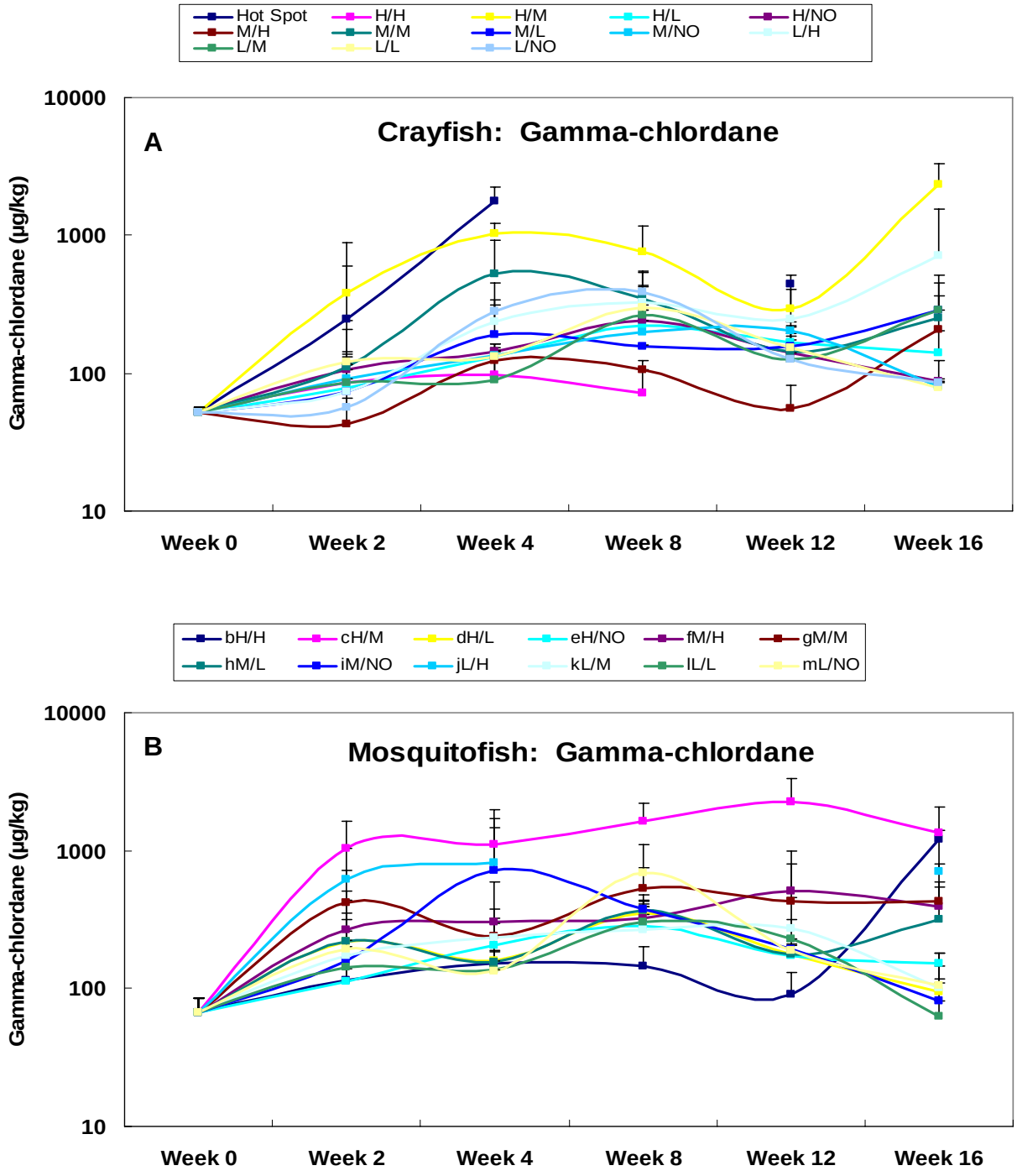


Figure 6-20B. Mean ± SD of gamma-chlordane lipid-normalized concentrations (µg/kg lipid) in crayfish (A) and mosquitofish (B) over the length of the experiment (week 0 = Nov. 5 – 7, 2001 through week 16 = Feb. 25 – 27, 2002) by treatment.

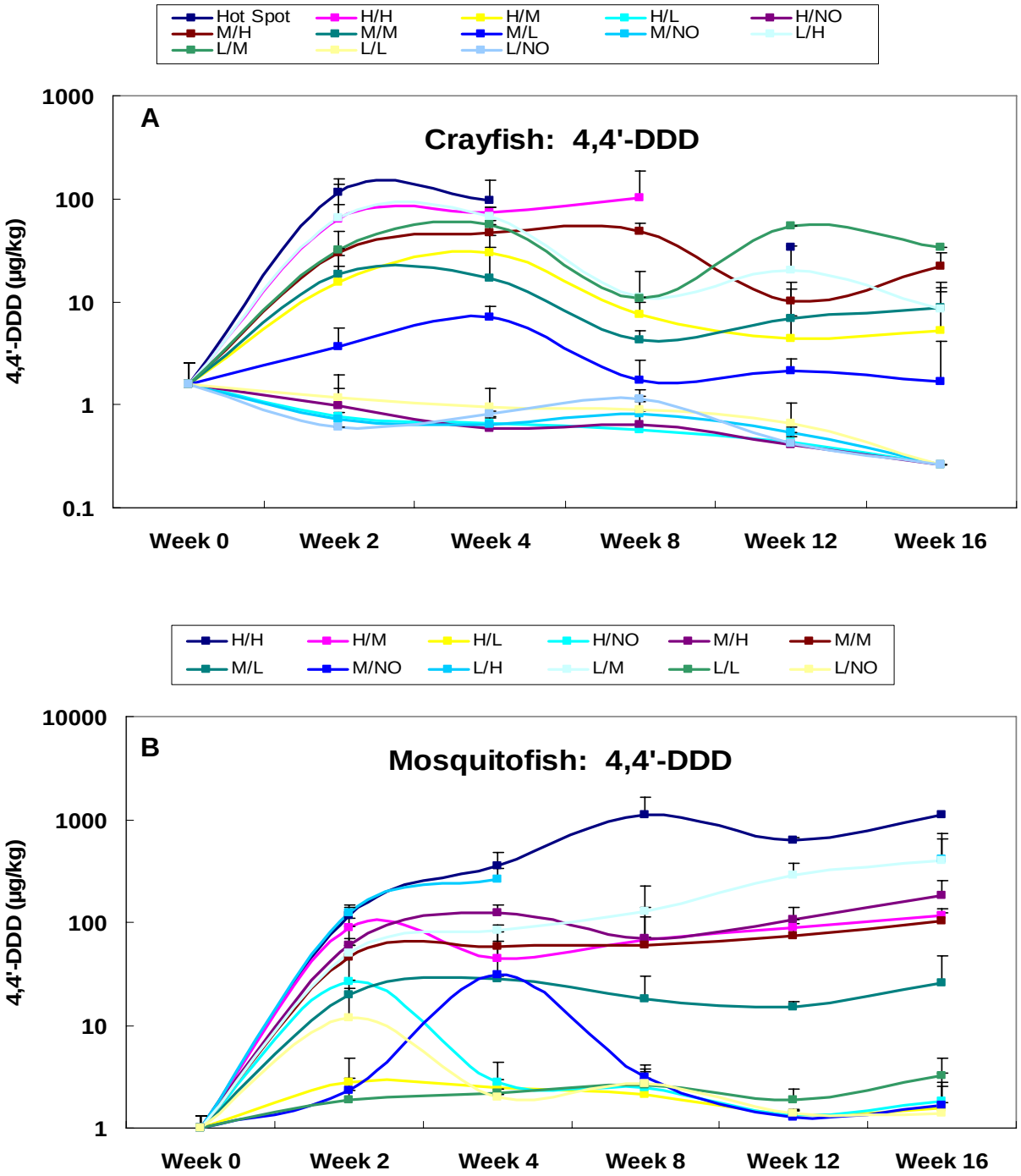


Figure 6-21A. Mean ± SD of 4,4'-DDD non-lipid-normalized concentrations (µg/kg, ww) in crayfish (A) and mosquitofish (B) over the length of the experiment (week 0 = Nov. 5 – 7, 2001 through week 16 = Feb. 25 – 27, 2002) by treatment.

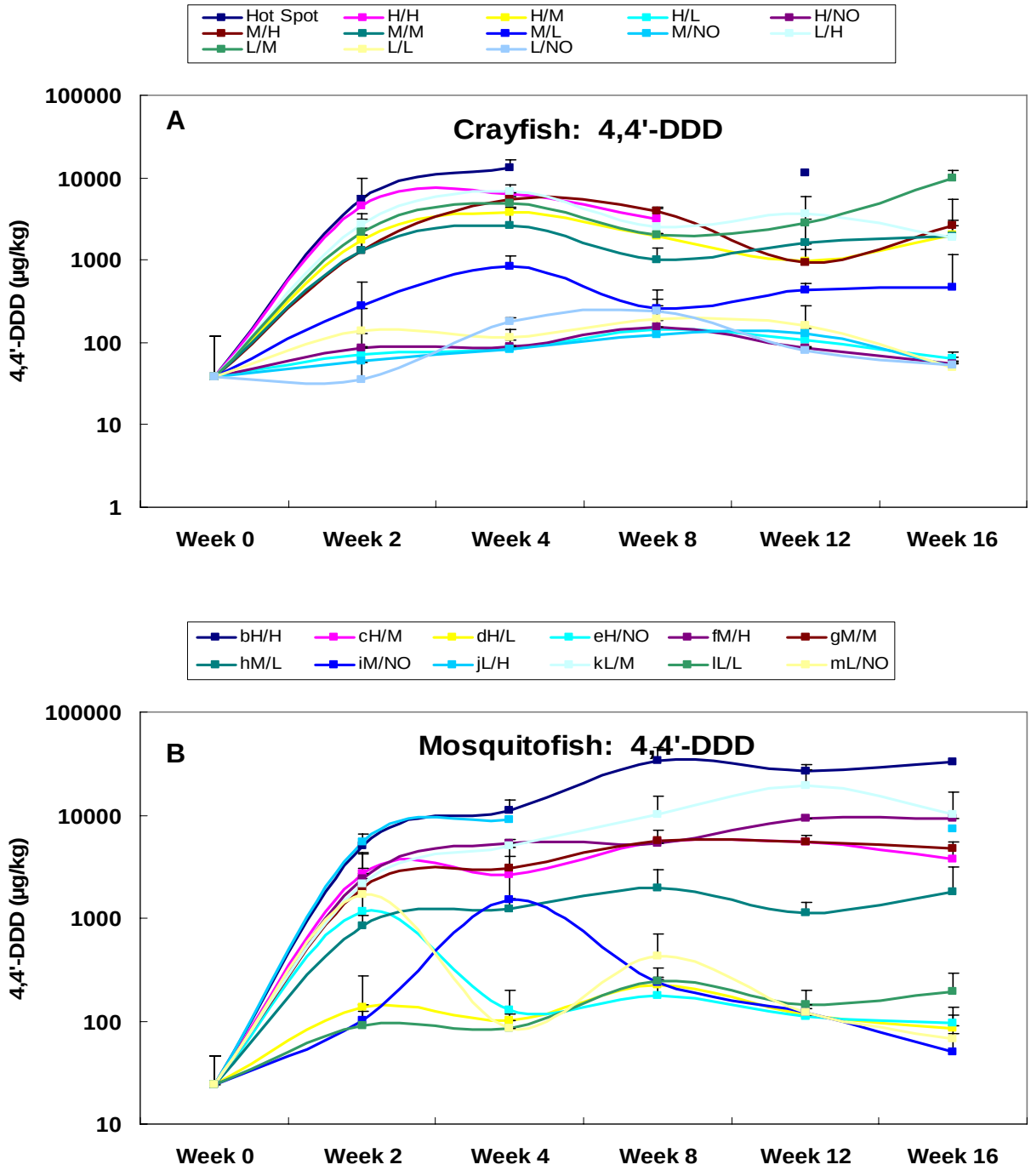


Figure 6-21B. Mean ± SD of 4,4'-DDD lipid-normalized concentrations (µg/kg lipid) in crayfish (A) and mosquitofish (B) over the length of the experiment (week 0 = Nov. 5 – 7, 2001 through week 16 = Feb. 25 – 27, 2002) by treatment.

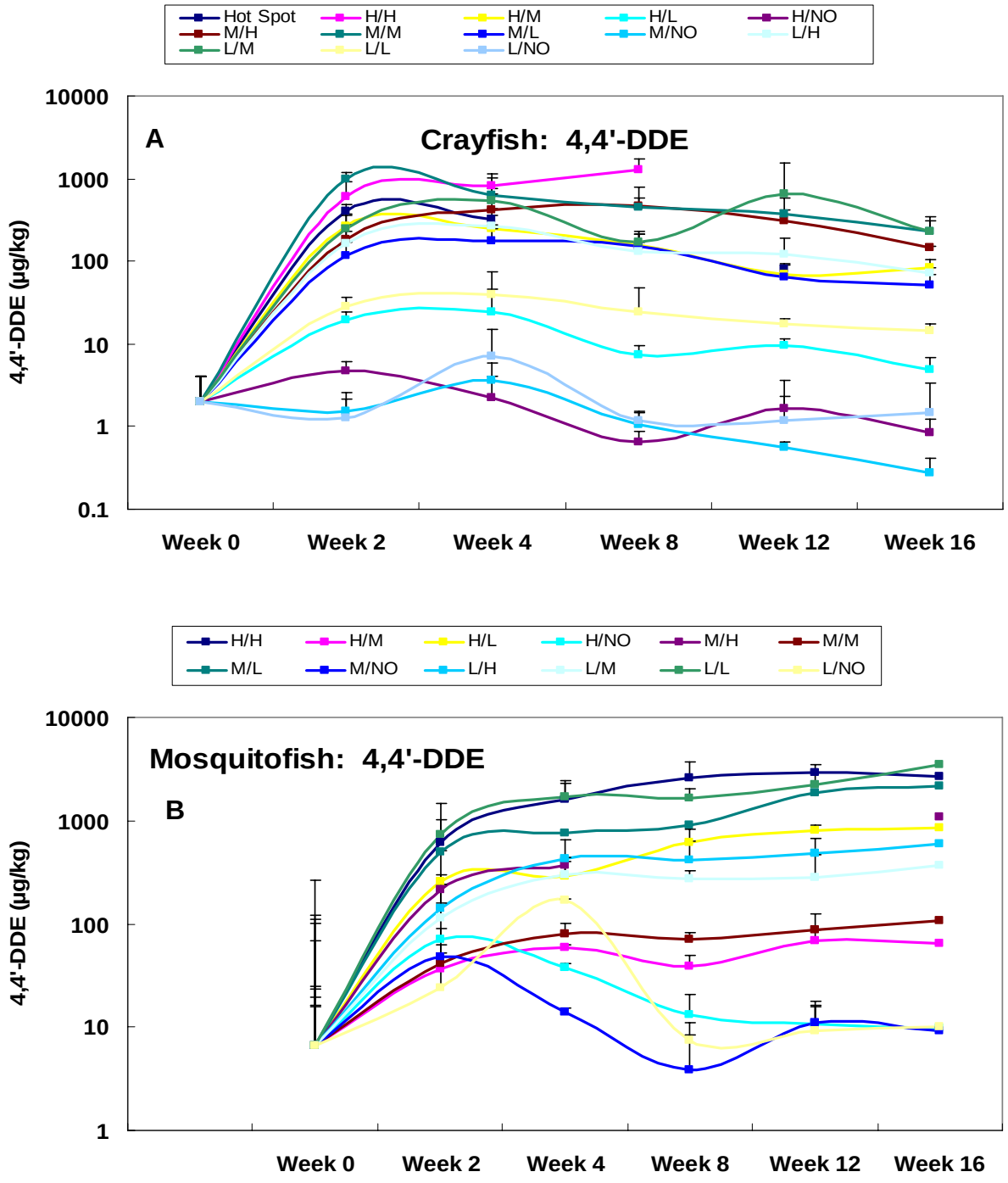


Figure 6-22A. Mean ± SD of non-4,4'-DDE non-lipid-normalized concentrations (µg/kg, ww) in crayfish (A) and mosquitofish (B) over the length of the experiment (week 0 = Nov. 5 – 7, 2001 through week 16 = Feb. 25 – 27, 2002) by treatment.

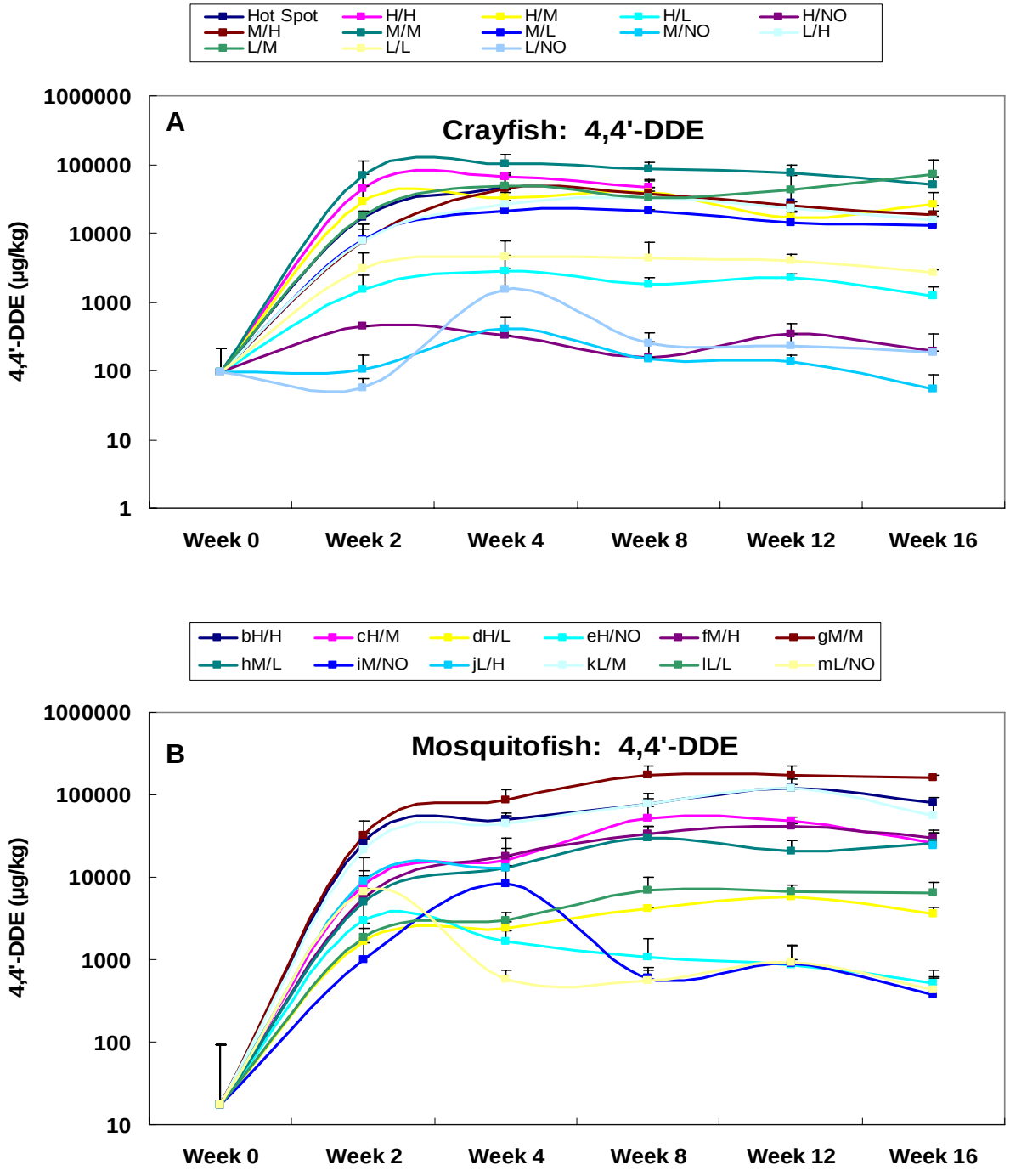


Figure 6-22B. Mean ± SD of 4,4'-DDE lipid-normalized concentrations (µg/kg lipid) in crayfish (A) and mosquitofish (B) over the length of the experiment (week 0 = Nov. 5 – 7, 2001 through week 16 = Feb. 25 – 27, 2002) by treatment.

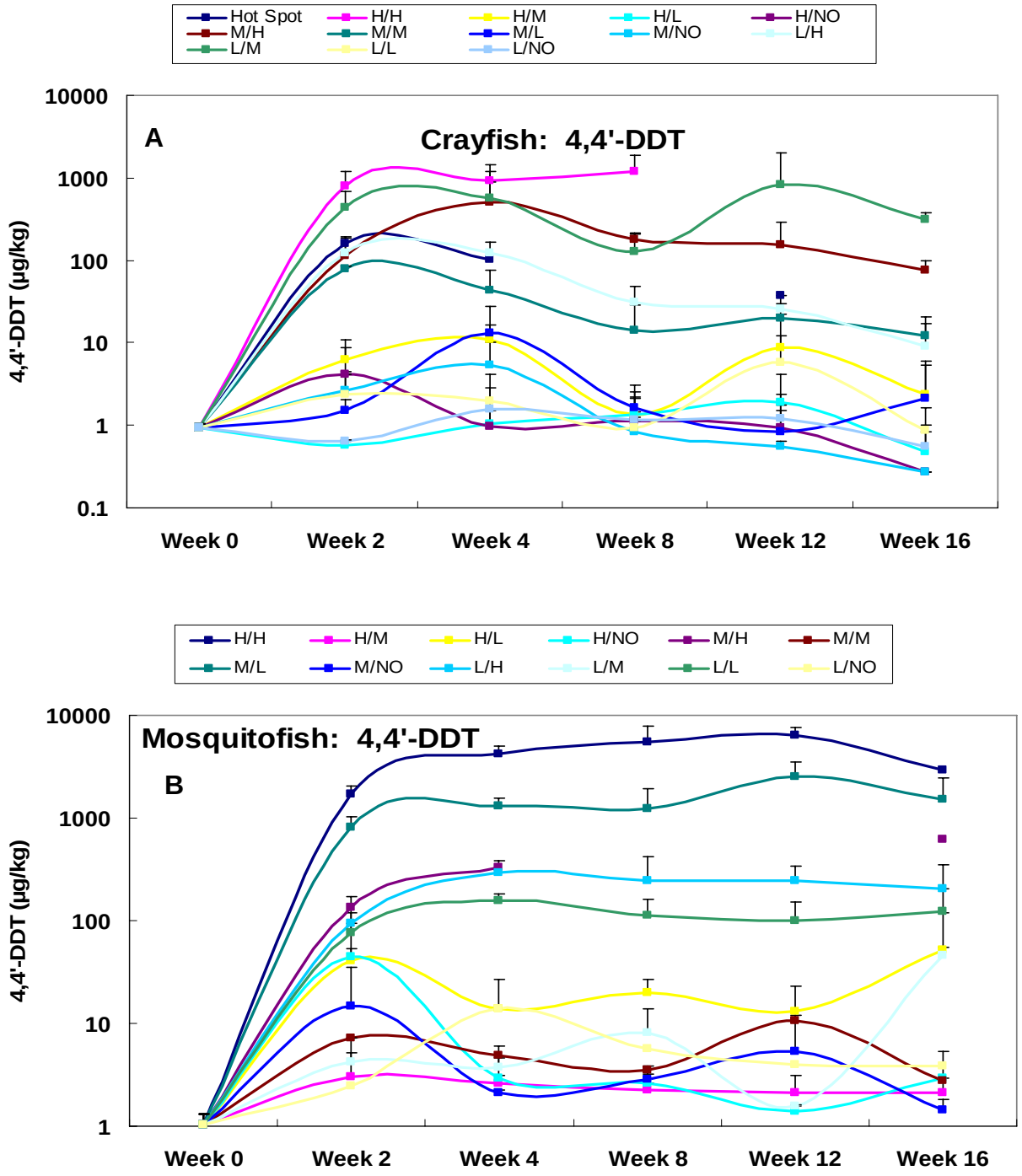


Figure 6-23A. Mean ± SD of 4,4'-DDT non-lipid-normalized concentrations (µg/kg, ww) in crayfish (A) and mosquitofish (B) over the length of the experiment (week 0 = Nov. 5 – 7, 2001 through week 16 = Feb. 25 – 27, 2002) by treatment.

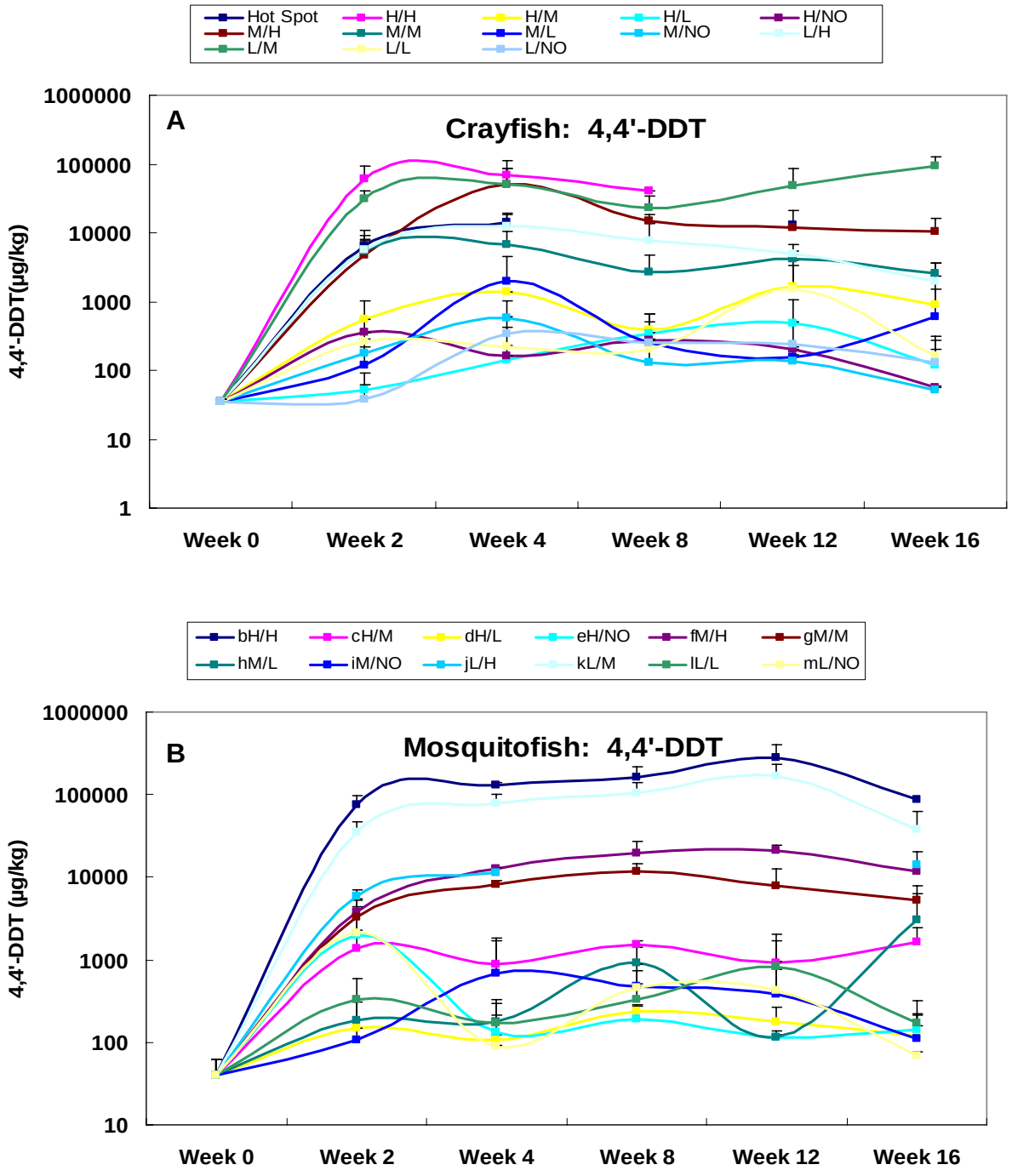


Figure 6-23B. Mean ± SD of 4,4'-DDT non-lipid-normalized concentrations (µg/kg lipid) in crayfish (A) and mosquitofish (B) over the length of the experiment (week 0 = Nov. 5 – 7, 2001 through week 16 = Feb. 25 – 27, 2002) by treatment.

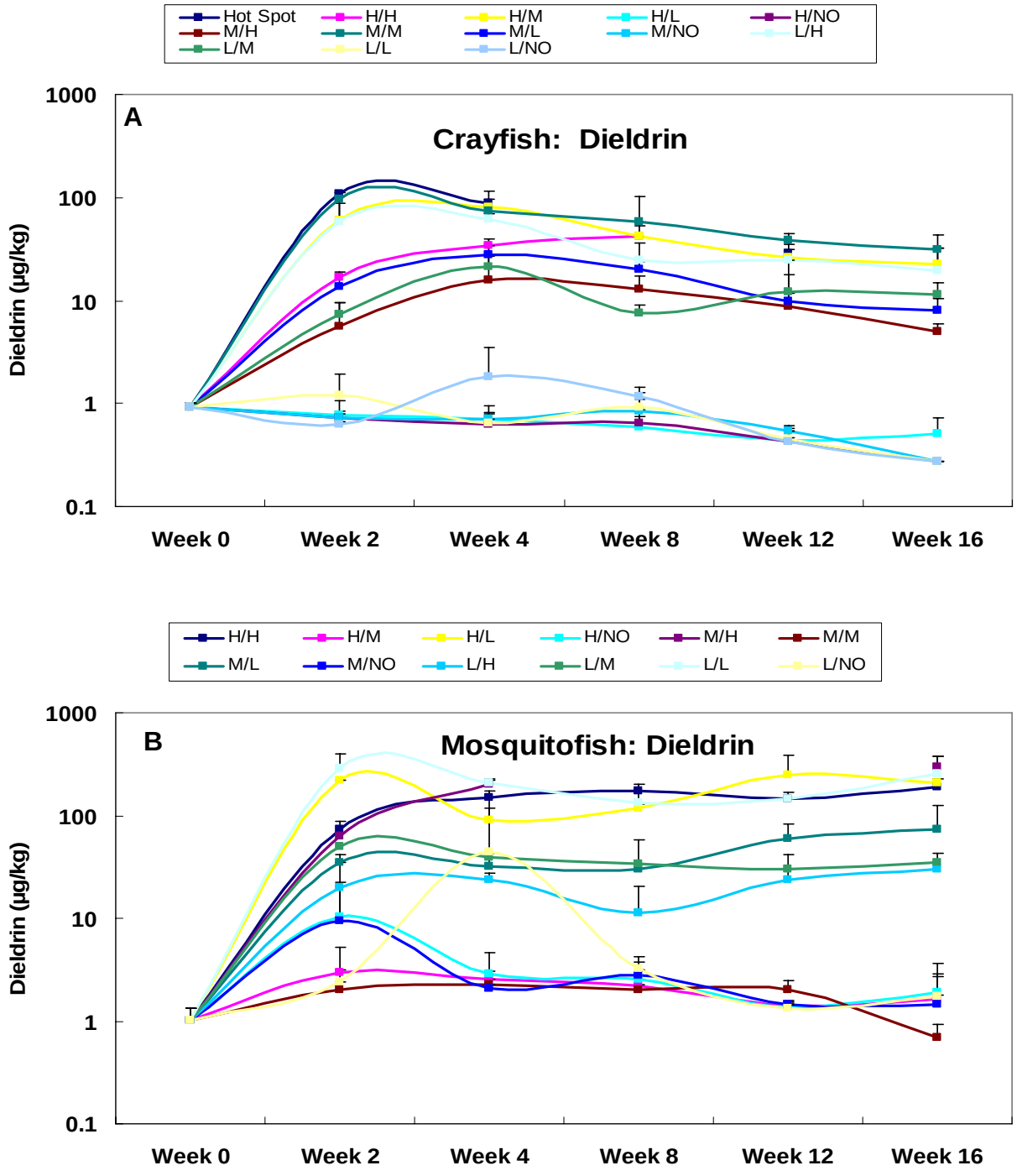


Figure 6-24A. Mean ± SD of dieldrin non-lipid-normalized concentrations (µg/kg, ww) in crayfish (A) and mosquitofish (B) over the length of the experiment (week 0 = Nov. 5 – 7, 2001 through week 16 = Feb. 25 – 27, 2002) by treatment.

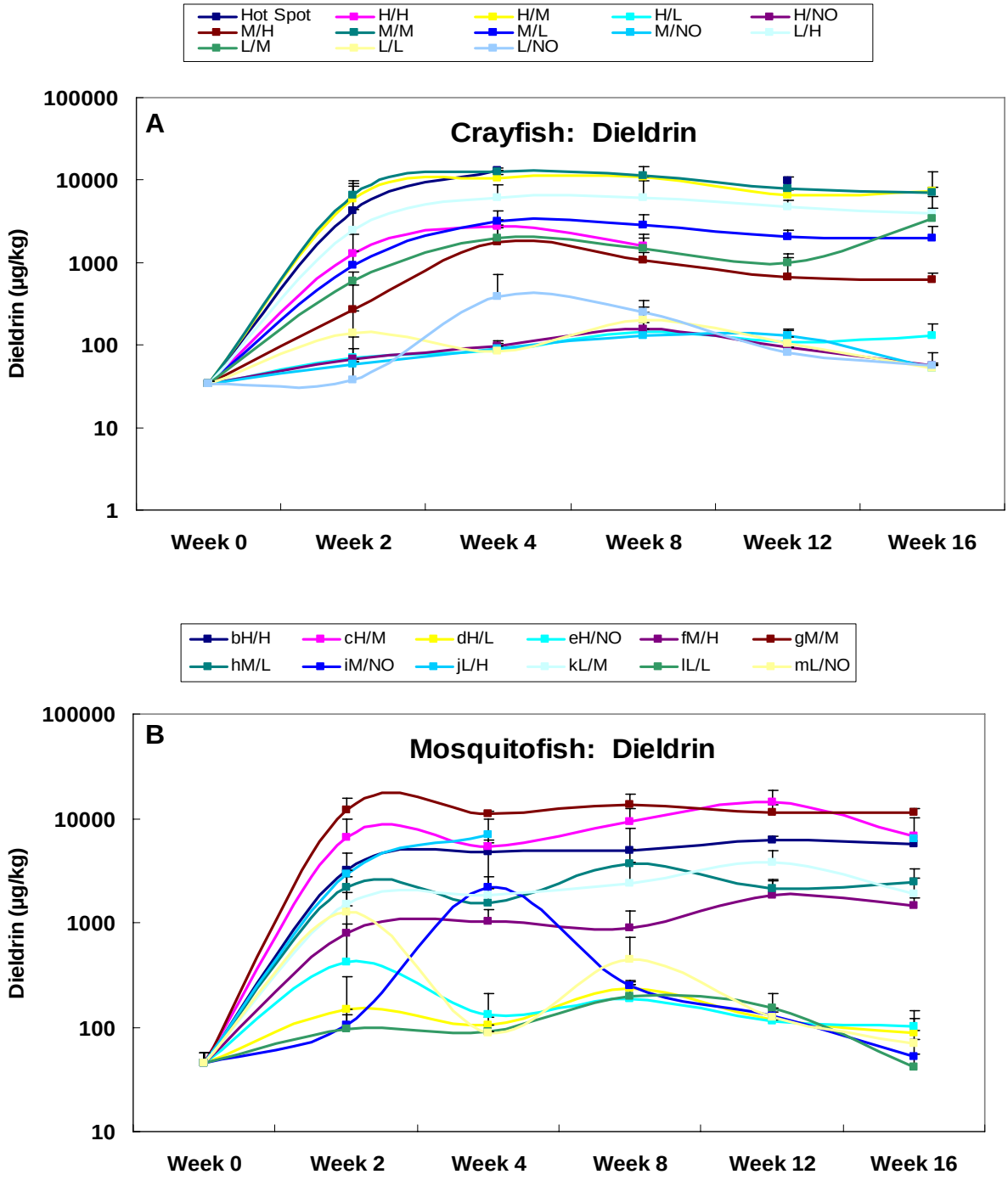


Figure 6-24B. Mean ± SD of dieldrin lipid-normalized concentrations (µg/kg lipid) in crayfish (A) and mosquitofish (B) over the length of the experiment (week 0 = Nov. 5 – 7, 2001 through week 16 = Feb. 25 – 27, 2002) by treatment.

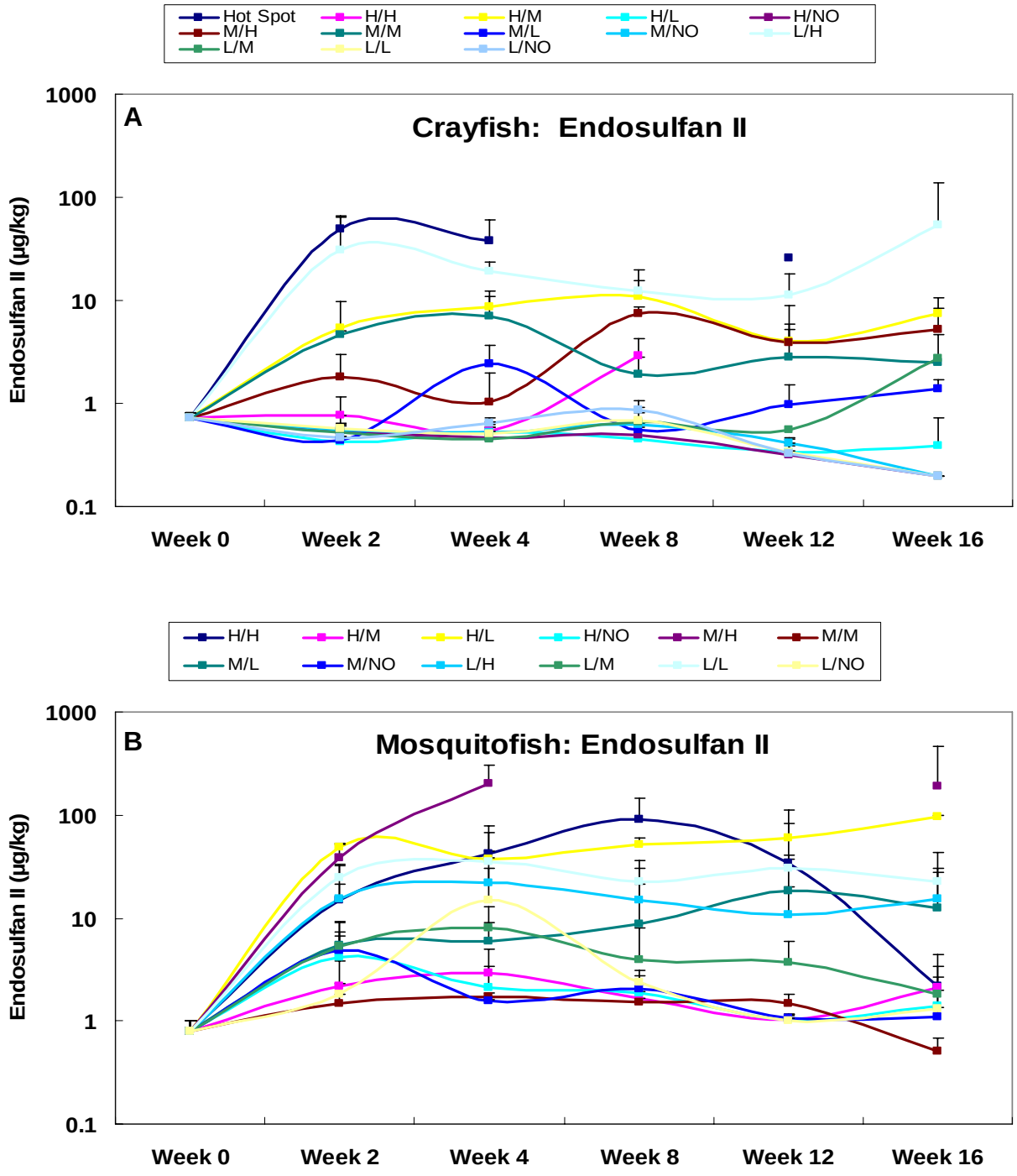


Figure 6-25A. Mean ± SD of endosulfan II non-lipid-normalized concentrations (µg/kg, ww) in crayfish (A) and mosquitofish (B) over the length of the experiment (week 0 = Nov. 5 – 7, 2001 through week 16 = Feb. 25 – 27, 2002) by treatment.

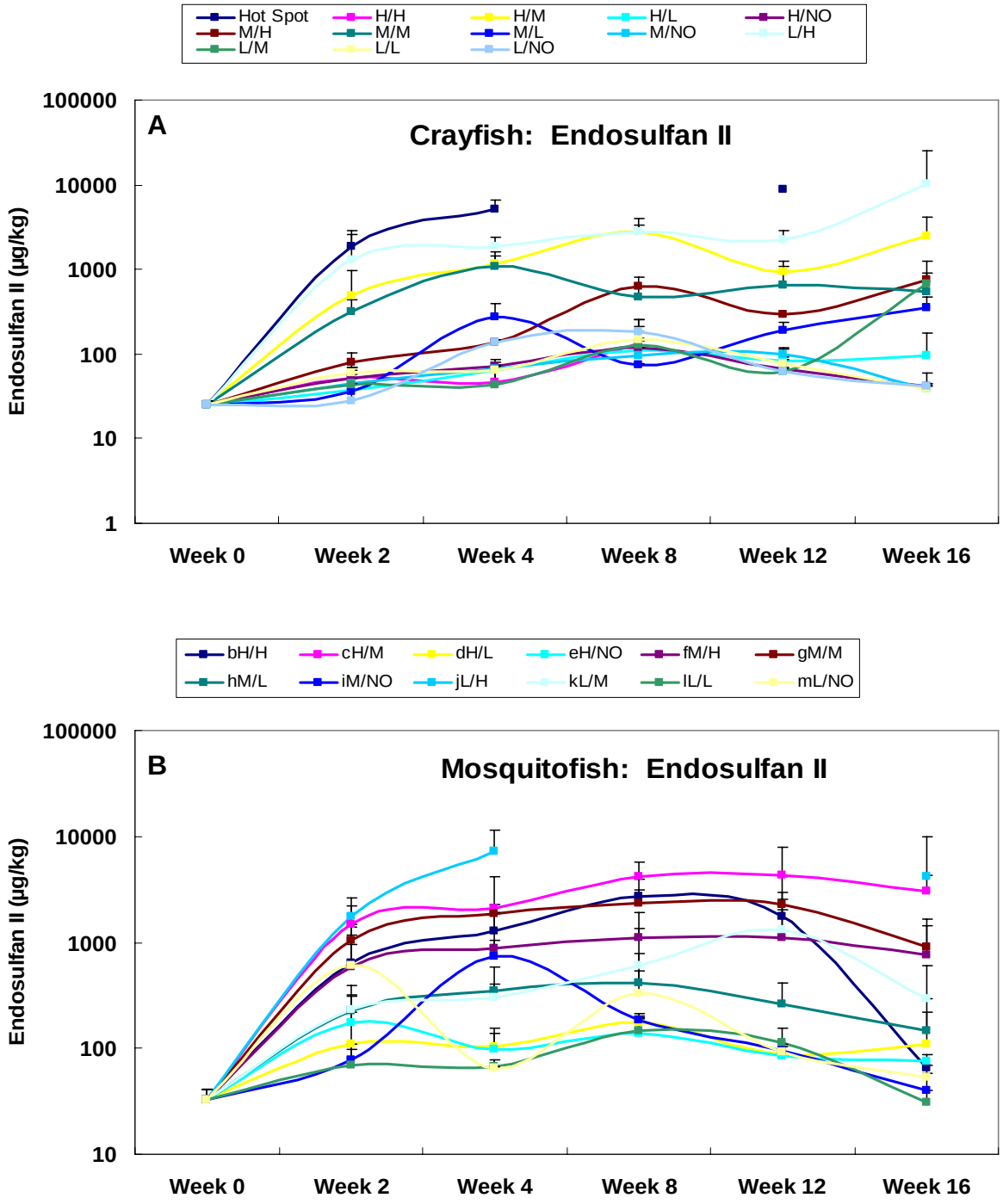


Figure 6-25B. Mean ± SD of endosulfan II lipid-normalized concentrations (µg/kg lipid) in crayfish (A) and mosquitofish (B) over the length of the experiment (week 0 = Nov. 5 – 7, 2001 through week 16 = Feb. 25 – 27, 2002) by treatment.

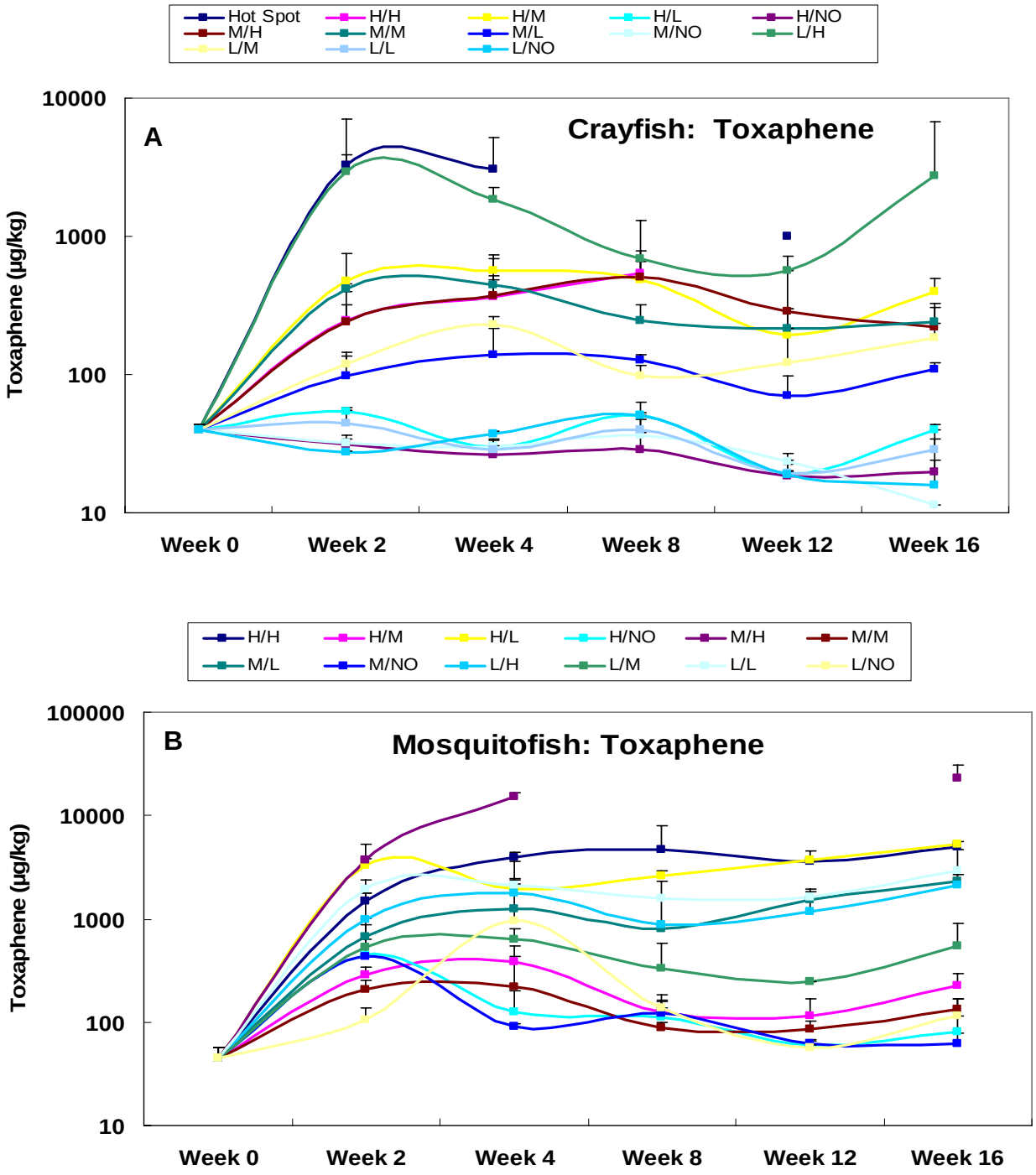


Figure 6-26A. Mean ± SD of toxaphene non-lipid-normalized concentrations (µg/kg, ww) in crayfish (A) and mosquitofish (B) over the length of the experiment (week 0 = Nov. 5 – 7, 2001 through week 16 = Feb. 25 – 27, 2002) by treatment.

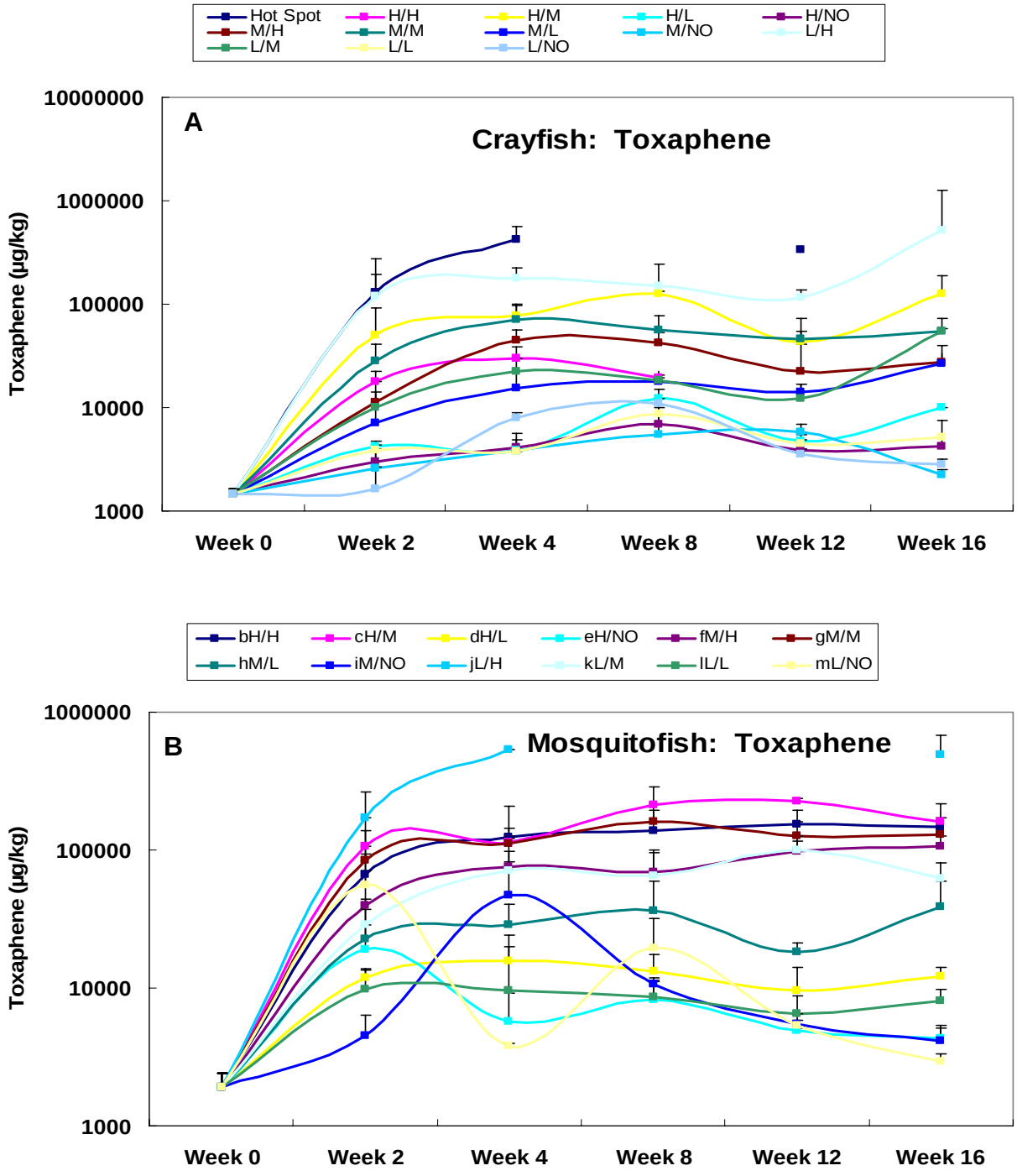


Figure 6-26B. Mean ± SD of toxaphene lipid-normalized concentrations (µg/kg lipid) in crayfish (A) and mosquitofish (B) over the length of the experiment (week 0 = Nov. 5 – 7, 2001 through week 16 = Feb. 25 – 27, 2002) by treatment.

CHAPTER 7
RESULTS AND DISCUSSION: BIOTA SEDIMENT ACCUMULATION FACTORS IN MOSQUITOFISH AND CRAYFISH

This chapter will summarize biota sediment accumulation factors (BSAFs) for crayfish and mosquitofish. As explained in detail in Chapter 2, BSAFs were calculated by dividing OCP lipid-normalized values from biota by their respective soil OCP TOC-normalized values. For OCP values in biota, it was assumed steady state had occurred by 8 weeks of exposure, and thus the average of OCP values attained between weeks 8 and 16 was used in these calculations. For OCP values in soil, the average observed between weeks 0 and 16 was used. These values were averaged, since very few significant differences in soil OCP concentrations were observed between these two sampling points (Chapter 4). Because BSAFs are ratios, concentrations equal to half the MDL have no information value in these calculations, and thus values with a "U" qualifier were excluded from these analyses.

To more easily determine the relationship between soil TOC, soil OCP and tissue OCP concentrations, a theoretical Accumulation Potential (AP) was also calculated. This is simply the ratio between the OCP concentrations in whole tissue to those observed in whole soil. If a chemical has an AP of 2, for instance, it means that it will be found in tissues at twice the concentration to that in soils independent of lipid and

organic carbon content. Regression equations were developed that will assist in future calculations of AP based on soil TOC values. Estimations of BSAFs (dependent variable) based on soil TOC values (independent variable) would be inherently biased because BSAFs are calculated using soil TOC values. In other words, there is autocorrelation between the dependent and independent variables.

Similar to previous chapters, data analysis was focused only on a subset of OCPs (i.e. 4,4'-DDD, 4,4'-DDE, and 4,4'-DDT; alpha and gamma-chlordane; dieldrin; endosulfan II; and toxaphene) (see Chapter 2 for a justification on why these OCPs were chosen for a more detailed analysis). Nevertheless, summary Tables (7-1 and 7-2) are given with BSAFs on the remaining chemicals analyzed in this study.

Biota Sediment Accumulation Factors

Effect of Species on BSAF

Biota sediment accumulation factors are presented for the OCPs of interest in Figures 7-1 through 7-8, by biota type and treatment. Overall, BSAFs were higher (1.5 to 2.6 times) in mosquitofish compared to crayfish. Exceptions to this pattern were observed with dieldrin, gamma-chlordane, and 4,4'-DDT for which no significant differences were found between the two biota types.

Overall BSAFs by chemical and biota type are summarized in Figure 7-9. When all treatments were combined, mosquitofish showed again higher BSAFs compared to crayfish, regardless of treatment. Higher BSAFs in mosquitofish agree with previous results on OCP bioaccumulation values reported in Chapter 6.

The effect of species on BSAF depends on several factors, including routes of exposure and amount of lipid contents in tissues. Crayfish are mostly benthic organisms, spending most of their time in contact with sediments, and thus are

potentially getting exposed to chemicals not only through direct contact but also through ingestion. In addition, crayfish are at a higher level in the food chain compared to mosquitofish, and thus the possibility of biomagnification is higher in this species. Because of these factors, we had hypothesized that BSAFs would be higher in crayfish compared to mosquitofish. However, the opposite was true, and mosquitofish accumulated higher concentrations of pesticides (Chapter 6) which was translated into higher BSAFs. As already discussed in the previous Chapter, crayfish had almost half the amount of body lipids compared to mosquitofish, and it declined constantly over the course of the experiment. This could have been an indication of stress and general lack of adaptation to captive conditions compared to mosquitofish. At this point, we can not rule out that the differences observed in BSAFs are indeed real, and that under the conditions tested here, mosquitofish will develop higher BSAFs compared to crayfish. In a previous study, mosquitofish also had much higher BSAFs when compared to several other fish species, including catfish, carp, and trout (Wong et al., 2001).

Effect of Chemical Type on BSAF

A physiochemical property known to affect BSAFs is lipophilicity illustrated by the log octanol/water partitioning coefficient or log K_{ow} (see Chapter 1). Since three distinct groupings of BSAFs values were observed in this study (i.e. chemicals with BSAFs > 3: 4,4'-DDE, dieldrin, endosulfan ketone; between 2.9 – 2.0: endosulfan's II and sulfate, 4,4'-DDD and 4,4'-DDT, methoxychlor, aldrin, endrin aldehyde, and heptachlor epoxide; and < 1.9: alpha and gamma chlordanes, toxaphene, and endosulfan I), we tested the hypothesis that these differences were being driven by the K_{ow} value of each chemical. We hypothesized that OCPs with higher K_{ow} would induce higher BSAFs in both species. The relationship between BSAF and K_{ow} is presented in Figure 7-10. As can

be seen from this Figure, log K_{ow} was not a driving factor in the BSAFs attained. The lack of a significant relationship could be attributed to the relatively low range of log K_{ow} in this study (from 3.7 for endosulfan sulfate to 6.8 for 4,4'-DDT). In addition, it is worth noting that the K_{ow} values used for this regression analysis were derived from literature values obtained from reagent grade chemicals.

Differences in mean BSAFs among the different OCPs studied (all treatments combined) are summarized in Figures 7-11 and 7-12 for mosquitofish and crayfish, respectively. In mosquitofish, the highest BSAF was observed with 4,4'-DDE (Figure 7-11). The BSAF for 4,4'-DDE (6.9) was significantly higher to those observed in 4,4'-DDT, toxaphene, endosulfan II, and chlordanes. In this species, the second highest BSAF was observed with dieldrin (5.7), which was also significantly higher to all other OCPs, except the DDTs (Figure 7-11). Alpha-chlordane showed the lowest BSAF (1.2). Similarly to what was observed in mosquitofish, the highest BSAF in crayfish was also recorded for 4,4'-DDE (4.2), which was significantly higher when compared to 4,4'-DDT, 4,4'-DDD, and toxaphene (Figure 7-12). As already discussed, in crayfish, the BSAFs values were lower and more variable to those observed in mosquitofish, which could explain the few significant differences observed in this species.

Interestingly, the OCPs with highest BSAF in this study were metabolites of 4,4'-DDT, namely 4,4'-DDE and 4,4'-DDD. The reason behind the higher BSAFs for metabolites as compared to that of parent compounds remains unknown at this time.

Effects of Treatment on BSAF

Biota sediment accumulation factors are plotted in relation to treatment in Figures 7-1 through 7-8. As expected, BSAFs could not be calculated for most of the treatments with "NO" soil OCPs. An extremely high BSAF value for the H/NO

treatment, however, was calculated for toxaphene (BSAF of 11.8, Figure 7-8A) and needs revision. On the other hand, treatments with "LOW" soil OCP, produced enough biota OCP values with no "U" qualifiers which allowed for calculation of BSAFs.

With the exception of 4,4'-DDT (Figure 7.5) and endosulfan II (Figure 7-7), BSAFs were significantly affected by treatment. In most instances, highest BSAFs were observed in treatments with either "LOW" or "MEDIUM" soil TOC. For example, treatment M/L induced highest BSAFs values for gamma-chlordane (BSAF of 3.4 vs. 0.5 remaining treatments, Figure 7-2B); 4,4'-DDE (8.7 vs.1.1, Figure 7-4), and dieldrin (7.5 vs. 0.9, Figure 7-6B). For 4,4'-DDD, the highest BSAF value was observed in treatment L/M (4.4 vs. 0.6, Figure 7-3B), and in toxaphene, if the abnormally high BSAF value from the H/NO treatment is excluded, highest BSAFs were found in the L/H tanks (3.3 vs. 1.6, Figure 7-8B). Alpha-chlordane followed a different pattern, with highest BSAFs observed in the H/H treatment (4.7 vs. 0.3, Figure 7-1B).

Relationship Between BSAFs and Soil TOC and OCPs

The relationship between BSAFs and soil TOC and OCPs are presented in Figures 7-13 through 7-28. For these analyses, relationships were fitted to either linear or second-order polynomial curves, whichever gave the largest R2 value. The highest R^2 values for BSAFs and TOC were observed for alpha-chlordane and 4,4'-DDD in crayfish (R^2 = 0.6) (Figures 7-13A and 7-17A, respectively). Intermediate R2 values (0.2 – 0.4) were observed for gamma-chlordane in both species (Figures 7-15A and 7-16A); 4,4'-DDD in mosquitofish (Figure 7-18A); 4,4'-DDE in crayfish (Figure 7-19A); and 4,4'-DDT (Figures 7-21A and 7-22A), dieldrin (Figures 7-23A and 7-24A), and toxaphene (Figures 7-27A and 7-28A) for both species. Statistically significant relationships (P<.05) were not detected or observed between BSAF and TOC for

alpha-chlordane and 4,4'-DDE in mosquitofish (Figures 7-14A and 7-20A, respectively), and for endosulfan II in both species (Figures 7-25A and 7-26A).

For both species, relationships among BSAFs and soil OCP were characterized only by intermediate R2 values (0.2 – 0.4) and these included: alpha chlordane (Figures 7-15B and 7-16B); 4,4'-DDD (Figures 7-17B and 7-18B); endosulfan II (Figures 7-25B and 7-26B); and toxaphene (Figures 7-27B and 7-28B). No significant relationships were observed between BSAF and soil OCP for alpha-chlordane (Figures 7-13B and 7-14B); 4,4'-DDE (Figures 7-19B and 7-20B); 4,4'-DDT (Figures 7-21B and 7-22B); and dieldrin (Figures 7-23B and 7-24B).

Relationship Between AP and Soil TOC

The relationship between AP and soil TOC for the OCPs of interest are presented in Figures 7-29 through 7-36. In addition, Tables 7-3 and 7-4 summarize the results from the regression analyses between AP and soil TOC for all chemicals studied. If we consider an α value of 0.05, there was a significantly negative relationship between soil TOC and AP for the majority of the OCPs studied. Exceptions were endosulfan sulfate and endrin aldehyde in crayfish (Table 7-3); and alpha-chlordane, aldrin, heptachlor epoxide, and methoxychlor in mosquitofish (Table 7-4).

In aquatic systems, the TOC fraction of sediments acts as a major repository for organic contaminants. As already discussed in Chapter 1, there is information suggesting that high TOC in sediments can result in lower BSAFs. For example, Ferraro et al. (1990) reported that bivalves inhabiting polluted sediments containing "high" organic content (> 3.7 %) had lower BSAF values (< 2) compared to bivalves collected from sediments with low TOC (< 0.86%). A similar trend was described by Lake et al. (1990), in that invertebrates collected from high TOC sediments had lower

BSAFs of PCBs. These authors hypothesized that this decline in BSAFs with increased TOC in sediment was a reflection of an increased sorption of contaminants by the organics present in the sediments. In a laboratory study, Nebeker et al. (1989) observed a decreased toxicity of spiked DDT soils to *Hyalella azteca* when the organic content of the soils was increased (from 3.0 to 10.5% TOC). It is important to mention, however, that the majority of BSAFs reported in the literature have been developed from sites containing sediments low in TOC (~ 10% or below). Based on the results obtained in the present study, it appears that soil TOC is an important factor driving the amount of bioaccumulation in biota, regardless of species and type of OCP.

Comparison of BSAFs Reported in this Study to Values Reported in the Literature

The range of values for BSAFs reported in the literature are generally highly variable and above the theoretical estimates of 1 to 2 (US EPA, 2000a) (see Table 7-5 for a summary compiled by the US Army Corp of Engineers on BSAFs calculated for many benthic and pelagic fish species). An explanation for this discrepancy is that besides “bioaccumulation”, OCPs are also being ”biomagnified” through the food chain. This would result in chemical concentrations in excess of the equilibrium concentrations expected from direct digestion. In addition, several other factors relating to contaminant kinetics, rates of metabolism, reproductive status, and lipid contents can cause errors in the estimation of bioaccumulation rates (Morrison et al., 1996; Wong et al., 2001).

Many BSAF values obtained in the present study were also high and tended to exceed the theoretical limit (Figure 7-9). This large range in BSAFs across studies may limit the use of this model as the only method for screening the bioaccumulative potential of sediments, and strengthens the need for determination of specific BSAF

values on an analyte by analyte, and species by species basis to be optimal. Nevertheless, the BSAFs reported in the present study, fall within ranges previously reported for other species and, despite all of the uncertainties, these values could still be used for estimating exposure of OCPs by fish-eating birds inhabiting NSRA flooded marshes. Furthermore, BSAFs in the present study were fairly consistent (regardless of percent TOC) which strengthens its use as a predictive model for determining bioaccumulation in biota at a wide range of sites within the NSRA.

Table 7-1. Summary of mean BSAFs (standard deviation) in crayfish, by analyte and treatment. BSAFs were calculated by dividing whole crayfish OCP lipid-normalized concentrations attained between weeks 8 and 16 of exposure, by sediment TOC-normalized OCP concentrations (average of weeks 0 and 16). OCP values with a "U" qualifier were not included in these analyses.

Analyte	Hot Spot	H/H	H/M	H/L	H/NO[a]	M/H	M/M	M/L	M/NO[a]	L/H	L/M	L/L	L/NO	Grand Total
4,4'-DDD	1.77	0.63 (0.3)	0.88 (0.7)	1.26 (0.7)		0.85 (0.4)	1.42 (0.7)	1.29 (1.2)		2.76 (1.3)	2.41 (1.8)	0.88 (1.1)		1.55
4,4'-DDE	2.88	1.82 (0.3)	3.52 (6.9)	1.85 (0.6)		5.52 (4.2)	3.53 (1.2)	6.62 (3.4)		10.03 (6.9)	5.02 (3.3)	2.37 (1.5)	1.59	4.65
4,4'-DDT	1.05	0.44 (0.02)	1.34 (1.8)	1.54 (1.3)		1.54 (0.7)	1.03 (0.6)	2.24 (1.8)		2.14 (1.6)	1.57 (1.3)	4.54 (4.2)	1.73	1.81
alpha-Chlordane	0.06	0.10	0.91 (0.7)	1.12 (0.9)		0.40 (1.8)	0.58 (0.5)	2.19 (1.8)		1.74 (1.5)	1.98 (2.1)	0.98 (0.3)		1.18
gamma-Chlordane	0.65		0.88 (0.7)	2.24 (1.2)		0.74 (0.5)	0.95 (0.3)	3.31 (1.9)		2.08 (1.7)		3.62 (3.6)		1.72
Aldrin			1.45 (1.4)							2.63 (1.6)				1.92
Dieldrin	1.57	1.24 (0.4)	3.24 (1.2)	1.96 (1.4)		1.35 (0.7)	4.57 (2.0)	7.05 (5.1)		6.14 (4.0)	5.31 (3.4)	0.19		4.26
Endrin aldehyde			1.64 (0.6)	2.4 (1.1)										2.02
Endrin ketone			1.73 (1.1)	6.22 (2.4)		1.72 (1.0)	1.29	7.00 (3.5)				2.05		3.67
Endosulfan I			0.26 (0.02)											0.26
Endosulfan II	0.94	0.24	1.30 (0.7)	1.23 (0.5)		0.88 (0.9)	1.10 (0.6)	1.42 (0.4)		5.36 (8.4)	1.87	0.57		1.92
Endosulfan sulfate	0.16	0.21				1.56				3.15	2.89			2.00
Heptachlor epoxide	0.70		1.28 (1.0)	4.41 (3.3)		0.84 (0.4)	2.54 (0.7)	4.40		2.03 (1.4)	3.09 (0.3)	0.95		2.14
Methoxychlor			2.72 (0.4)			1.24	1.83 (0.5)			3.44 (1.4)				2.58
Toxaphene	0.63	0.22 (0.02)	0.92 (0.4)	0.98 (0.3)		0.57 (0.3)	0.92 (0.4)	1.55 (0.9)		2.68 (4.4)	0.87 (0.7)	1.51 (0.1)		1.37

[a] No data was available for calculating BSAFs.

Table 7-2. Summary of mean BSAFs (standard deviation) in mosquitofish, by analyte and treatment. BSAFs were calculated by dividing whole mosquitofish OCP lipid-normalized concentrations attained between weeks 8 and 16 of exposure, by sediment TOC-normalized OCP concentrations (average of weeks 0 and 16). OCP values with a "U" qualifier were not included in these analyses.

Analyte	Hot Spot[a]	H/H	H/M	H/L	H/NO	M/H	M/M	M/L	M/NO[b]	L/H	L/M	L/L	L/NO	Grand Total
4,4'-DDD		5.42 (2.0)	2.21 (0.7)	0.81		2.36 (1.2)	4.32 (1.4)	3.47 (2.7)		7.64 (7.8)	6.13 (3.4)	1.59 (1.2)		3.88
4,4'-DDE		3.00 (1.0)	5.77 (2.2)	4.67 (1.2)		6.68 (4.2)	8.43 (2.1)	10.84 (5.9)		7.89 (6.5)	8.85 (4.7)	4.00 (1.2)	3.95 (0.9)	6.70
4,4'-DDT		1.77 (1.3)	1.22 (0.3)	0.81 (0.4)		2.15 (1.0)	2.3 (1.2)	6.55 (8.8)		4.51 (4.2)	2.98 (2.3)	3.25 (2.5)		2.74
alpha-Chlordane		6.25 (5.9)	1.44 (0.5)	0.61		1.34 (0.7)	1.45 (1.0)	1.95 (2.0)		7.28	2.38 (2.3)	0.64 (0.2)		2.27
gamma-Chlordane		1.87	1.25 (0.5)	1.42		2.00 (1.7)	1.66 (0.8)	3.67 (2.5)		2.59 (1.6)		2.72 (0.1)		1.99
Aldrin			2.45			3.84	4.50			2.92				3.43
Dieldrin		4.02 (1.7)	4.20 (1.4)	0.70		2.38 (1.4)	6.19 (1.5)	7.87 (4.1)		7.87 (3.4)	7.32 (3.8)	0.84		5.42
Endrin ketone		1.26	0.93 (0.6)	5.92		0.77 (0.3)	2.87 (1.9)	4.03 (0.3)						2.44
Endosulfan II		2.93 (1.5)	2.94 (1.1)	1.82		1.80 (0.8)	2.60 (1.1)	3.11 (2.0)		3.10 (10.8)	5.89			3.08
Endosulfan sulfate		1.48 (1.2)			7.61	2.21 (0.4)	4.19			1.16	9.12			3.68
Heptachlor epoxide			3.99 (3.4)	2.81 (1.8)		2.57 (2.1)	1.51 (1.3)	1.75		1.44 (1.7)	0.26			2.26
Methoxychlor		1.24 (0.5)	3.71 (0.7)			1.21 (0.3)	4.29			7.65 (3.5)	1.33 (0.8)			2.75
Toxaphene		1.55 (0.6)	1.96 (0.5)	1.08 (0.2)		1.67 (0.6)	2.39 (0.7)	2.53 (1.8)		5.30 (2.4)	2.35 (1.1)	1.53 (1.0)		2.14

[a] Very few fish survived from this treatment, and thus BSAFs could not be calculated.
[b] No data was available for calculating BSAFs.

Table 7-3. Summary of ANOVAs used to determine the relationship between crayfish Accumulation Potential (AP, dependent variable) and percent total organic carbon (TOC) in soil (independent variable). AP was calculated dividing tissue OCP by soil OCP. Regression equation was as follows: **Log AP = a (intercept) + X (Log TOC).**

CRAYFISH

Analyte	DF	Sum of Squares	F	P	a	X
alpha-Chlordane	1	20.2529	91.4847	0.000001	-5.7556	-1.3609
gamma-Chlordane	1	40.1311	62.9485	0.000002	-6.0240	-1.4348
4,4'-DDD	1	33.0169	36.8956	0.000012	-5.5787	-1.2350
4,4'-DDE	1	45.0346	67.5654	0.000000	-4.1604	-1.1204
4,4'-DDT	1	31.4106	35.7142	0.000012	-5.1941	-1.1298
Dieldrin	1	40.5234	85.1850	0.000000	-4.4823	-1.2195
Endosulfan II	1	31.5842	70.2340	0.000004	-6.2702	-1.8036
Heptachlor epoxide	1	4.1318	6.9331	0.038899	-4.1068	-0.5982
Toxaphene	1	46.2698	101.9080	0.000000	-5.7069	-1.2275

Table 7-4. Summary of ANOVAs used to determine the relationship between mosquitofish Accumulation Potential (AP, dependent variable) and percent total organic carbon (TOC) in soil (independent variable). AP was calculated dividing tissue OCP by soil OCP. Regression equation was as follows: **Log AP = a (intercept) + X (Log TOC).**

MOSQUITOFISH

Analyte	DF	Sum of Squares	F	P	a	X
alpha-Chlordane	1	16.8176	35.4241	0.000067	-4.3754	-1.2401
gamma-Chlordane	1	41.6404	150.4722	0.000000	-4.2438	-1.4615
4,4'-DDD	1	49.9851	41.8141	0.000006	-3.9107	-1.5196
4,4'-DDE	1	57.8840	160.7573	0.000000	-2.8279	-1.2702
4,4'-DDT	1	60.1370	90.6832	0.000000	-4.5209	-1.5633
Dieldrin	1	59.6498	104.9162	0.000000	-3.4341	-1.4796
Endosulfan II	1	8.9950	50.9964	0.000019	-3.3465	-0.9625
Heptachlor epoxide	1	19.1018	17.4039	0.005867	-3.6999	-1.2862
Toxaphene	1	65.0190	155.0690	0.000000	-4.1544	-1.4551

Table 7-5. Summary of grand means, min and max values for BSAFs on different fish species. Summary compiled by U. S. Army Corps of Engineers (http://www.wes.army.mil/el/bsaf/bsaf.html).

Analyte	Grand Mean	Min	Max	N
4,4'-DDD	2.20	0.108	4.4	11
4,4'-DDE	16.21	0.07	41.471	17
4,4'-DDT	1.32	0.018	5.2	14
trans-nonachlor	5.00	0.005	21	2
Dieldrin	7.61	0.64	14	13
Endosulfan sulfate	2.70	0.0009	5	2
Total Chlordane	2.38	1.53	23.2	9
Heptachlor epoxide	0.65	0.3	1	2
Methoxychlor	1.20	0.4	2	2
BHC-Alpha	0.70	0.4	1	2
BHC-Gamma	0.60	0.052	1	2
BHC-Delta	1.15	0.065	2	2

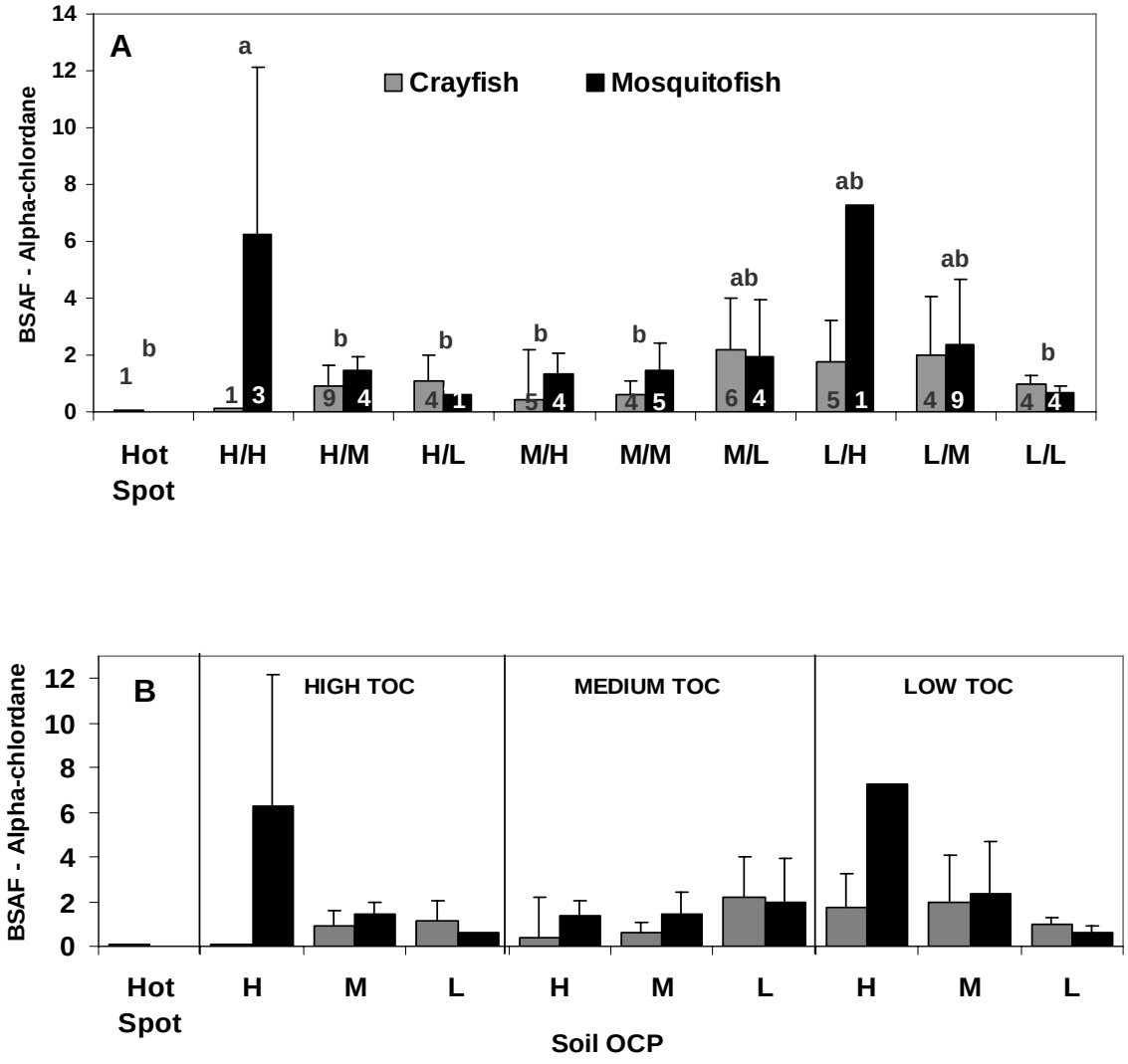


Figure 7-1. Mean ± SD of BSAFs for alpha-chlordane, by treatment and biota. Both panels are showing the same data, but in Panel B, BSAFs are grouped based on soil Total Organic Carbon (TOC). Numbers on bottom of bars represent sample sizes (i.e. number of tanks). BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16. Regardless of treatment, mosquitofish had higher BSAFs than crayfish (2-way ANOVA, p = 0.004, F= 9.3; overall means of 2.3 and 1.2 for mosquitofish and crayfish, respectively). For both species, different small letters indicate significant differences in BSAFs across treatments (2-way ANOVA, p = 0.04, F = 2.1).

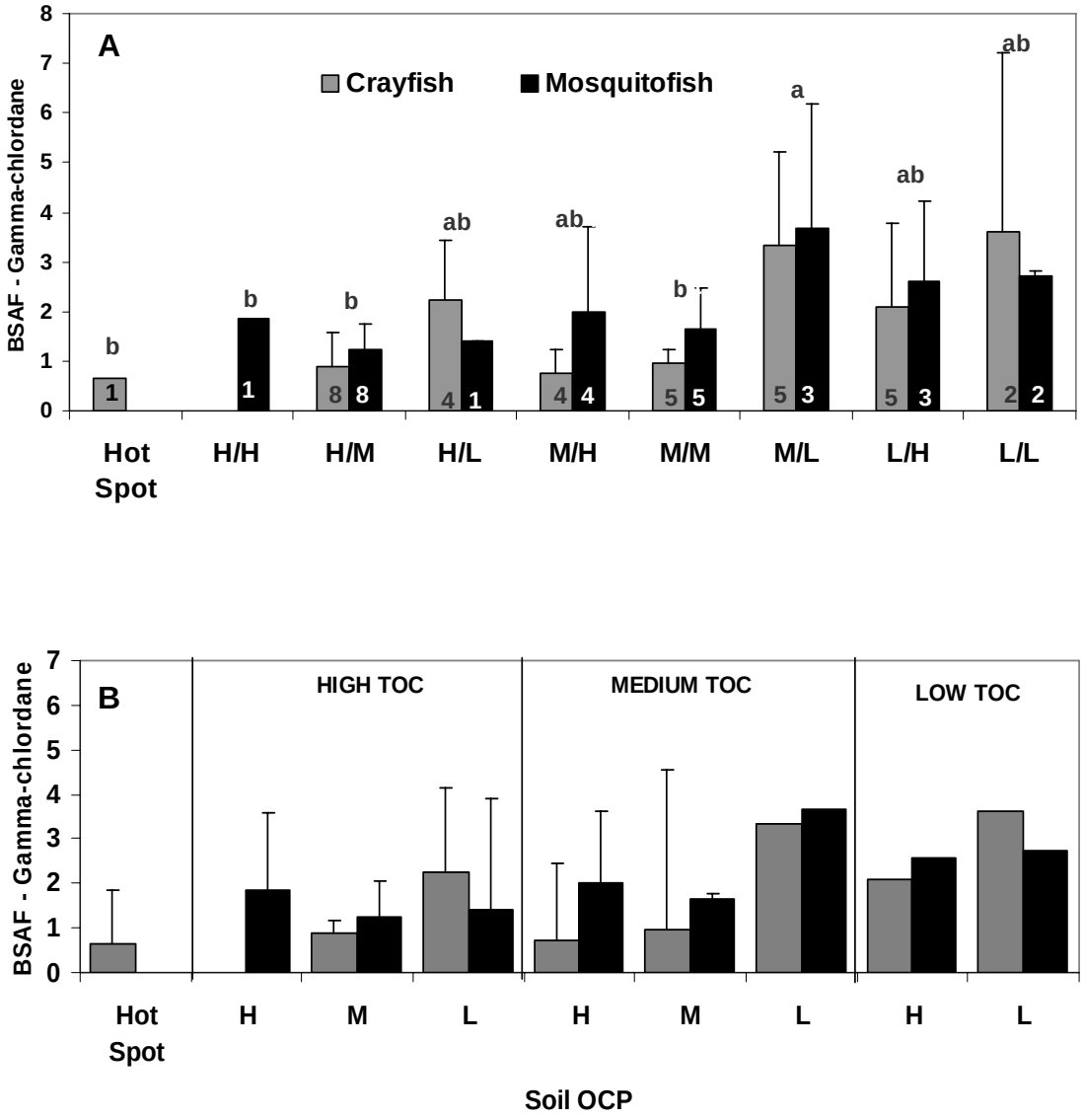


Figure 7-2. Mean ± SD of BSAFs for gamma-chlordane, by treatment and biota. Both panels are showing the same data, but in Panel B, BSAFs are grouped based on soil Total Organic Carbon (TOC). Numbers on bottom of bars represent sample sizes (i.e. number of tanks). BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16. There was no difference in BSAFs between mosquitofish and crayfish. For both species, different small letters indicate significant differences in BSAFs across treatments (2-way ANOVA, p = 0.006, F = 3.2).

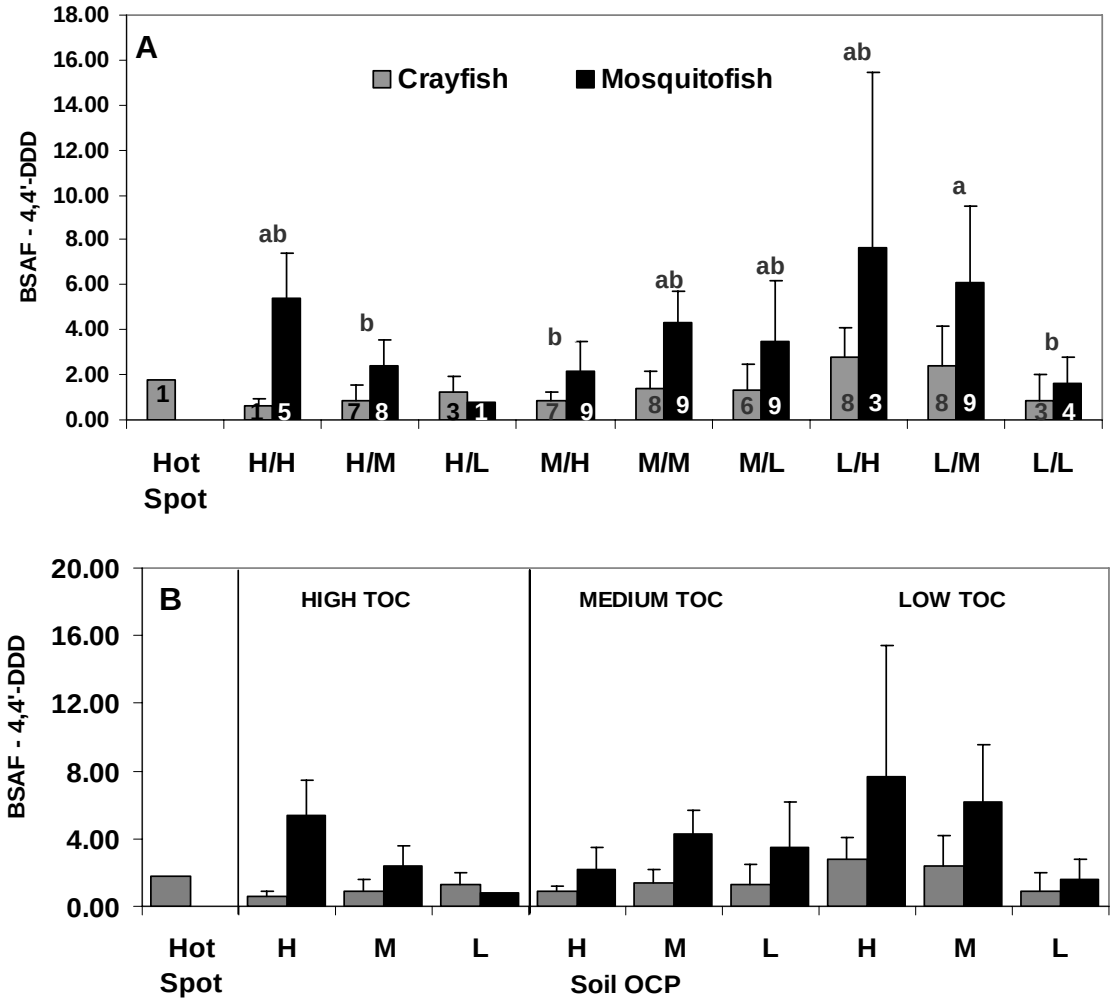


Figure 7-3. Mean ± SD of BSAFs for 4,4'-DDD, by treatment and biota. Both panels are showing the same data, but in Panel B, BSAFs are grouped based on soil Total Organic Carbon (TOC). Numbers on bottom of bars represent sample sizes (i.e. number of tanks). BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16. Regardless of treatment, mosquitofish had higher BSAFs than crayfish (2-way ANOVA, $p < 0.0001$, $F = 26$; overall means of 3.9 and 1.5 for mosquitofish and crayfish, respectively). For both species, different small letters indicate significant differences in BSAFs across treatments (2-way ANOVA, $p < 0.0001$, $F = 4.4$).

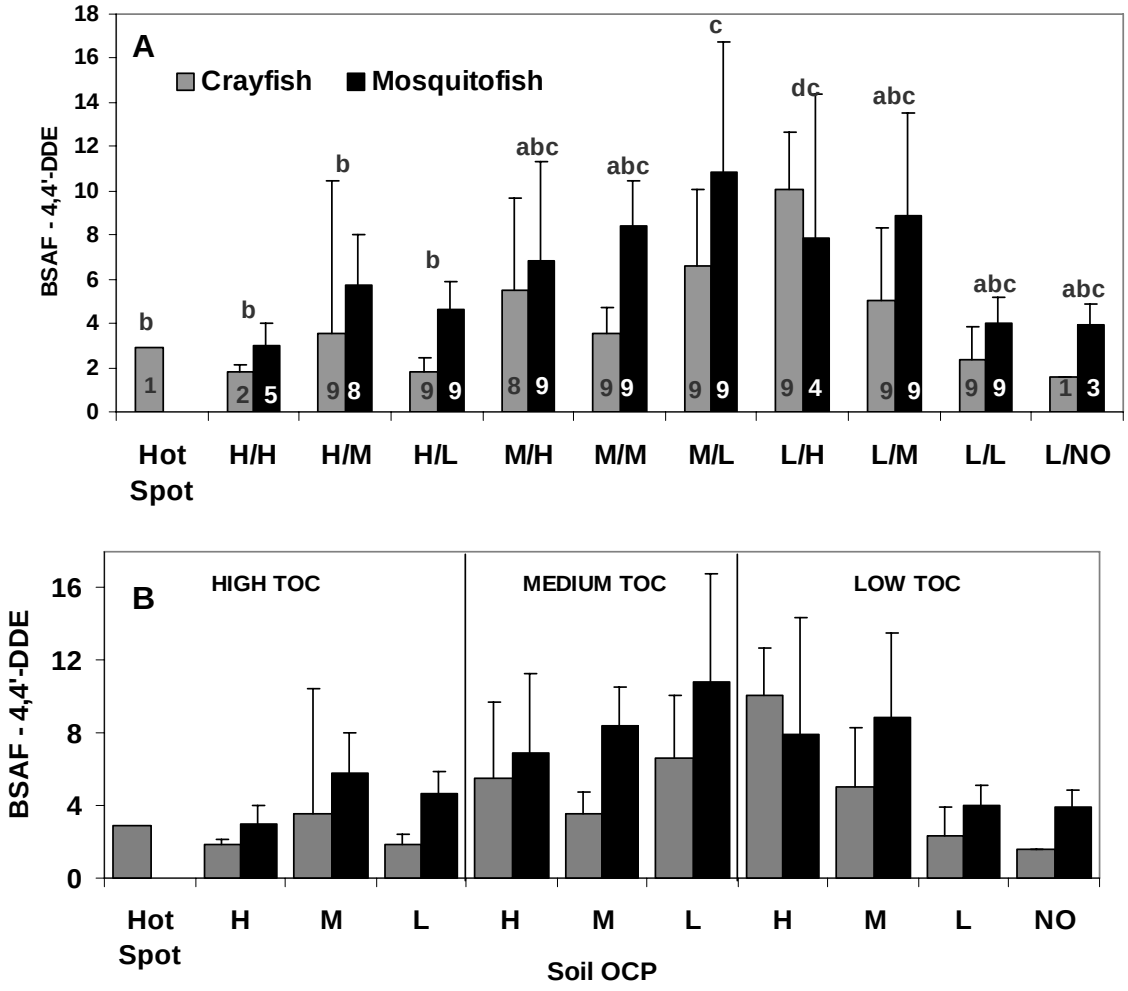


Figure 7-4. Mean ± SD of BSAFs for 4,4'-DDE, by treatment and biota. Both panels are showing the same data, but in Panel B, BSAFs are grouped based on soil Total Organic Carbon (TOC). Numbers on bottom of bars represent sample sizes (i.e. number of tanks). BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16. Regardless of treatment, mosquitofish had higher BSAFs than crayfish (2-way ANOVA, $p < 0.0001$, $F = 10.2$; overall means of 6.7 and 4.6 for mosquitofish and crayfish, respectively). For both species, different small letters indicate significant differences in BSAFs across treatments (2-way ANOVA, $p < 0.0001$, $F = 5.3$).

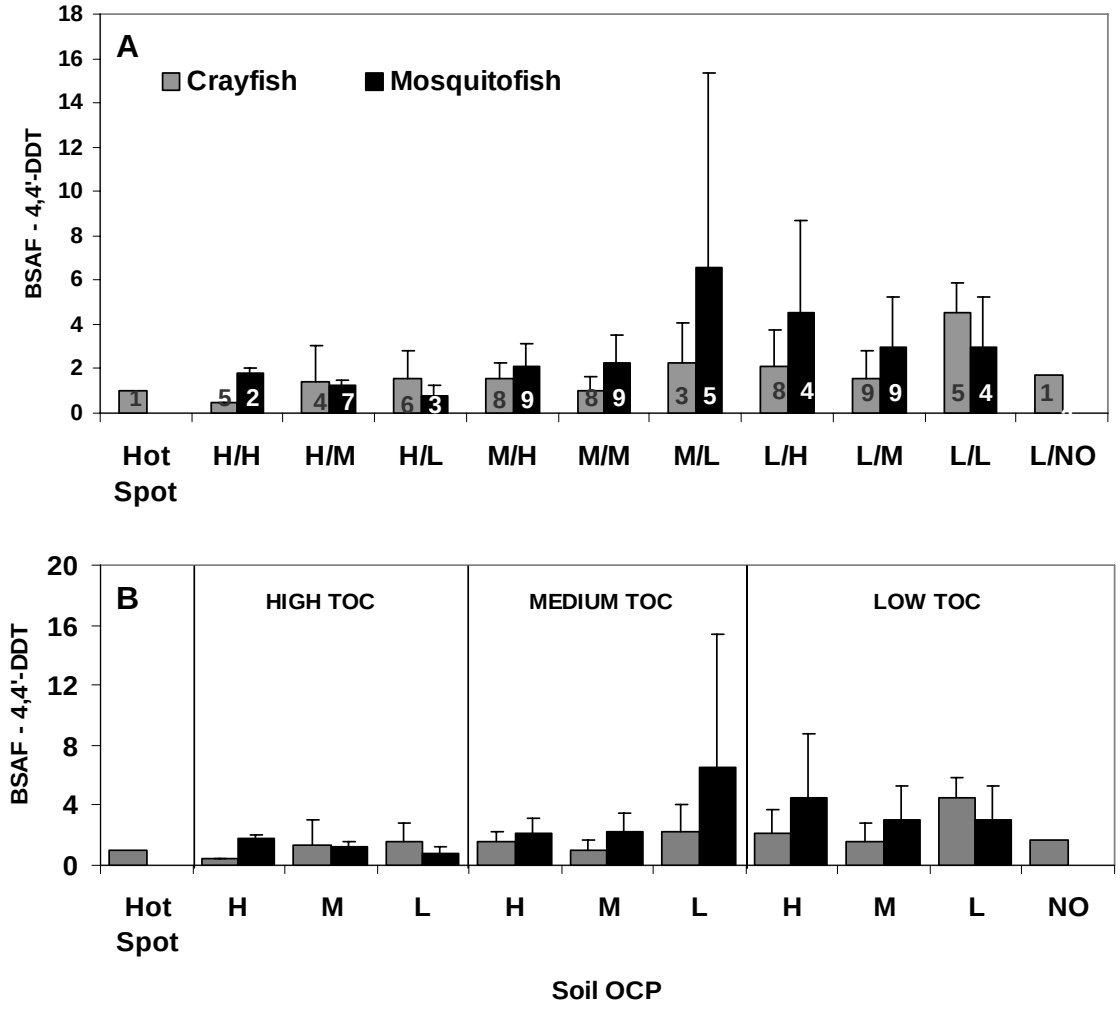


Figure 7-5. Mean ± SD of BSAFs for 4,4'-DDT, by treatment and biota. Both panels are showing the same data, but in Panel B, BSAFs are grouped based on soil Total Organic Carbon (TOC). Numbers on bottom of bars represent sample sizes (i.e. number of tanks). BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16. There were no biota or treatment effects on BSAFs.

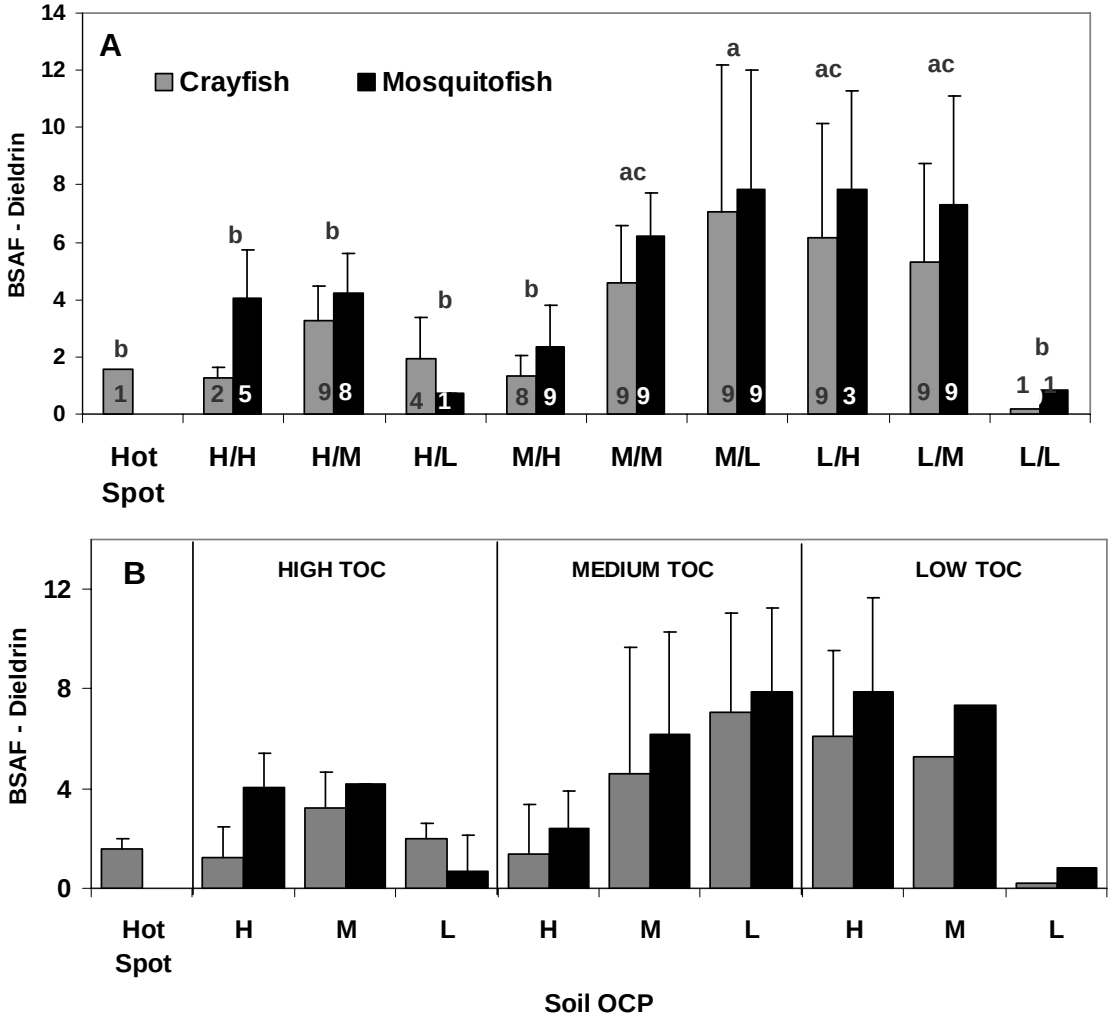


Figure 7-6. Mean ± SD of BSAFs for dieldrin, by treatment and biota. Both panels are showing the same data, but in Panel B, BSAFs are grouped based on soil Total Organic Carbon (TOC). Numbers on bottom of bars represent sample sizes (i.e. number of tanks). BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16. There were no differences in BSAFs between crayfish and mosquitofish. For both species, different small letters indicate significant differences in BSAFs across treatments (2-way ANOVA, $p < 0.0001$, $F = 6.4$).

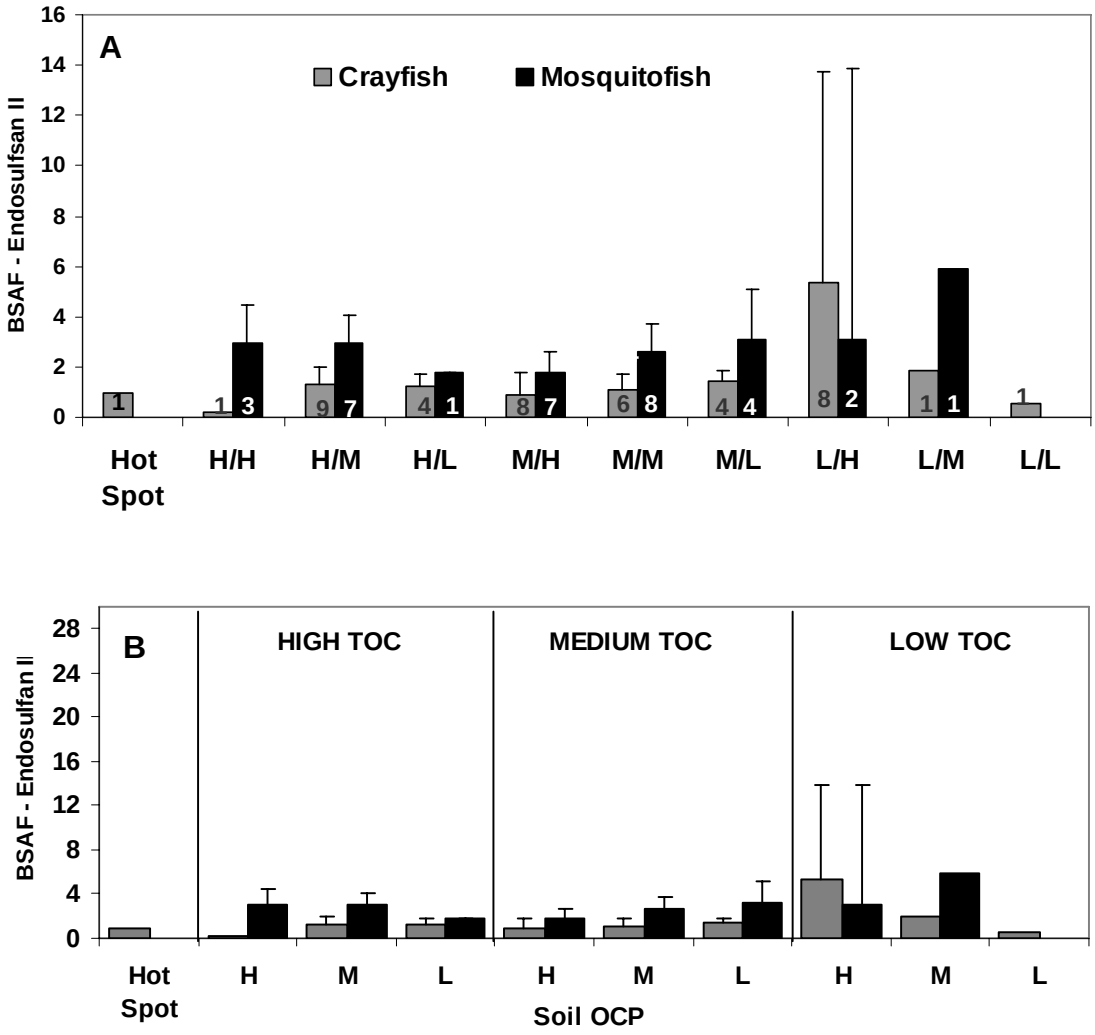


Figure 7-7. Mean ± SD of BSAFs for endosulfan II, by treatment and biota. Both panels are showing the same data, but in Panel B, BSAFs are grouped based on soil Total Organic Carbon (TOC). Numbers on bottom of bars represent sample sizes (i.e. number of tanks). BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16. Regardless of treatment, mosquitofish had higher BSAFs than crayfish (2-way ANOVA, p = 0.05, F= 3.9; overall means of 3.1 and 1.9 for mosquitofish and crayfish, respectively). Treatment had no effect on BSAFs.

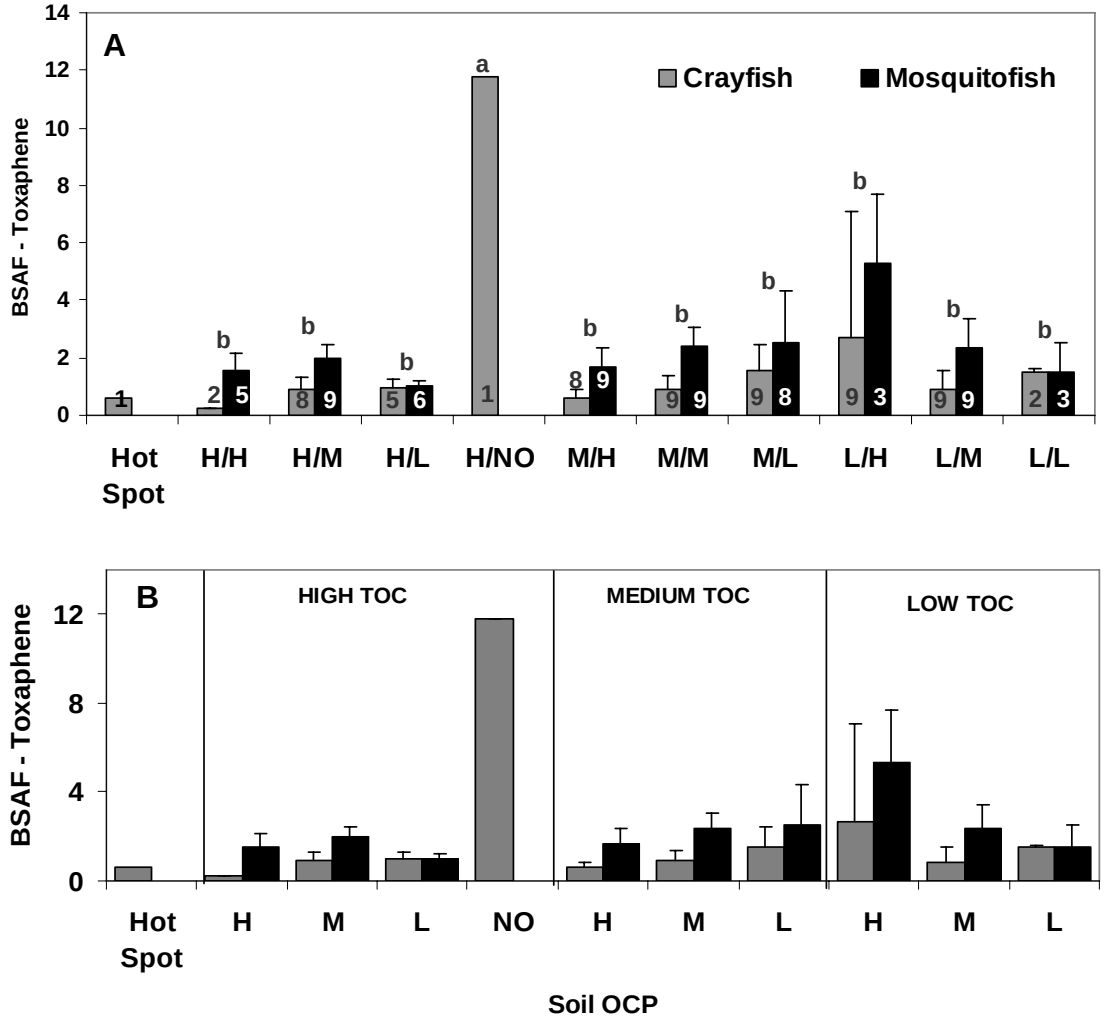


Figure 7-8. Mean ± SD of BSAFs for toxaphene, by treatment and biota. Both panels are showing the same data, but in Panel B, BSAFs are grouped based on soil Total Organic Carbon (TOC). Numbers on bottom of bars represent sample sizes (i.e. number of tanks). BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16. Regardless of treatment, mosquitofish had higher BSAFs than crayfish (2-way ANOVA, $p = 0.0003$, $F = 13.9$; overall means of 2.1 and 1.4 for mosquitofish and crayfish, respectively). For both species, different small letters indicate significant differences in BSAFs across treatments (2-way ANOVA, $p < 0.0001$, $F = 8.2$).

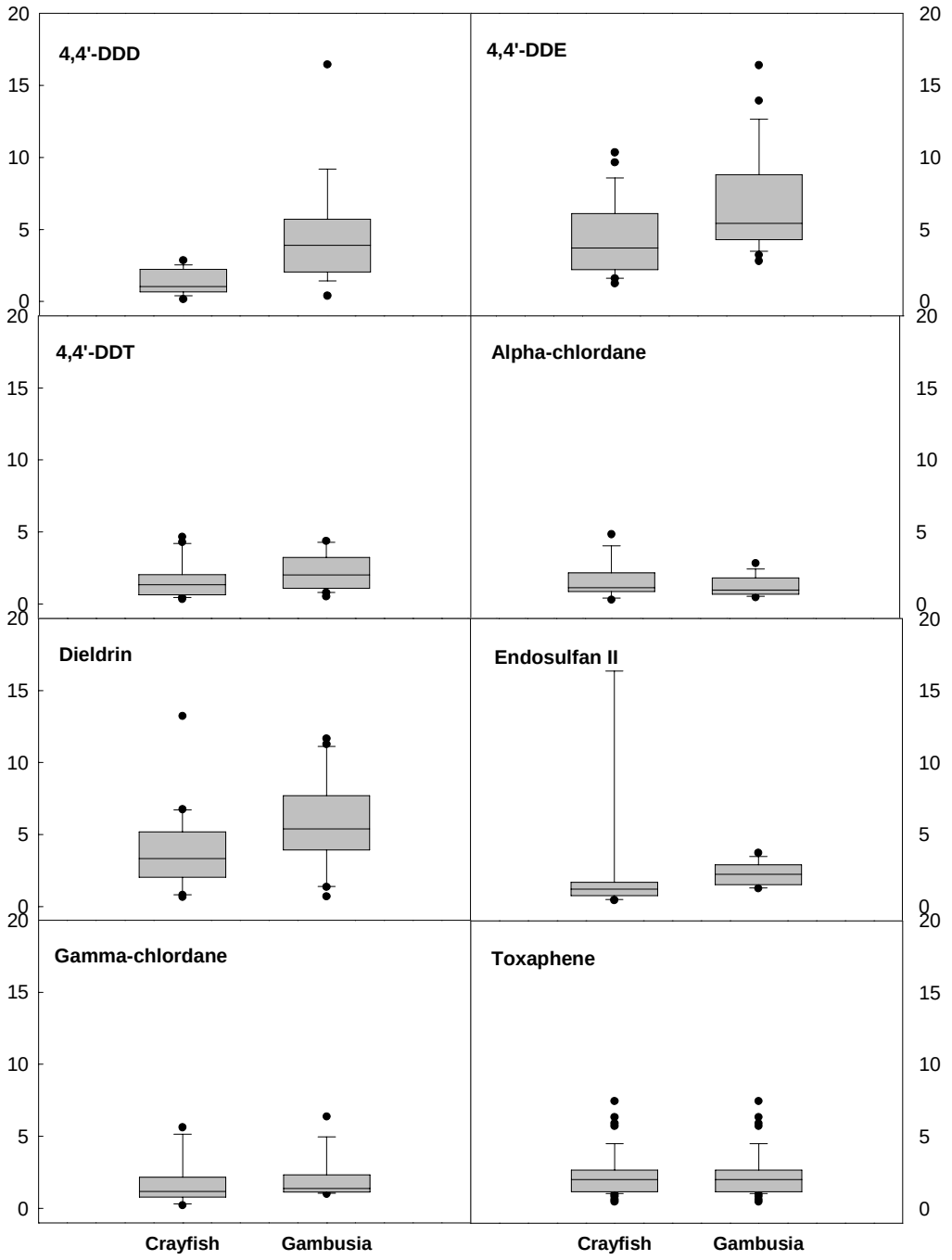


Figure 7-9. Boxplots showing BSAFs, by analyte and biota type. Regardless of chemical, mosquitofish had higher BSAFs than crayfish (2-way ANOVA, $p < 0.0001$, $F = 16.5$). There was also a significant difference in BSAFs across the different OCPs examined (2-way ANOVA, $p < 0.0001$, $F = 15.9$).

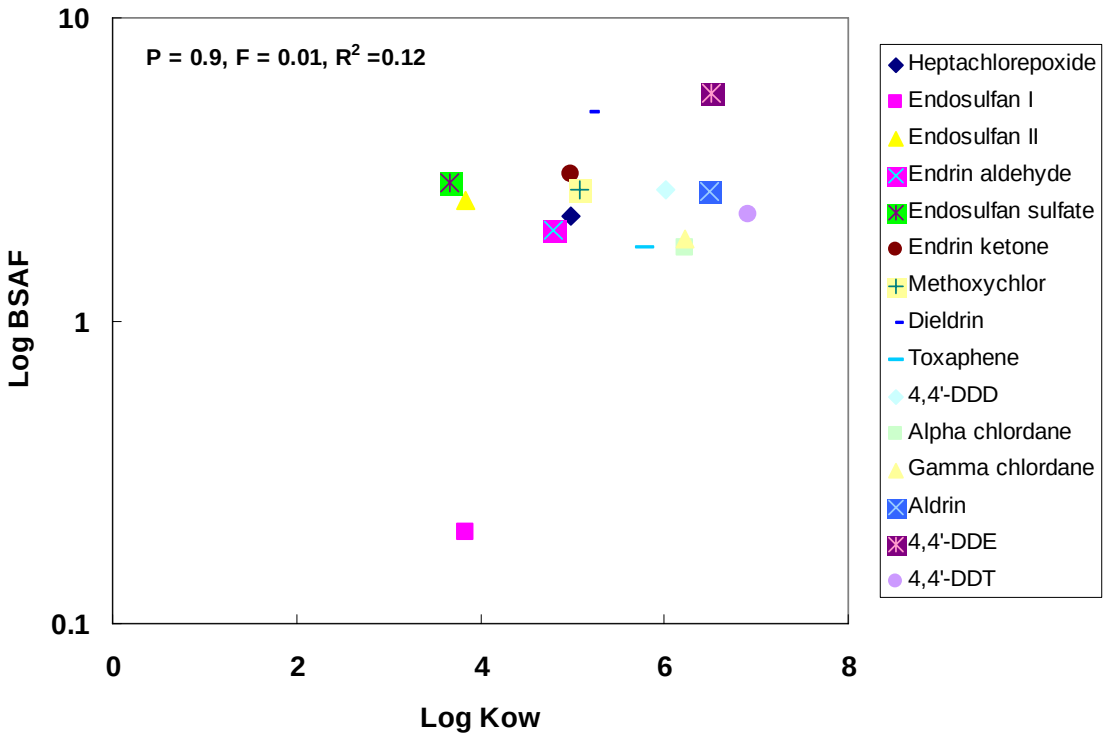


Figure 7-10. Relationship between overall log BSAF obtained in this study (mosquitofish and crayfish combined) and log K_{ow}, by analyte. There was no clear relationship between log BSAF and log K_{ow}.

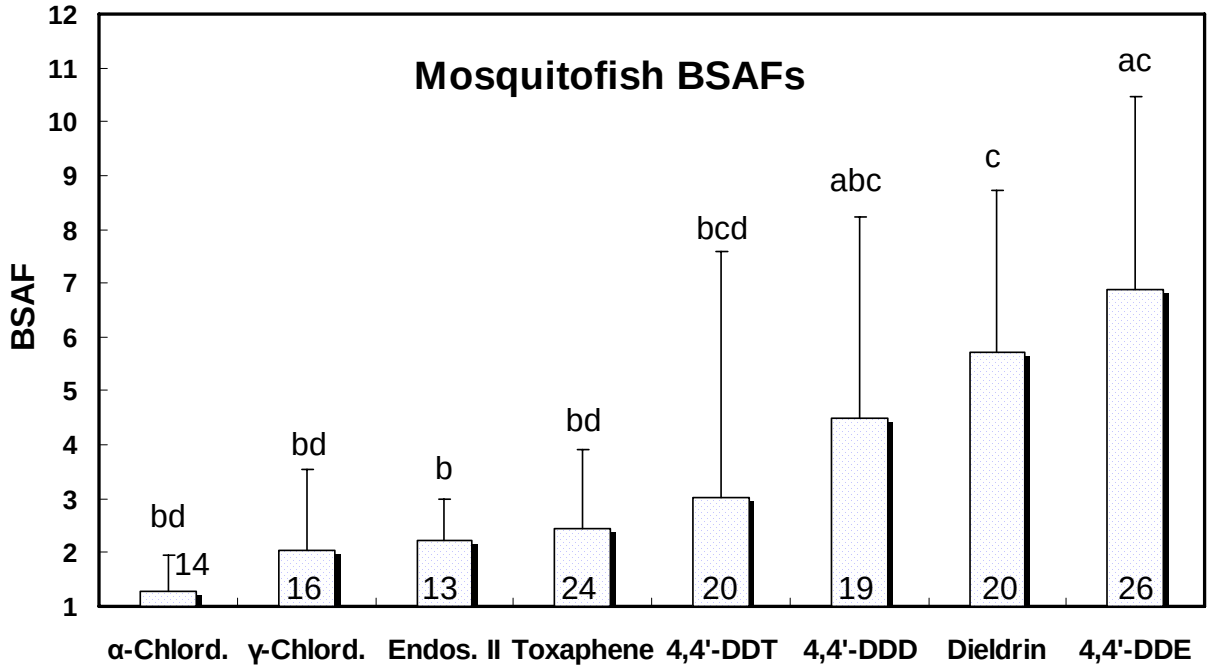


Figure 7-11. Mean ± SD of BSAFs in mosquitofish for the different OCPs studied (all treatments combined). Numbers on bottom of bars represent sample sizes (i.e. number of fish samples for which a BSAF was calculated). BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16. Different small letters indicate significant differences in BSAFs across OCPs (1-way ANOVA, $p < 0.0001$, $F = 6.7$).

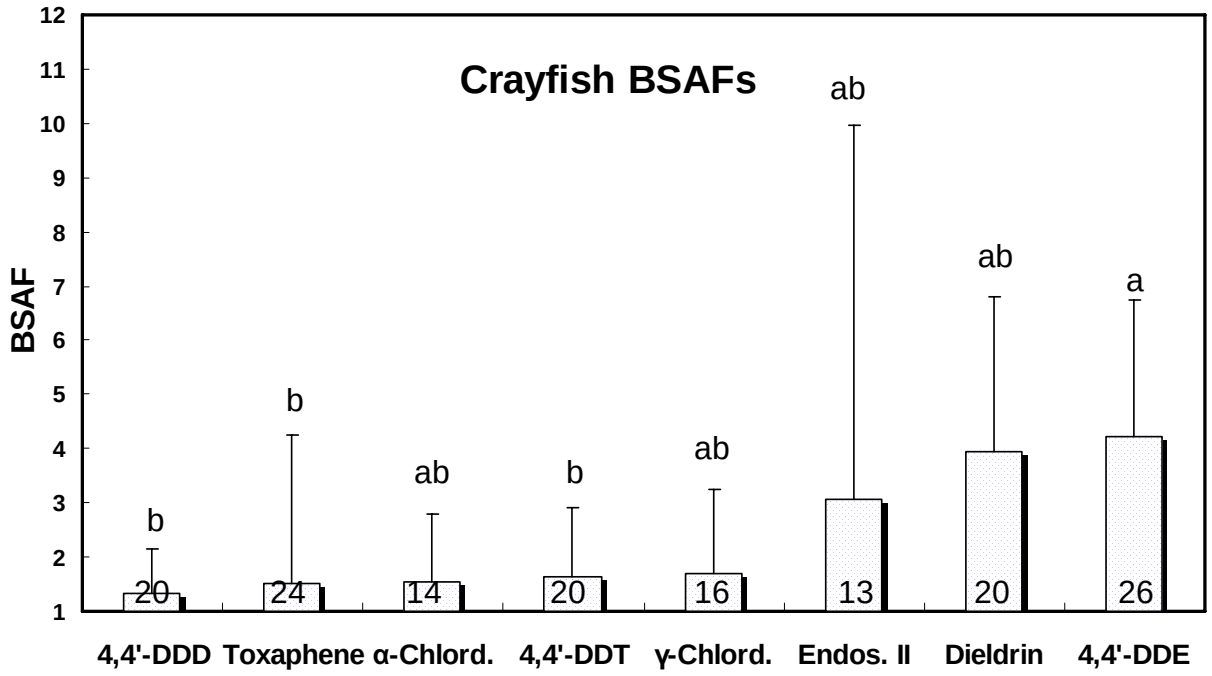


Figure 7-12. Mean ± SD of BSAFs in crayfish for the different OCPs studied (all treatments combined). Numbers on bottom of bars represent sample sizes (i.e. number of crayfish samples for which a BSAF was calculated). BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16. Different small letters indicate significant differences in BSAFs across OCPs (1-way ANOVA, $p < 0.001$, $F = 2.9$).

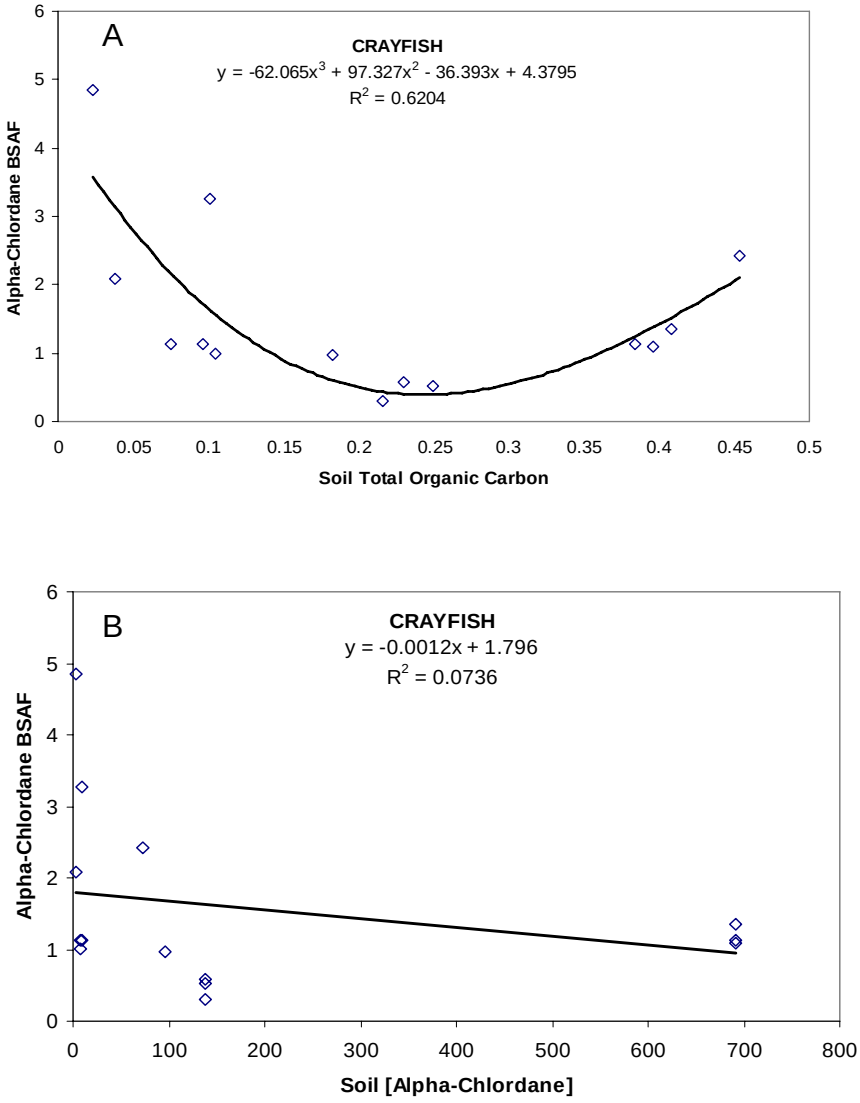


Figure 7-13. Relationship among BSAF for alpha-chlordane and soil total organic carbon (A) and soil alpha-chlordane concentration (µg/kg, dry weight) (B) in crayfish. BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16.

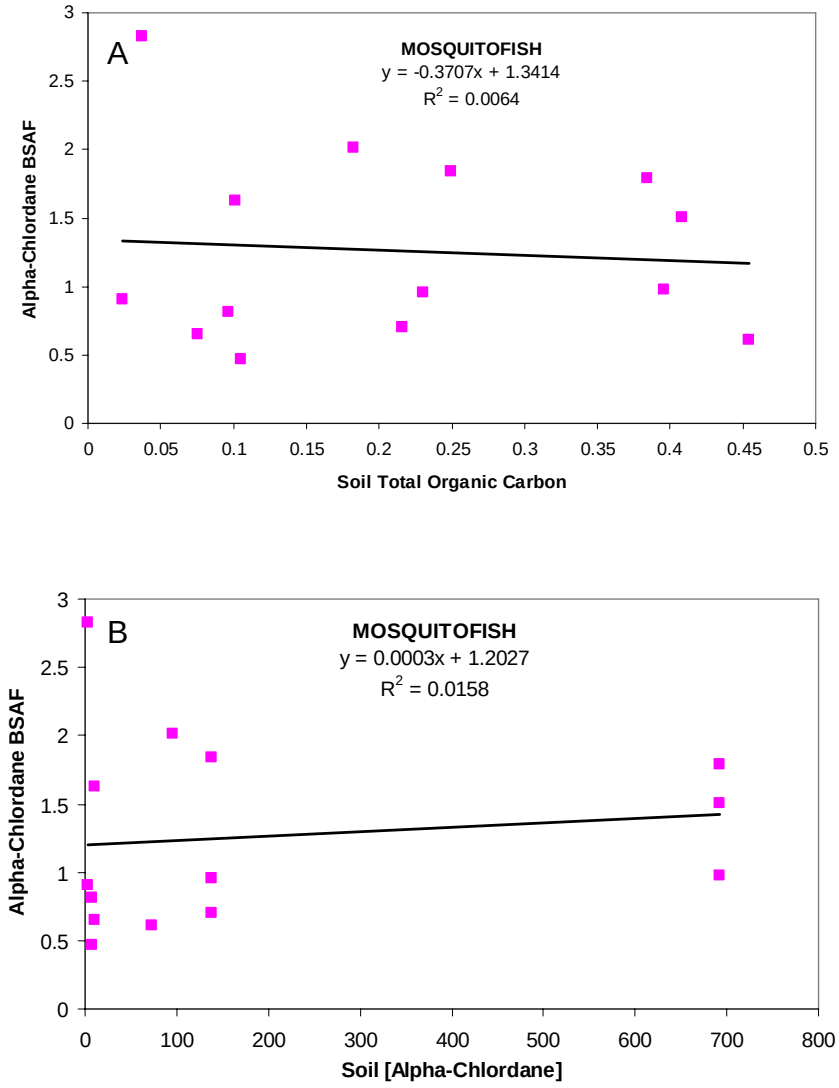


Figure 7-14. Relationship among BSAF for alpha-chlordane and soil total organic carbon (A) and soil alpha-chlordane concentration (µg/kg, dry weight) (B) in mosquitofish. BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16.

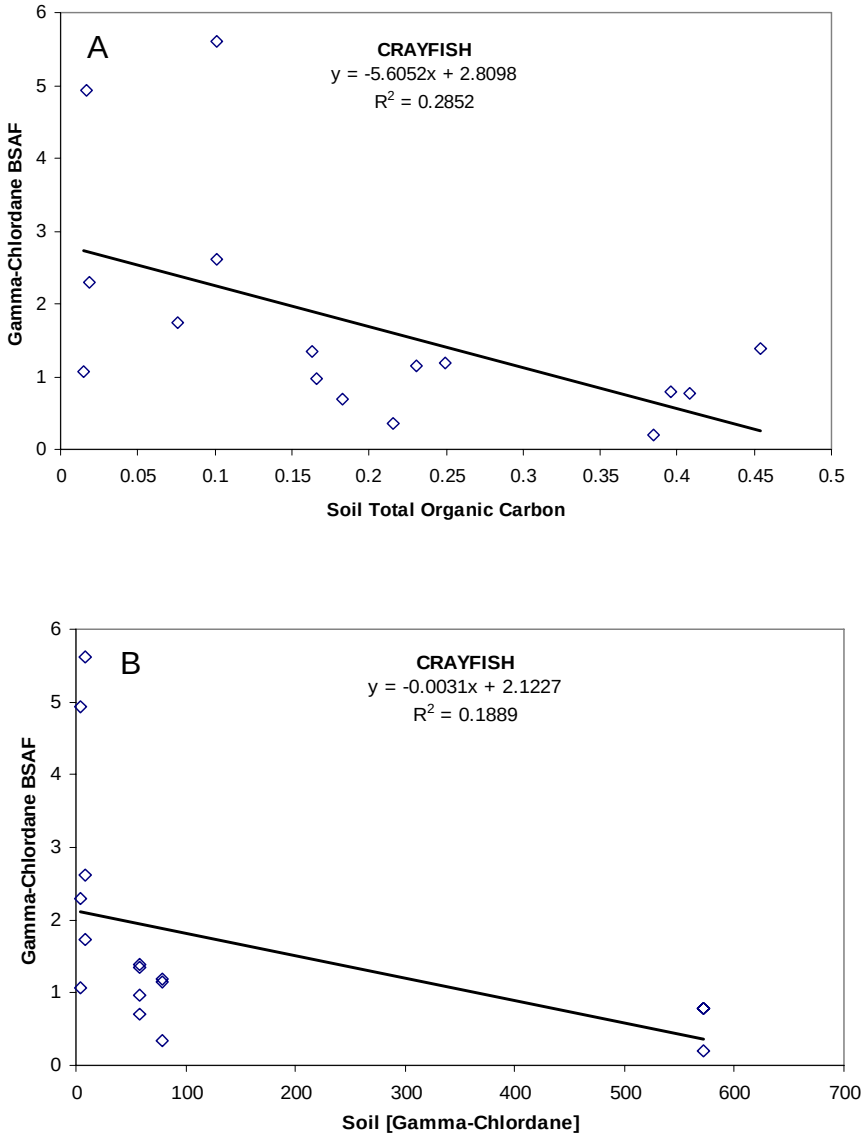


Figure 7-15. Relationship among BSAF for gamma-chlordane and soil total organic carbon (A) and soil gamma-chlordane concentration (µg/kg, dry weight) (B) in crayfish. BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16.

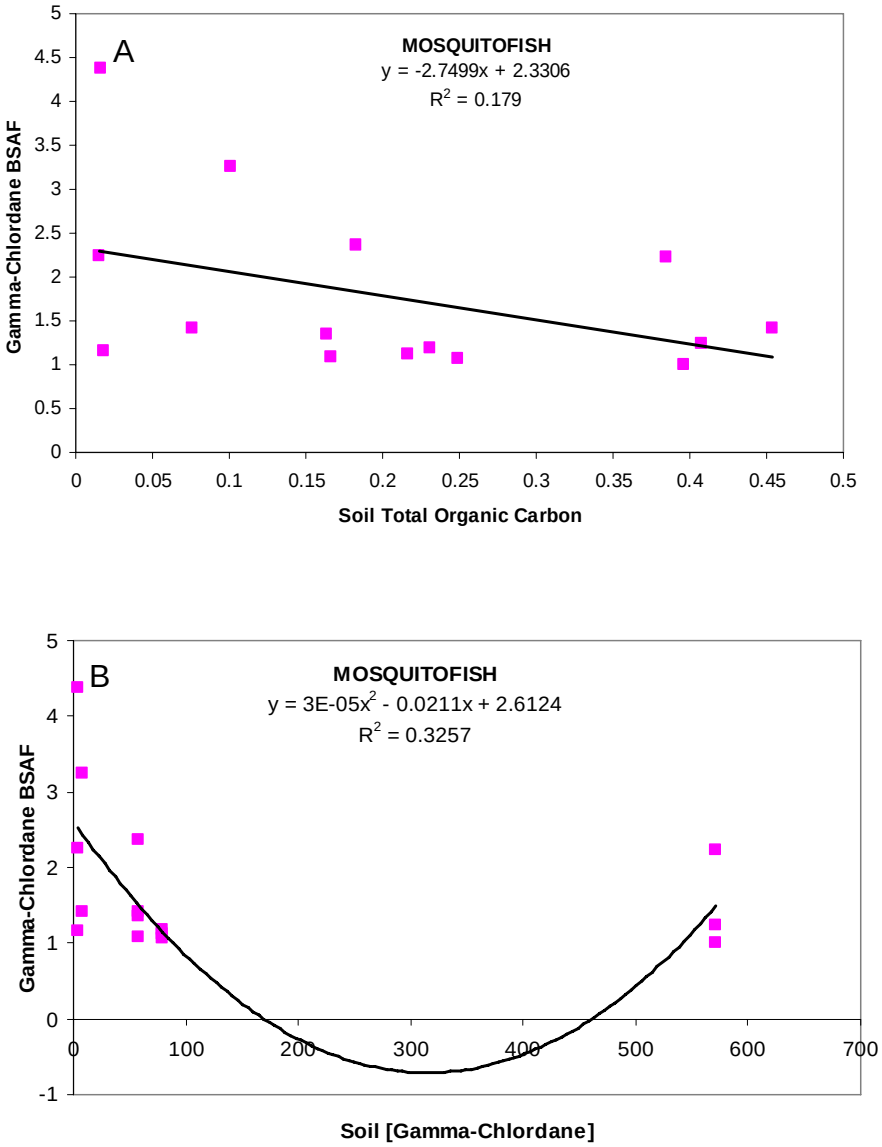


Figure 7-16. Relationship among BSAF for gamma-chlordane and soil total organic carbon (A) and soil gamma-chlordane concentration (µg/kg, dry weight) (B) in mosquitofish. BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16.

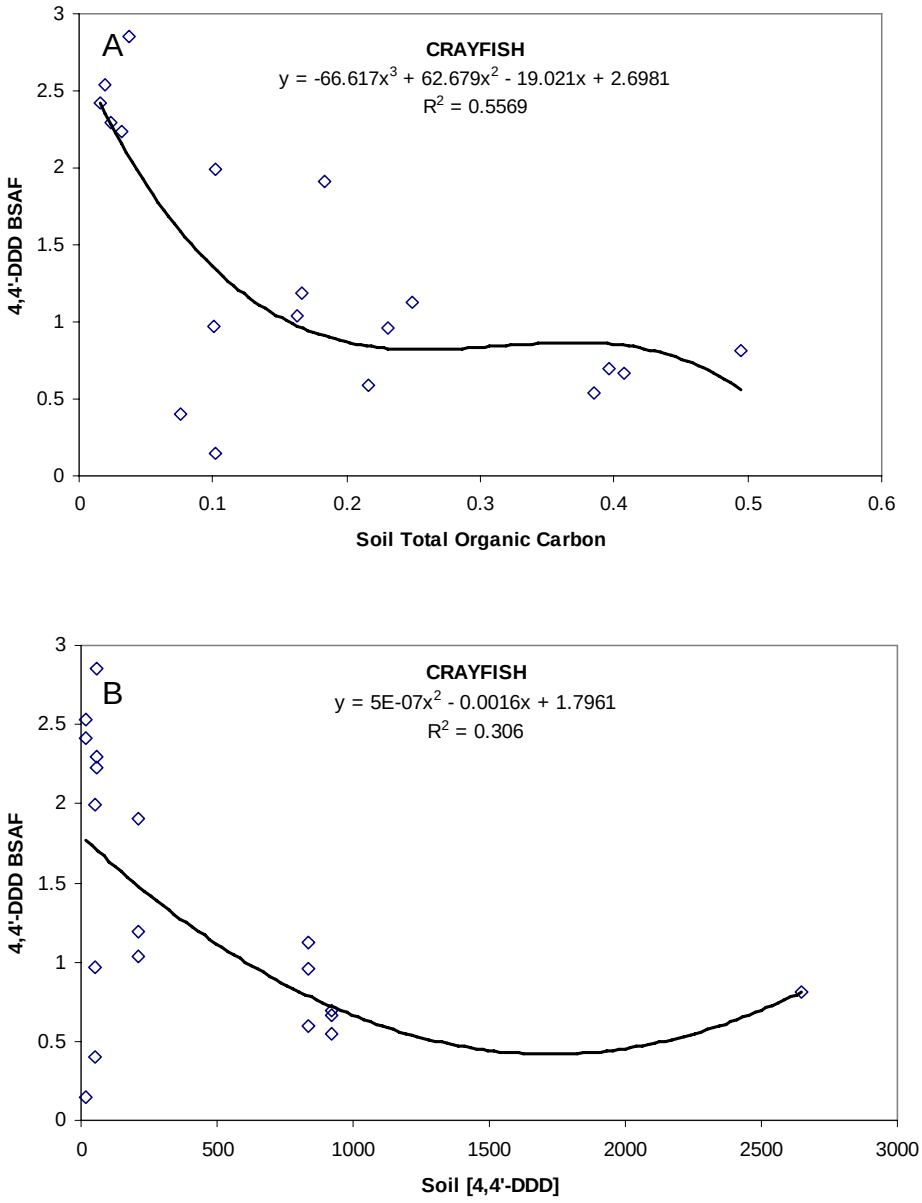


Figure 7-17. Relationship among BSAF for 4,4'-DDD and soil total organic carbon (A) and soil 4,4'-DDD concentration (µg/kg, dry weight) (B) in crayfish. BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16.

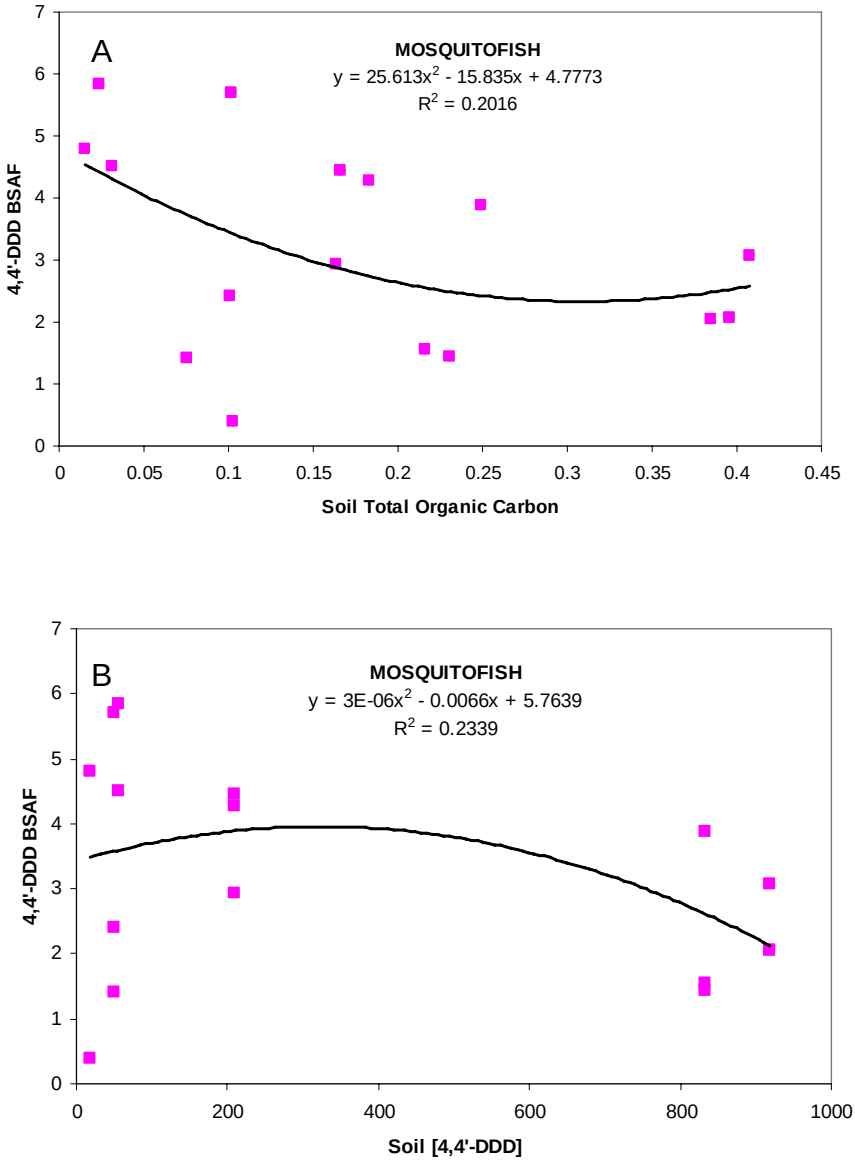


Figure 7-18. Relationship among BSAF for 4,4'-DDD and soil total organic carbon (A) and soil 4,4'-DDD concentration (µg/kg, dry weight) (B) in mosquitofish. BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16.

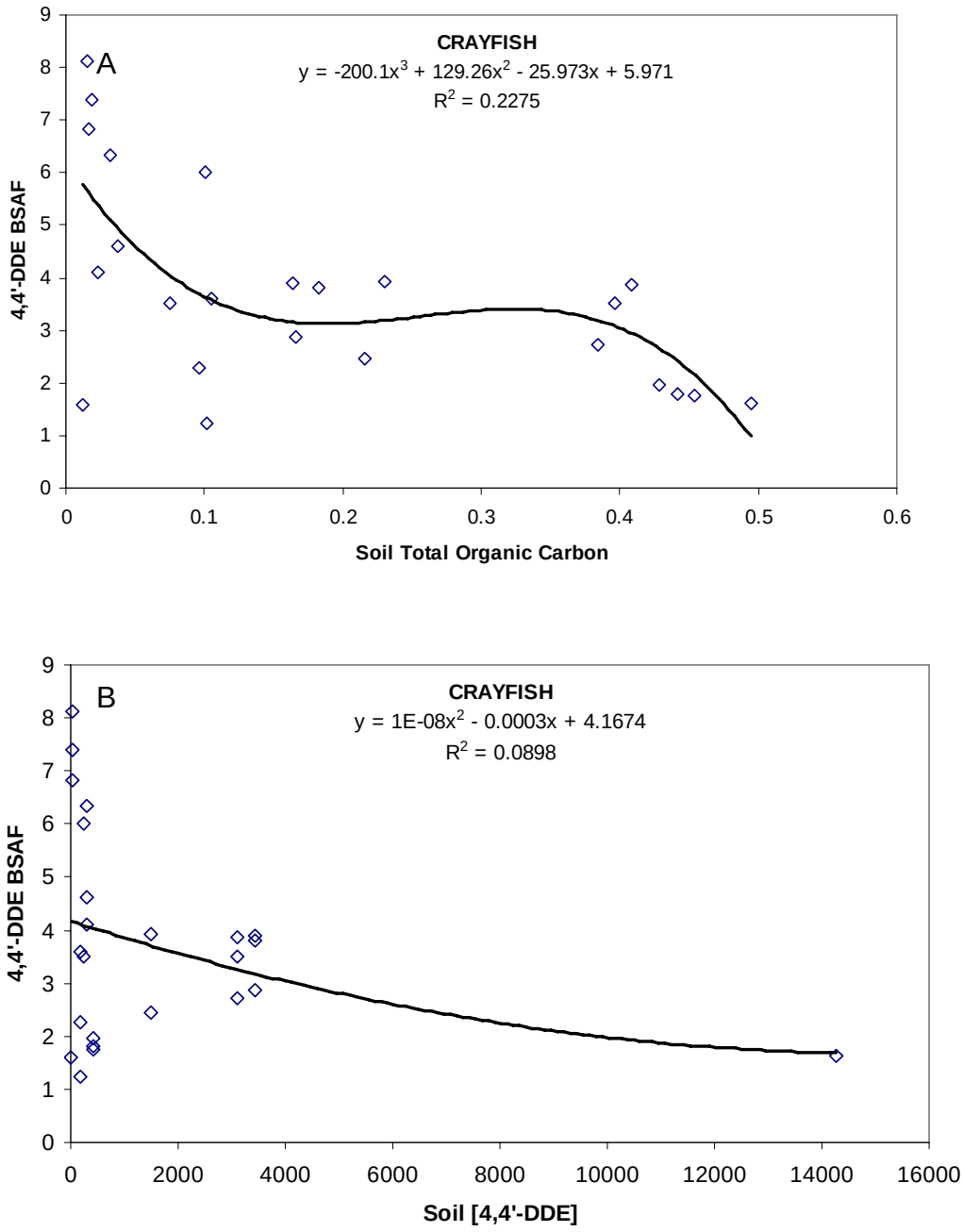


Figure 7-19. Relationship among BSAF for 4,4'-DDE and soil total organic carbon (A) and soil 4,4'-DDE concentration (µg/kg, dry weight) (B) in crayfish. BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16.

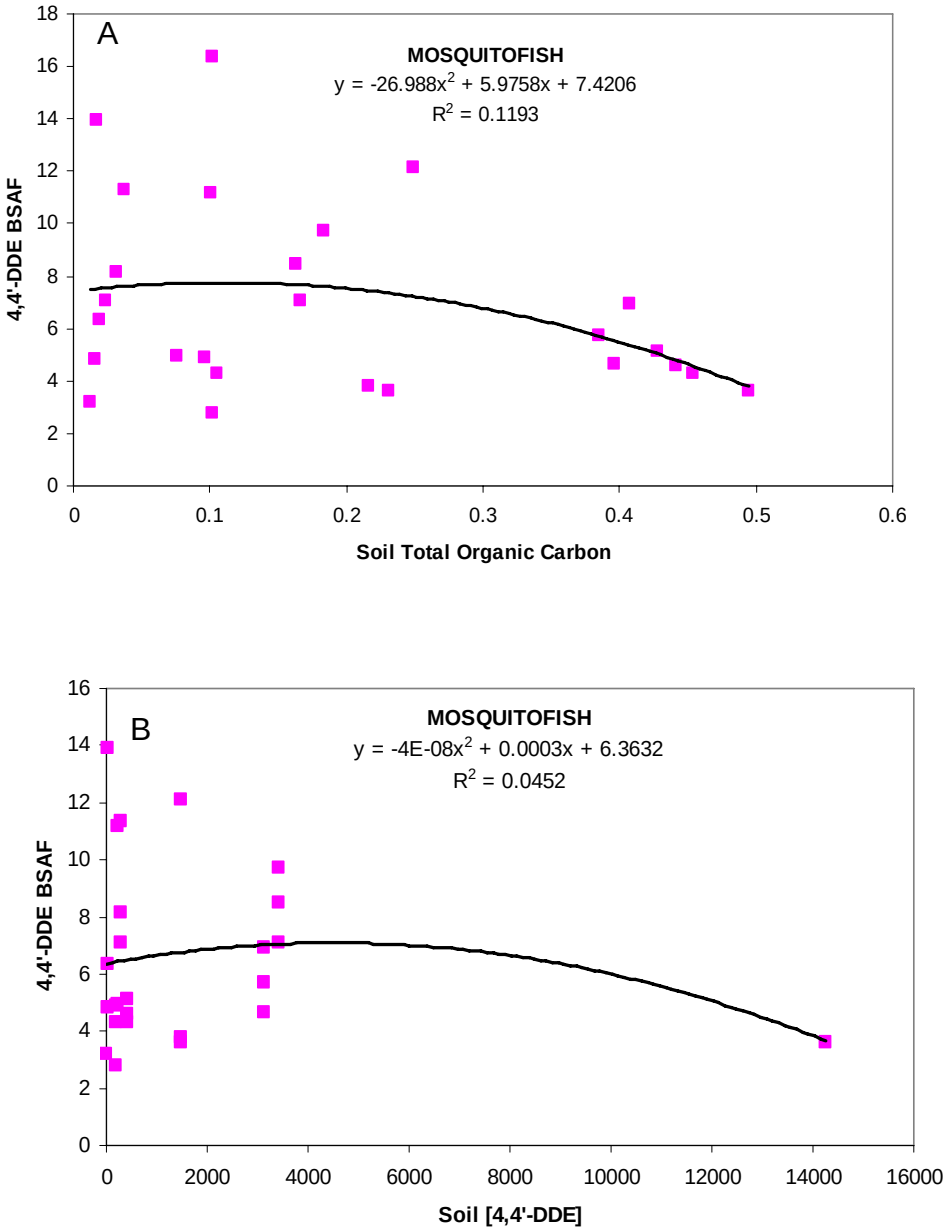


Figure 7-20. Relationship among BSAF for 4,4'-DDE and soil total organic carbon (A) and soil 4,4'-DDE concentration (µg/kg, dry weight) (B) in mosquitofish. BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16.

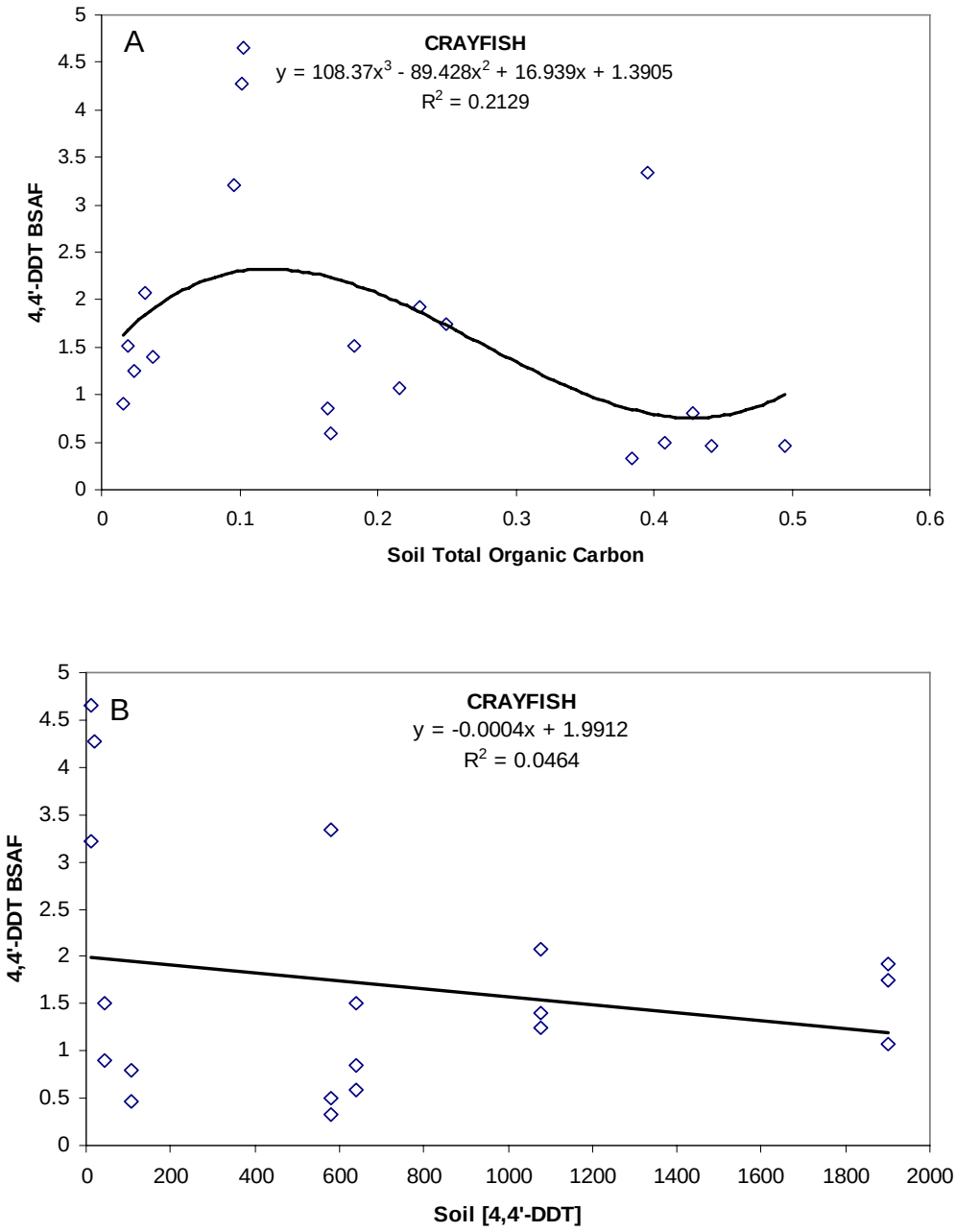


Figure 7-21. Relationship among BSAF for 4,4'-DDT and soil total organic carbon (A) and soil 4,4'-DDT concentration (µg/kg, dry weight) (B) in crayfish. BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16.

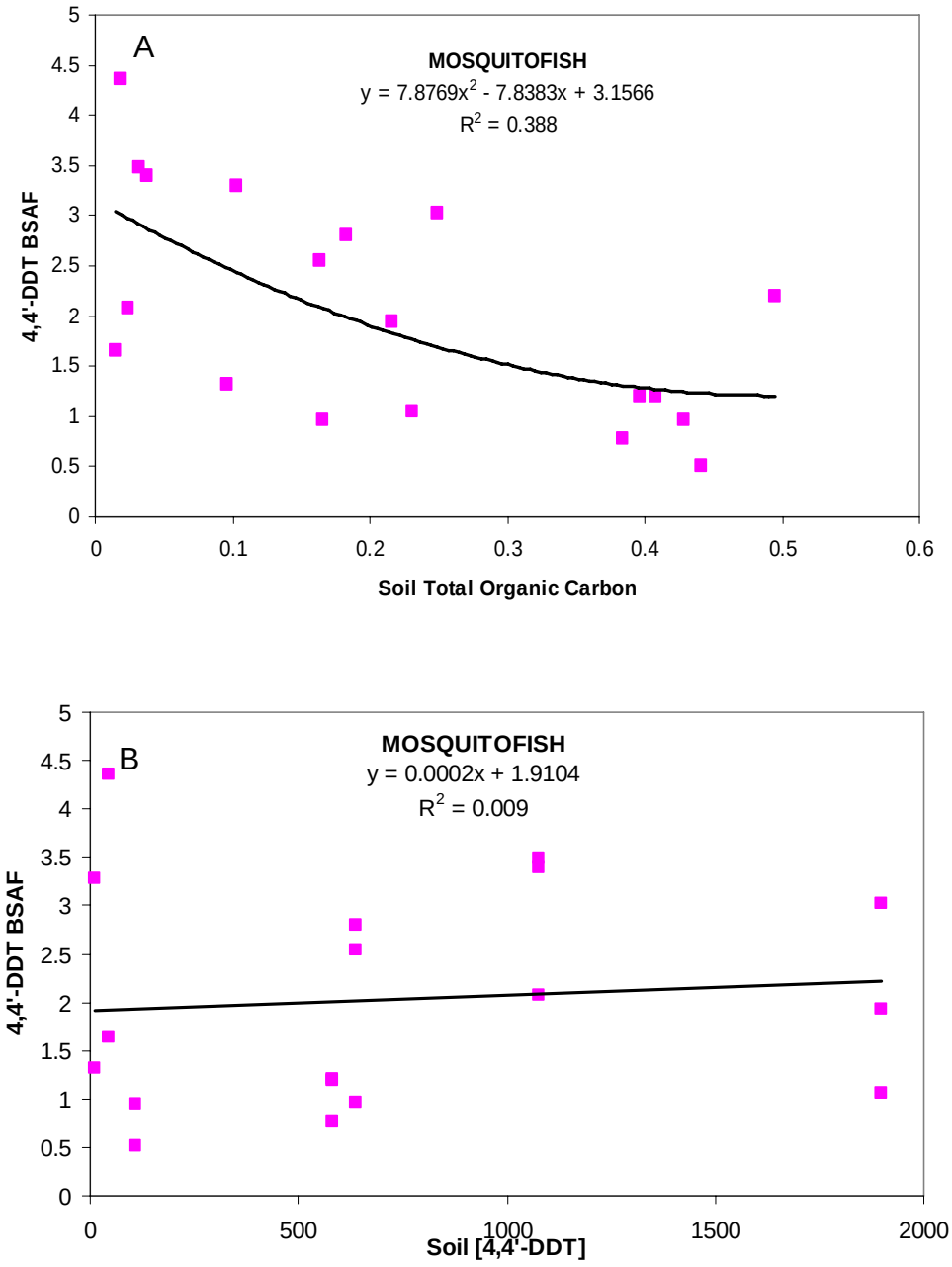


Figure 7-22. Relationship among BSAF for 4,4'-DDT and soil total organic carbon (A) and soil 4,4'-DDT concentration (µg/kg, dry weight) (B) in mosquitofish. BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16.

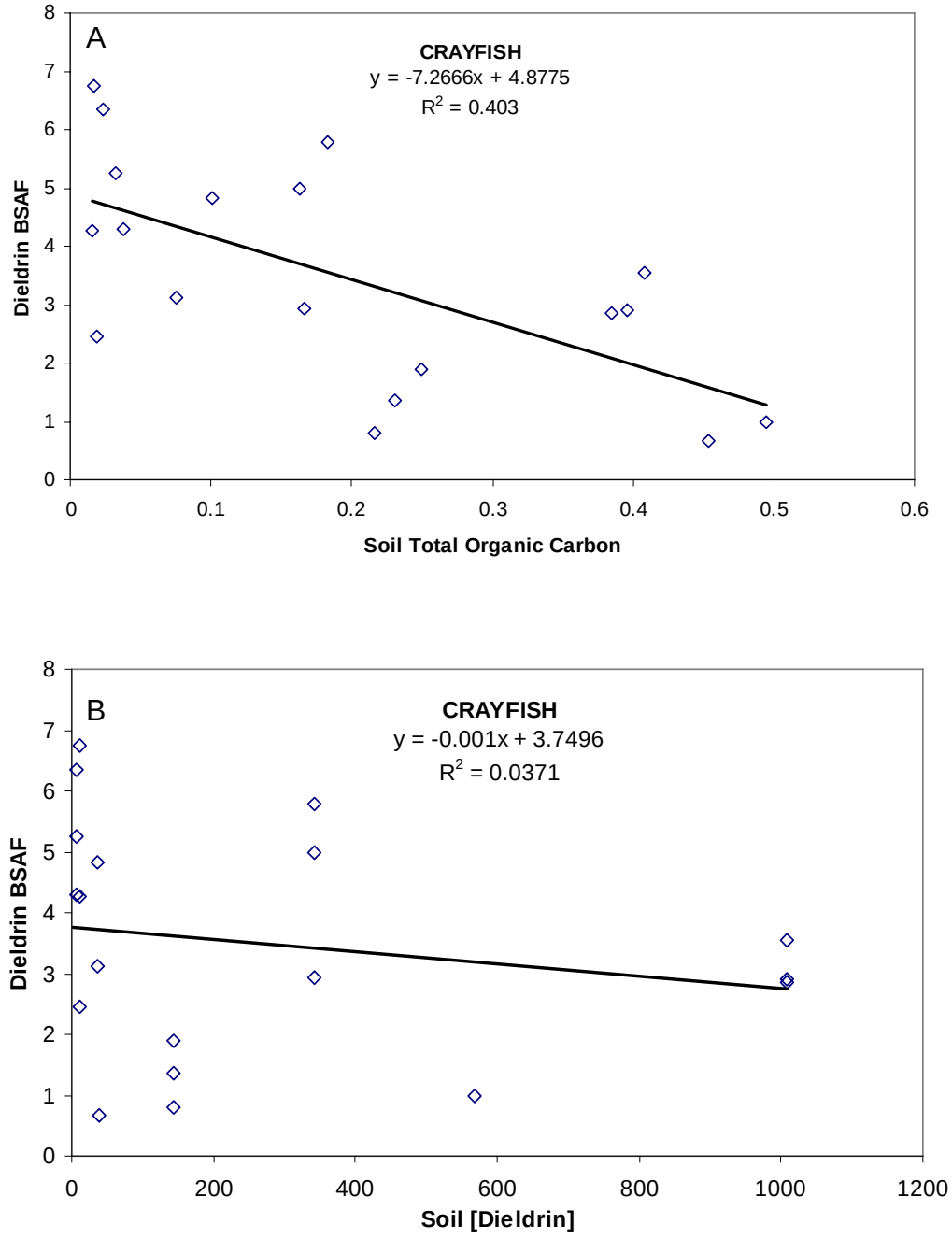


Figure 7-23. Relationship among BSAF for dieldrin and soil total organic carbon (A) and soil dieldrin concentration (µg/kg, dry weight) (B) in crayfish. BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16.

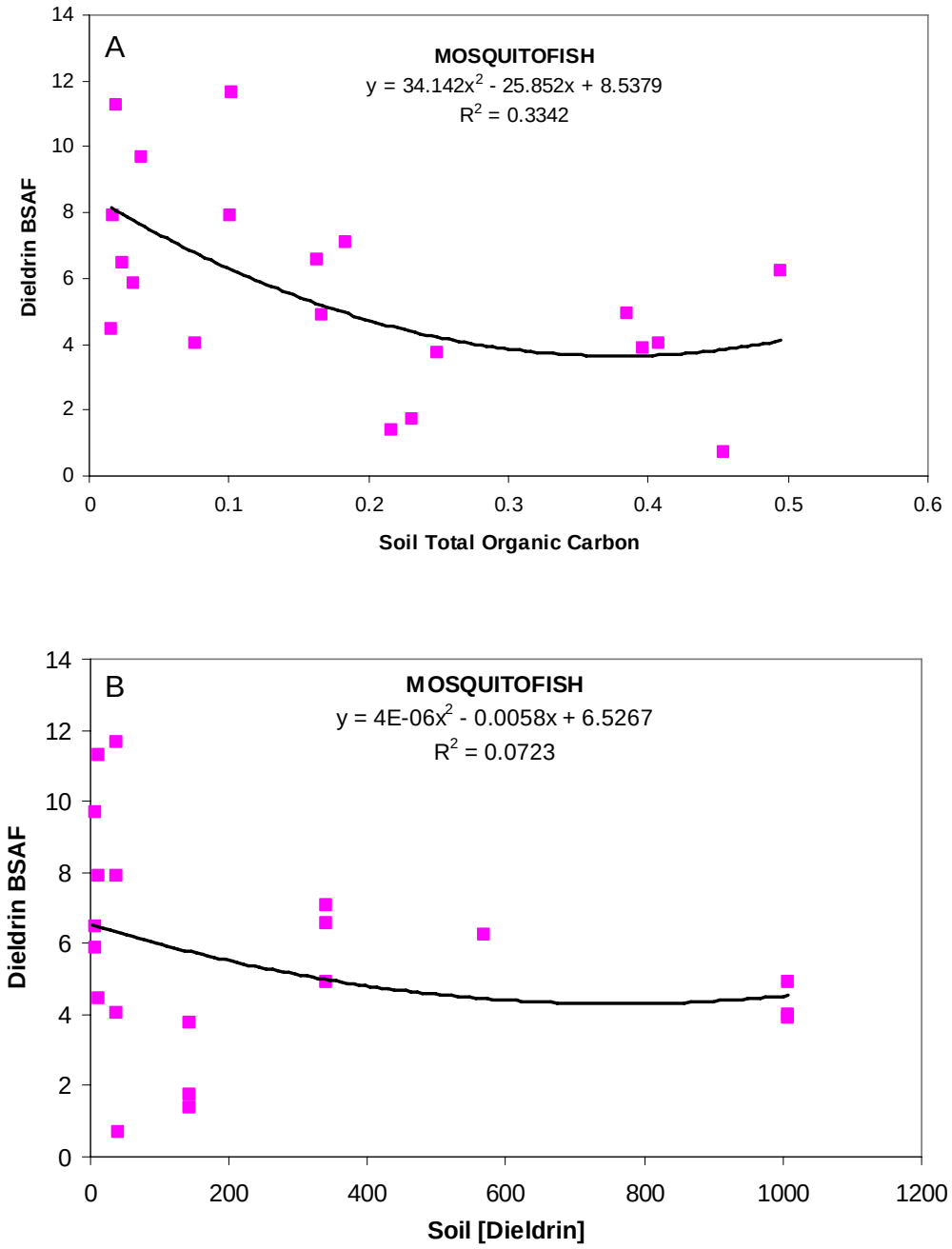


Figure 7-24. Relationship among BSAF for dieldrin and soil total organic carbon (A) and soil dieldrin concentration (µg/kg, dry weight) (B) in mosquitofish. BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16.

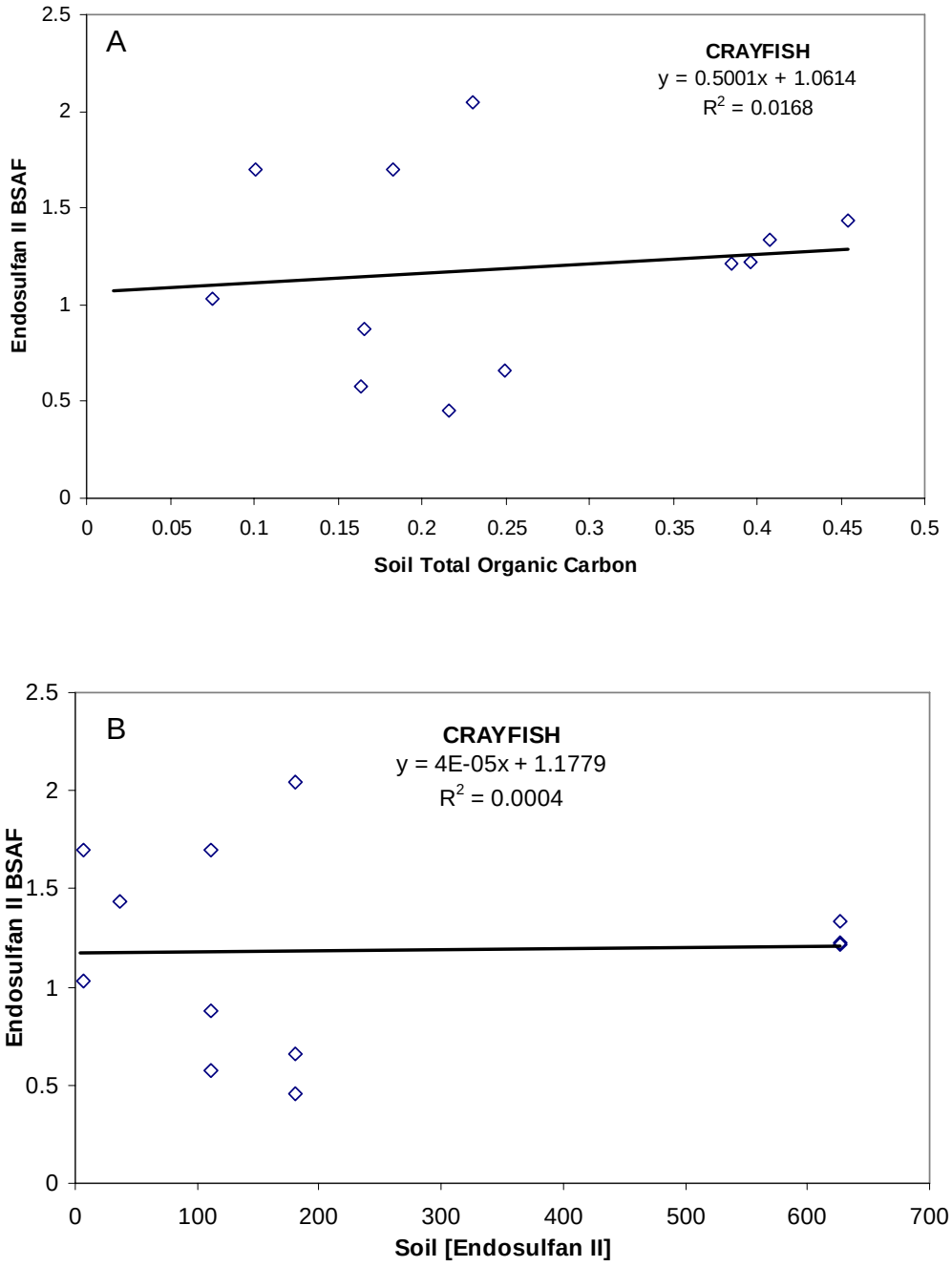


Figure 7-25. Relationship among BSAF for endosulfan II and soil total organic carbon (A) and soil endosulfan II concentration (µg/kg, dry weight) (B) in crayfish. BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16.

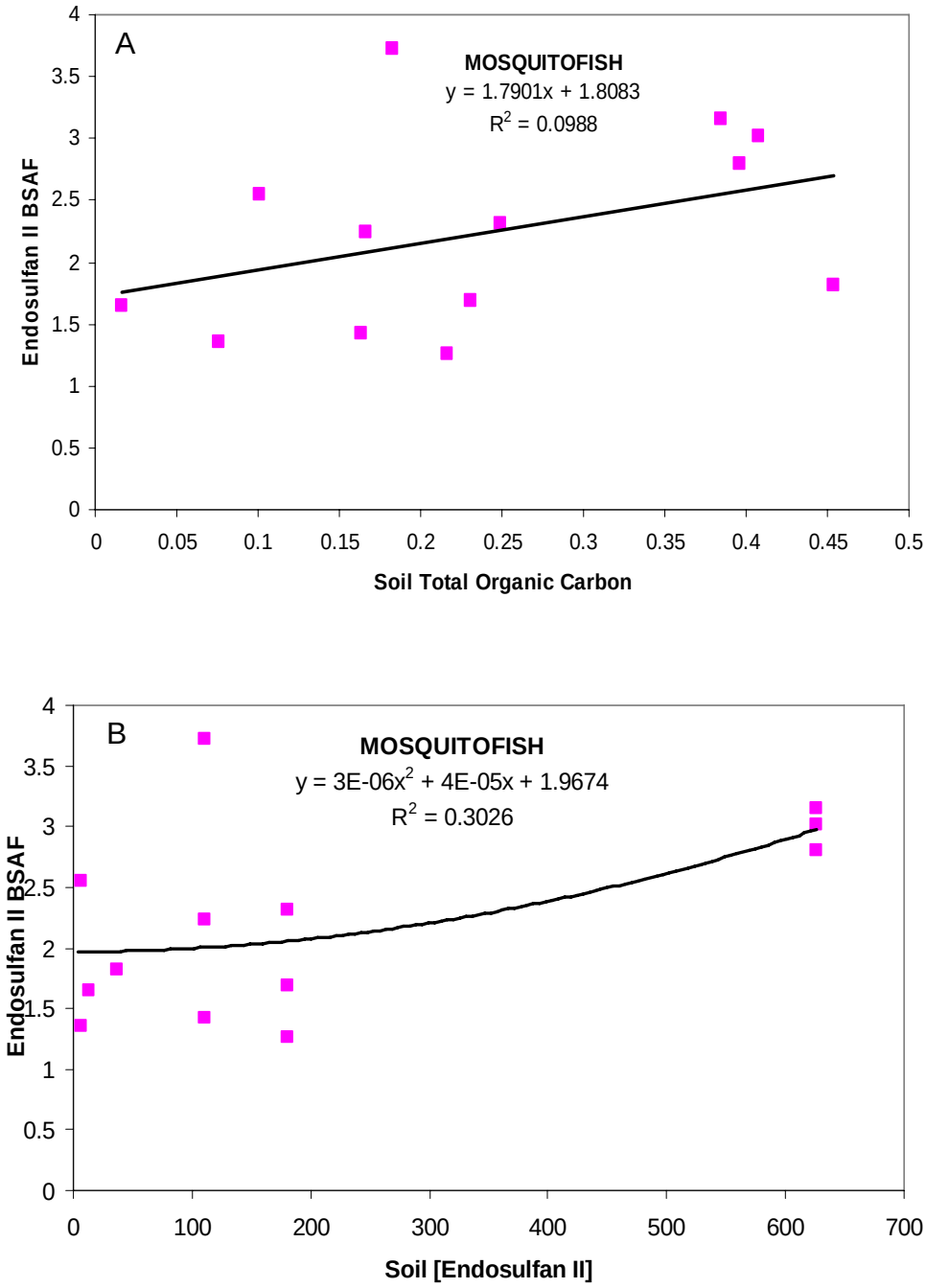


Figure 7-26. Relationship among BSAF for endosulfan II and soil total organic carbon (A) and soil endosulfan II concentration (µg/kg, dry weight) (B) in mosquitofish. BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16.

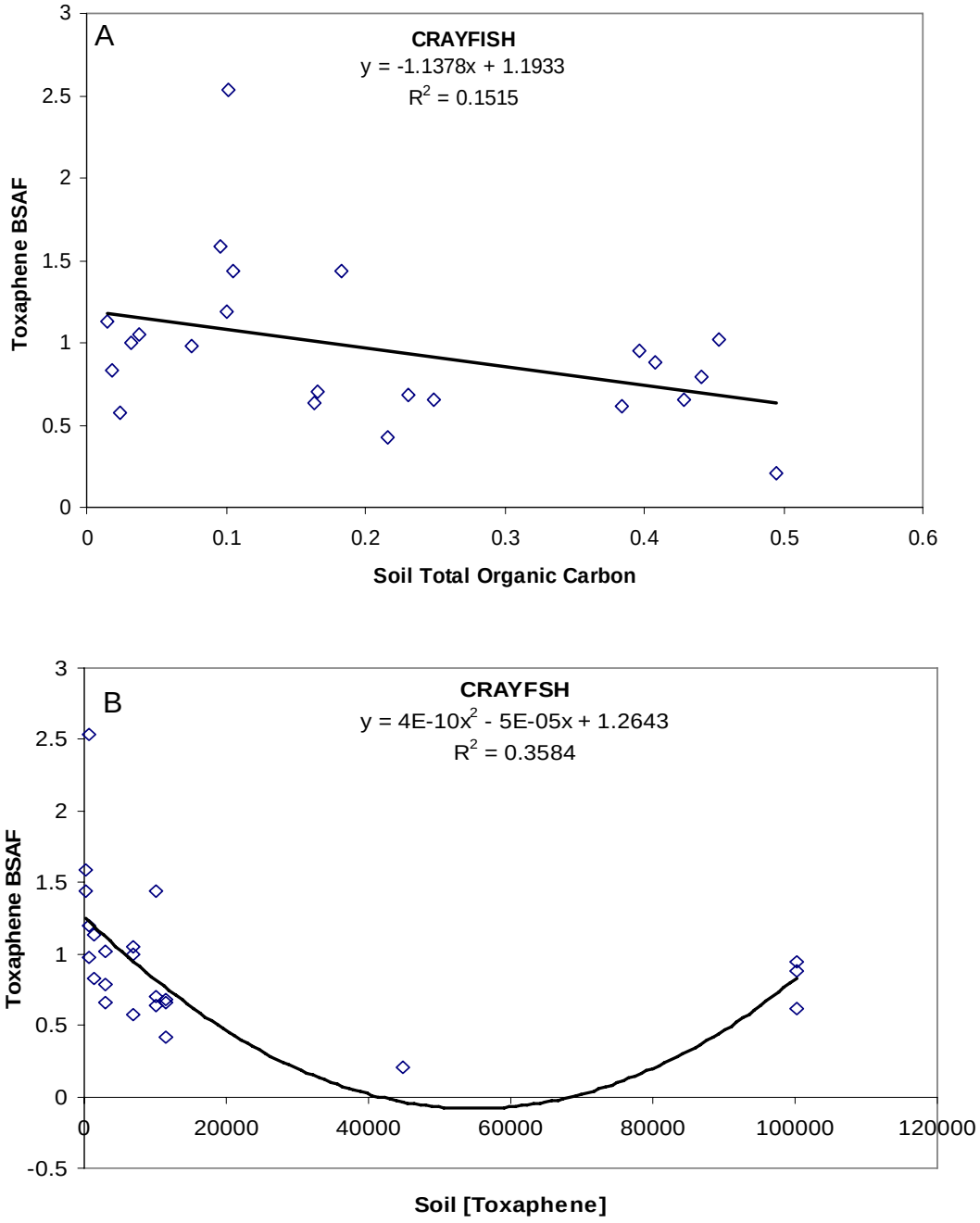


Figure 7-27. Relationship among BSAF for toxaphene and soil total organic carbon (A) and soil toxaphene concentration (µg/kg, dry weight) (B) in crayfish. BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16.

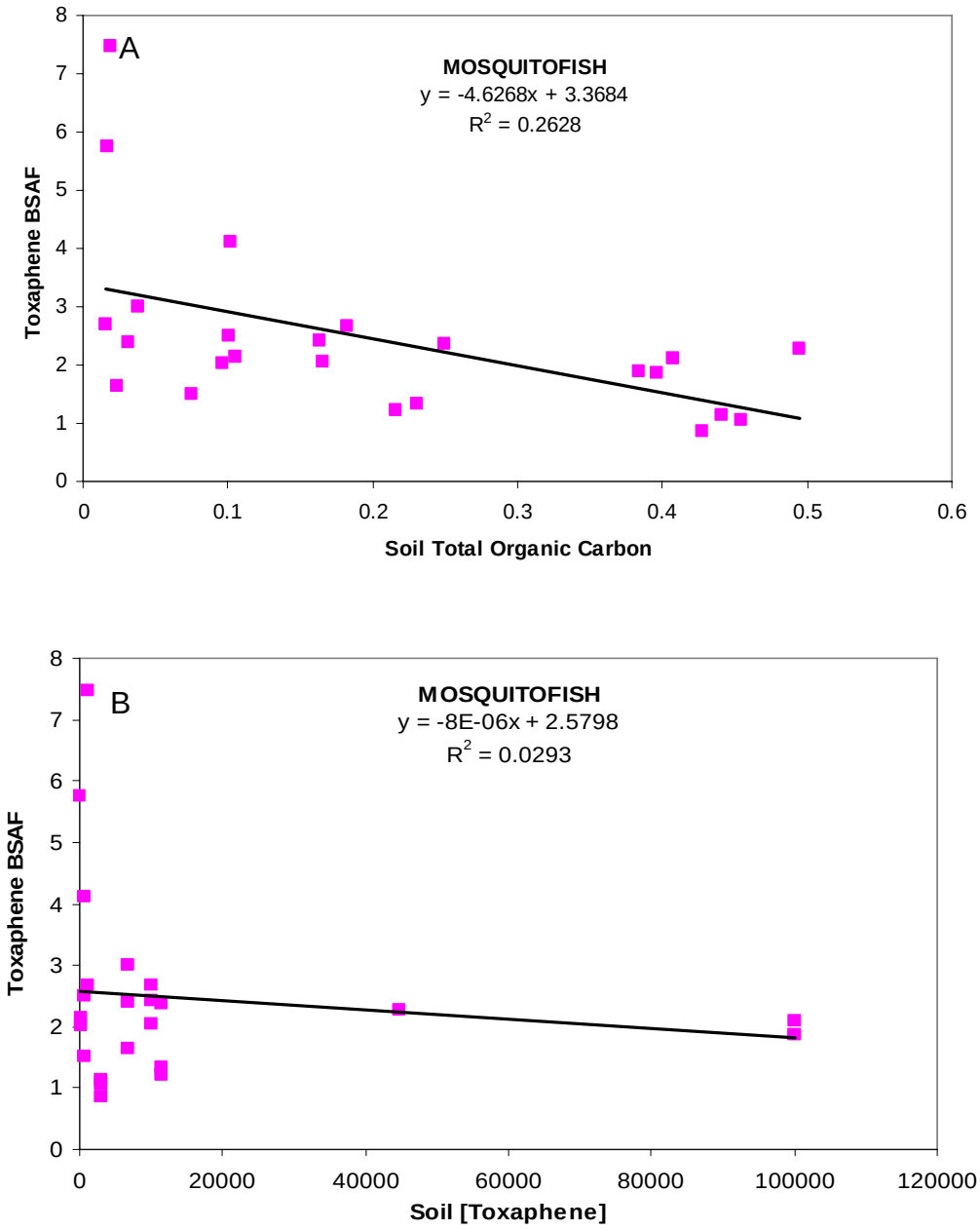


Figure 7-28. Relationship among BSAF for toxaphene and soil total organic carbon (A) and soil toxaphene concentration (µg/kg, dry weight) (B) in mosquitofish. BSAFs were calculated using the average of biota OCP lipid-normalized data obtained between weeks 8 and 16 of exposure, and the average of soil TOC-normalized OCP data obtained between weeks 0 and 16.

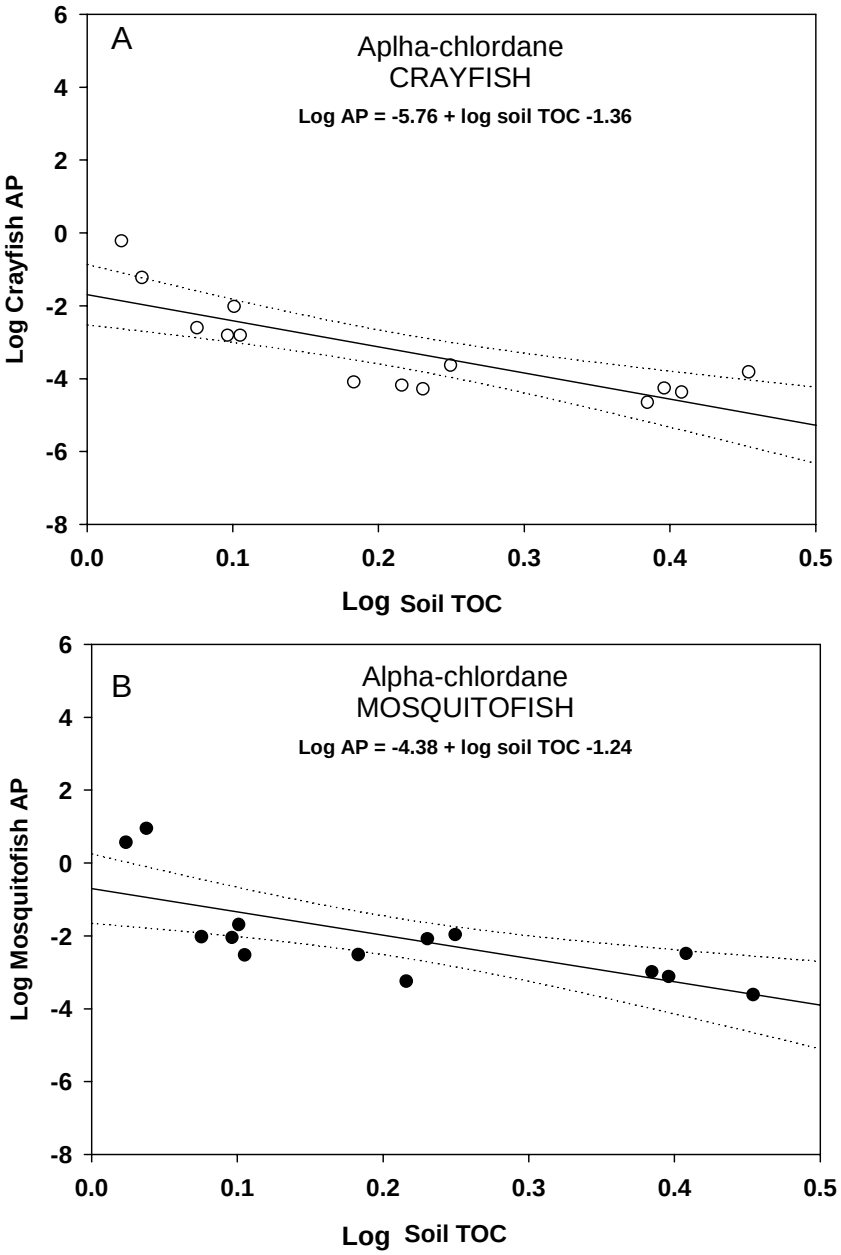


Figure 7-29. Relationship between log Accumulation Potential (AP, independent variable) and log soil total organic carbon (TOC, dependent variable) for alpha-chlordane in crayfish (A) and mosquitofish (B). There was a significant negative relationship between both variables in crayfish and mosquitofish (see Tables 7-3 and 7-4). Regression equations are given.

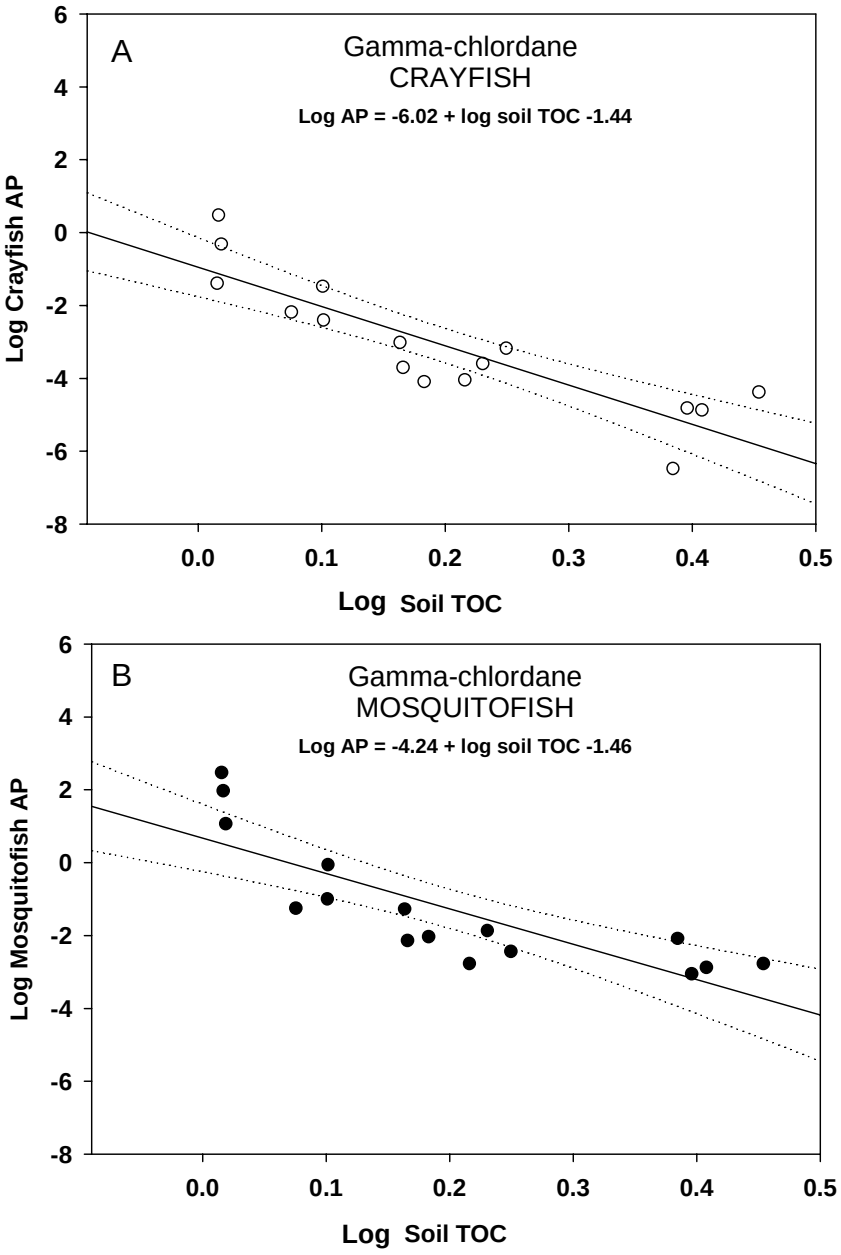


Figure 7-30. Relationship between log Accumulation Potential (AP, independent variable) and log soil total organic carbon (TOC, dependent variable) for gamma-chlordane in crayfish (A) and mosquitofish (B). There was a significant negative relationship between both variables in crayfish and mosquitofish (see Tables 7-3 and 7-4). Regression equations are given.

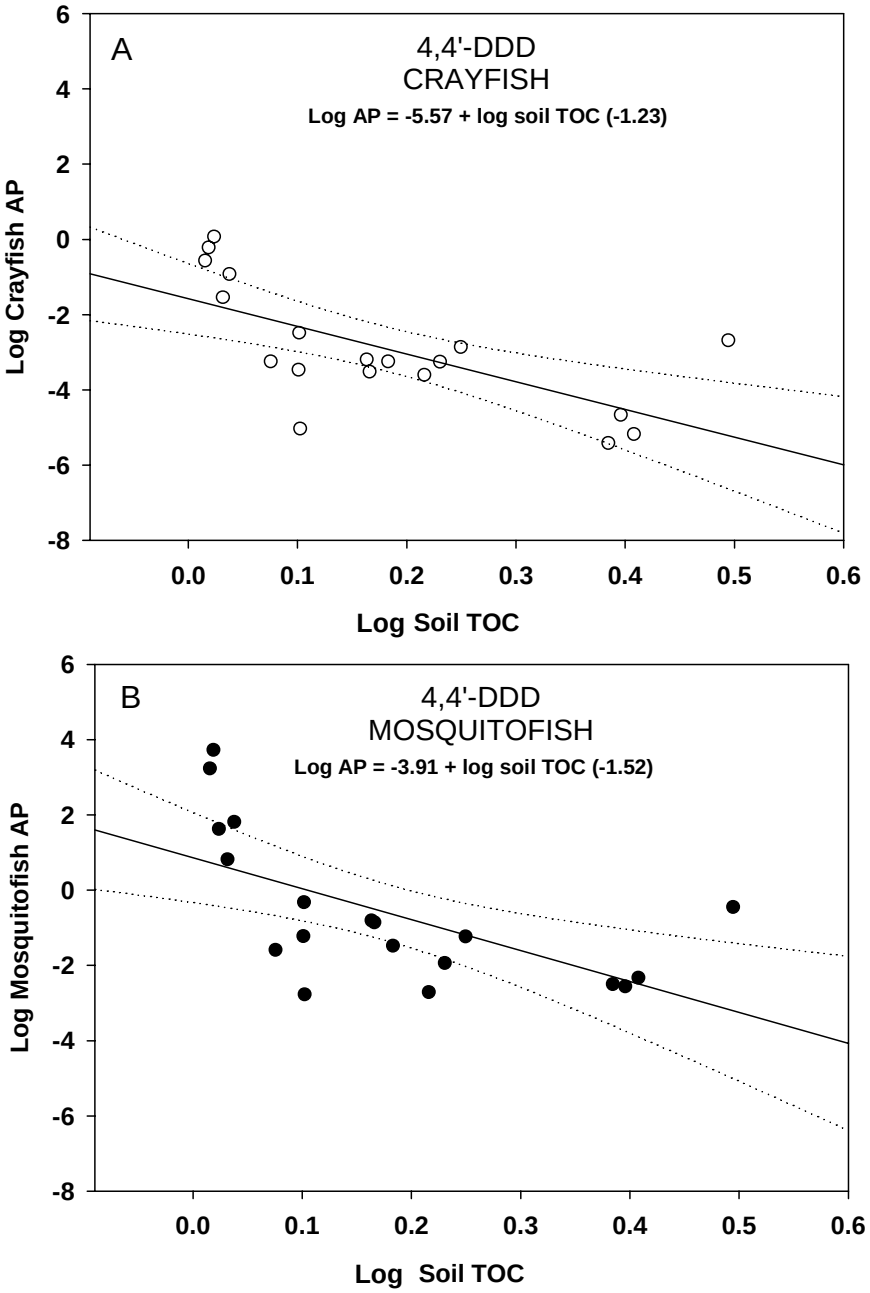


Figure 7-31. Relationship between log Accumulation Potential (AP, independent variable) and log soil total organic carbon (TOC, dependent variable) for 4,4'-DDD in crayfish (A) and mosquitofish (B). There was a significant negative relationship between both variables in crayfish and mosquitofish (see Tables 7-3 and 7-4). Regression equations are given.

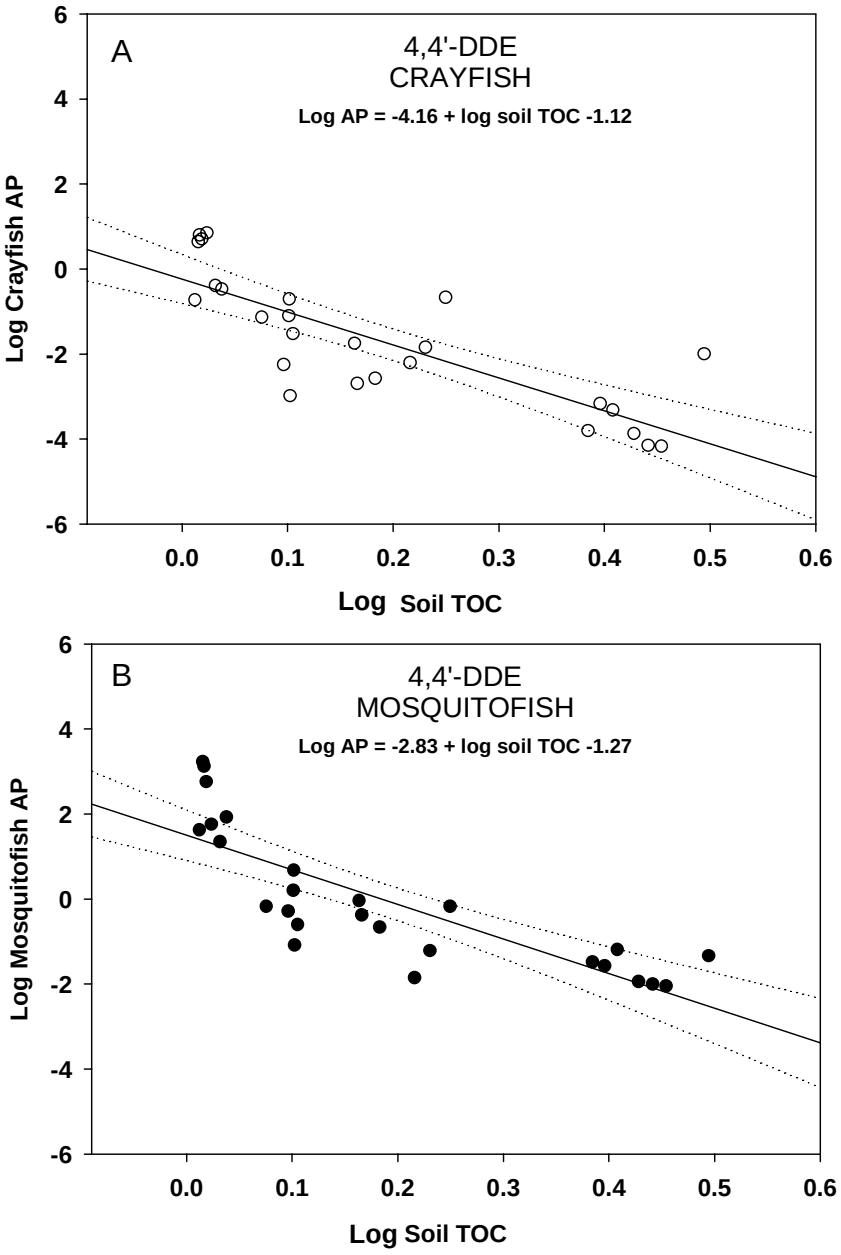


Figure 7-32. Relationship between log Accumulation Potential (AP, independent variable) and log soil total organic carbon (TOC, dependent variable) for 4,4'-DDE in crayfish (A) and mosquitofish (B). There was a significant negative relationship between both variables in crayfish and mosquitofish (see Tables 7-3 and 7-4). Regression equations are given.

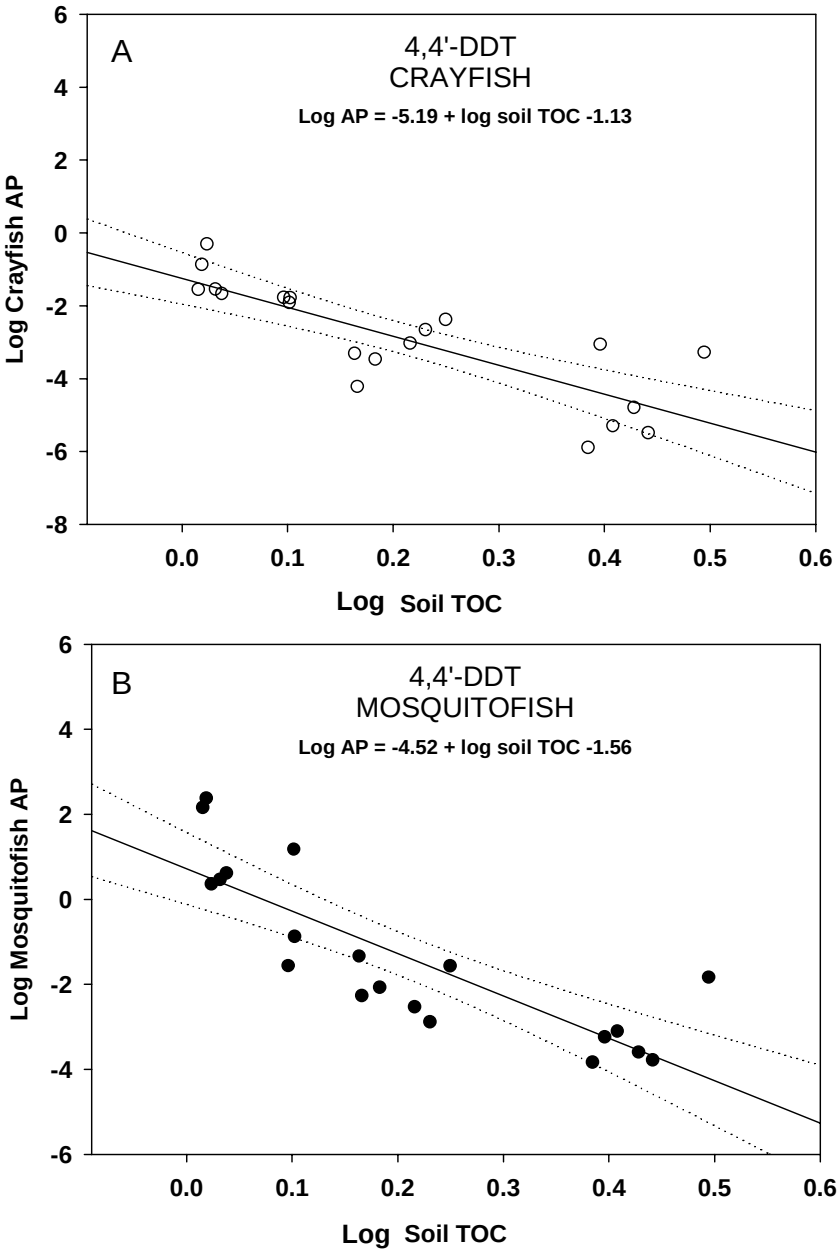


Figure 7-33. Relationship between log Accumulation Potential (AP, independent variable) and log soil total organic carbon (TOC, dependent variable) for 4,4'-DDT in crayfish (A) and mosquitofish (B). There was a significant negative relationship between both variables in crayfish and mosquitofish (see Tables 7-3 and 7-4). Regression equations are given.

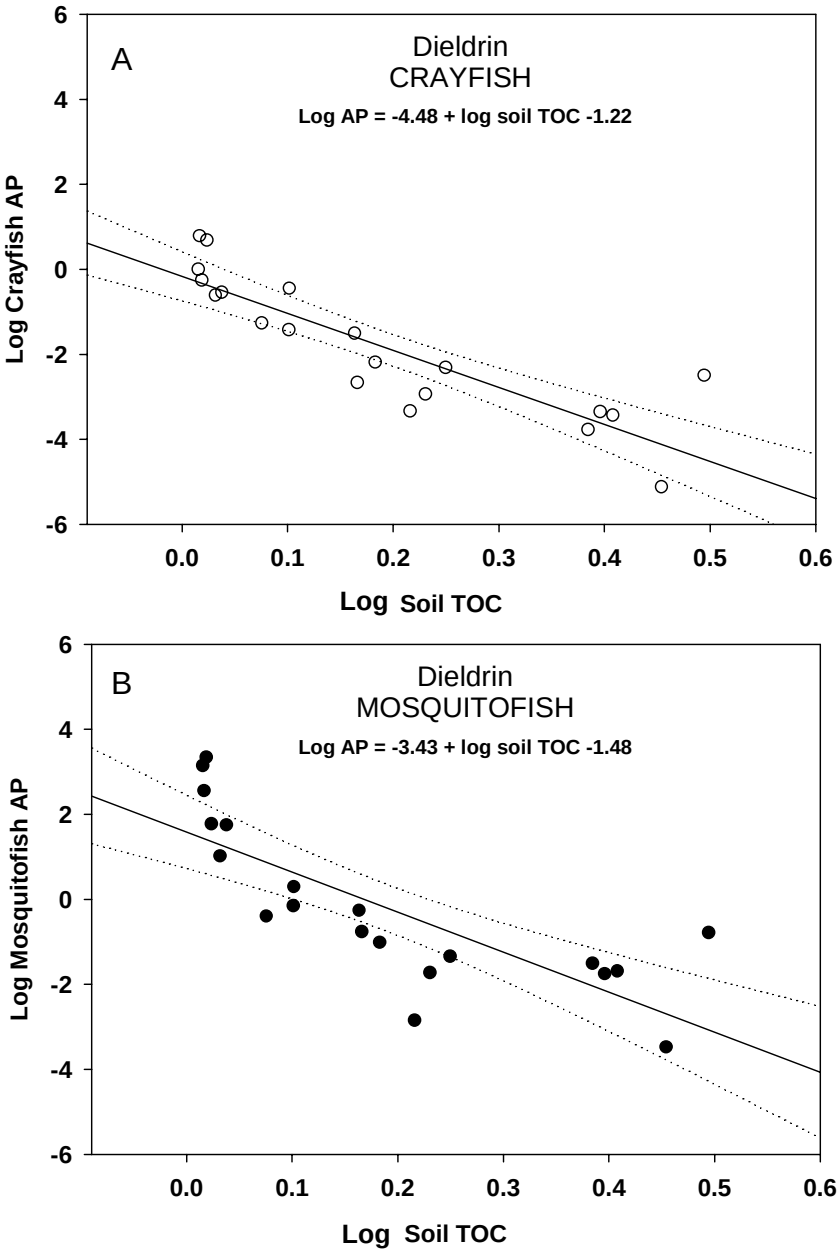


Figure 7-34. Relationship between log Accumulation Potential (AP, independent variable) and log soil total organic carbon (TOC, dependent variable) for dieldrin in crayfish (A) and mosquitofish (B). There was a significant negative relationship between both variables in crayfish and mosquitofish (see Tables 7-3 and 7-4). Regression equations are given.

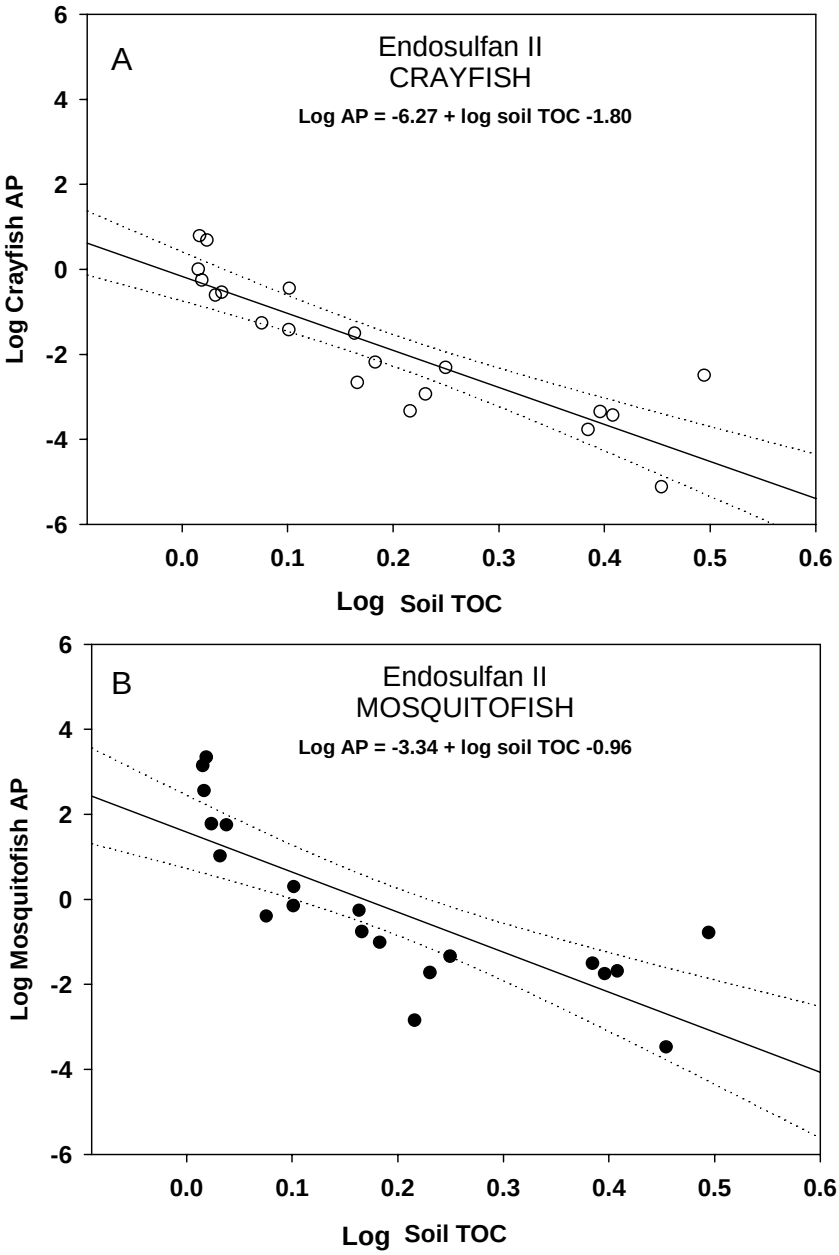


Figure 7-35. Relationship between log Accumulation Potential (AP, independent variable) and log soil total organic carbon (TOC, dependent variable) for endosulfan II in crayfish (A) and mosquitofish (B). There was a significant negative relationship between both variables in crayfish and mosquitofish (see Tables 7-3 and 7-4). Regression equations are given.

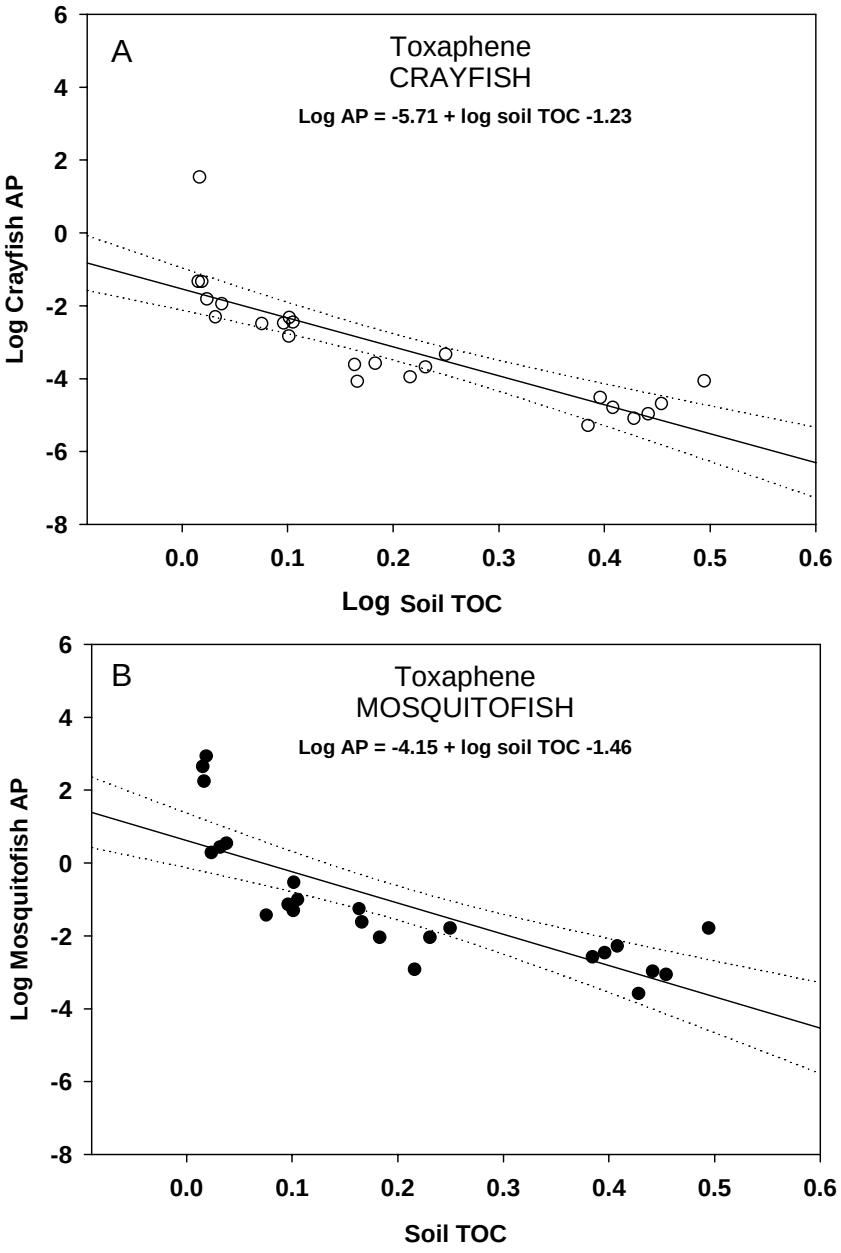


Figure 7-36. Relationship between log Accumulation Potential (AP, independent variable) and log soil total organic carbon (TOC, dependent variable) for toxaphene in crayfish (A) and mosquitofish (B). There was a significant negative relationship between both variables in crayfish and mosquitofish (see Tables 7-3 and 7-4). Regression equations are given.

CHAPTER 8
UNCERTAINTY EVALUATION

The main objective of this study was to calculate Biota Sediment Accumulation Factors (BSAFs) for different OCPs in two aquatic species exposed to contaminated sediments from the NSRA, Lake Apopka, Florida. Although several important conclusions were derived from these studies (see next section), these should be interpreted bearing in mind some potential sources of uncertainty. The most critical sources of uncertainty that could have affected the calculated BSAFs include: a) Inability of controlling some basic water quality parameters in the microcosms (Chapter 3); b) Heterogeneity of sediments across replicates (Chapter 4); c) Sensitivity of the analytical chemistry laboratory (Chapter 6); and d) Temporal variation in biota body composition and general health status due to captivity (Chapter 6). These are discussed in more detail below.

As already discussed, the amount of chemical bioaccumulation will depend on the *bioavailability* of contaminants as well as on *species-specific uptake* and *elimination* processes. There are several important chemical characteristics that influence bioavailability. The most important ones are molecular size and polarity. These will largely determine the extent and type of association with particles (e.g. degree of sorption, desorption, and precipitation). Large, nonpolar chemicals (such as OCPs) have low water solubility and a strong tendency to be associated with organic matter, and thus are less bioavailable. Uptake and elimination of chemicals from animals will

be affected by the organism's lipid content, its size, growth rate, gender, diet, and ability to metabolize or transform a given contaminant. In addition, environmental factors (such as temperature and dissolved oxygen, DO) can indirectly affect the uptake of chemicals by altering the metabolic rate and growth of organisms. For instance, low water temperatures can lead to decreased food consumption, and thus a decrease uptake of chemicals. In contrast, low DO concentrations can lead to increased ventilation rates and increased rates of chemical uptake.

Inability to Control Water Quality Parameters

Because microcosm tanks were kept outdoors, several water quality parameters were not controlled for and were subjected to changes over time. For instance, water temperature was highest during the fall (October) and lowest during the winter months (mid December to early January). Similarly, dissolved oxygen varied significantly over time. This temporal change followed an almost opposite trend to what was observed with temperature, with lowest and highest values observed during the fall and winter months, respectively. Other water quality parameters, such as pH, conductivity, turbidity (Chapter 3) and chlorophyll a (Chapter 5) also varied throughout the experiment. As already discussed, changes in water quality parameters over time could have affected the metabolic rate and general activity of fish and crayfish, and thus could have impacted the rate of uptake and/or elimination of contaminants and thus BSAFs.

Heterogeneity of Sediments Across Replicates

Another source of uncertainty relates to the evaluation of BSAFs using "artificial" soils created in the laboratory by mixing NSRA soils with either sand (to decrease TOC contents and OCP concentrations) or with peat (to decrease OCP concentrations) (see Table 2-2 and 2-3). Because of the large volume of soils needed for each microcosm

tank, and because mixing was done manually, some heterogeneity was observed across replicates within the same treatment (see Chapter 4, Figures 4-1 through 4-9). Variation across replicates was also observed in "naturally" occurring NSRA sediments. In addition, because of reasons not totally understood, concentrations in soil TOC and OCP also differed with time (weeks 0 and 16). Again, since most of the variance was observed in sediment treatments created by mixing peat and sand in the laboratory, it is possible that the observed differences were due to incomplete mixing and/or homogenization prior to analysis. This heterogeneity in sediments could be, at least in part, responsible for the variance observed in BSAFs across replicates, and introduces another source of error to these calculations.

Sensitivity of the Analytical Chemistry Laboratory

During the course of this study, a significant amount of OCP values were reported by the chemical analytical laboratory (EN CHEM) with a "U" data qualifier (see Figure 6-8). These are "non-detect" values and as such get assigned the laboratory method detection limit or MDL instead. For a significant group of OCPs (16 total), most of the values reported by En CHEM were non-detects (mean of 82 % of the values were "U", range of 60 – 99 %) (Figure 6-8). Because of this uncertainty, these chemicals were left out of more detailed analyses in this report. For the remaining OCPs (alpha and gamma-chlordane; 4,4'-DDD; 4,4'-DDE; 4,4'-DDT; endosulfan II; dieldrin; and toxaphene), "U" values were reported on average in 42 % of the samples submitted (range of 8 – 70 %). For these analytes, we chose to use half the concentration of these non-detect values for analyzes of OCP burdens in fish and crayfish, however, all these values were *excluded* in BSAF calculations. Although the objective of this approach was to decrease uncertainty, it is still of concern that many values could not

be included for BSAFs calculations, which if available, would have increased the sample size and thus decreased the variation observed in BSAFs.

Temporal Variation in Biota Body Composition and General Health Status due to Captivity

Temporal changes in lipid content were evident for crayfish and mosquitofish. Lipid contents steadily decreased over time in crayfish, and in mosquitofish lowest values were observed approximately in the middle of the study, with values increasing towards the end. This differential rate of lipid accumulation across species could due to several factors. First, since crayfish were much harder to retrieve from the tanks, the last sampling event (week 16) had a proportionally higher number of individuals (between 3 and 5/tank) than the previous time points (single animal/tank). This large sample size could have "skewed" the lipid determination towards a more "diluted" concentration. Second, crayfish could have been eating less than their fish counterparts, and thus accumulating less fat. This possibility is supported by the relatively small increase in the mass of crayfish in relationship to amount stocked, compared to mosquitofish. Lower food consumption could have been the result of behavioral differences between species. For instance, it is possible that crayfish did not adapt as well to captive conditions compared to mosquito fish, and thus not only ate less, but used more energy to deal with the increased stress conditions. Second, the type of food present (mostly phyto and zooplankton, see Chapter 5) likely were not adequate for a higher top predator such as the crayfish. Decreased food consumption could also have been a subtle effect induced by the OCPs themselves. And thirdly, crayfish could have been overstocked compared to mosquitofish. Overcrowding could have led to a decrease in food consumption due to a combination of stress and lack of

enough food. As already discussed, the amount of lipid present in an organisms is an important driving factor in determining the total uptake of lipophilic contaminants, and it's change over time due to weather and/or captivity constraints should have directly affected the BSAFs calculated here.

CHAPTER 9
GENERAL CONCLUSIONS AND FUTURE RESEARCH NEEDS

General Conclusions

The main objective of this study was to calculate Biota Sediment Accumulation Factors (BSAFs) for different OCPs in two aquatic species exposed to contaminated sediments from the NSRA, Lake Apopka, Florida. The results from the present study support the following conclusions:

1. In general, the experimental design of creating microcosm environments with different concentrations of soil OCPs and TOCs was achieved. Four distinctive and non-overlapping soil OCP and TOC groups were created by appropriate use of different NSRA soils, peat, and mixtures of NSRA soils with peat and/or sand. In addition, three true replicates per treatment were established.
2. Overall, the water quality parameters created in the microcosms were suitable for sustaining populations of crayfish and mosquitofish.
3. Crayfish, however, appeared to have adapted less than mosquitofish to captive conditions, and showed a decline in lipid content over the course of the study. Lipid contents in crayfish were also half compared to those measured in mosquitofish.

4. Exposure of crayfish and mosquitofish to contaminated soils resulted in measurable tissue concentrations of chemicals for most of the OCPs studied. Regardless of species, over 98% of all the OCPs bioaccumulated consisted of toxaphene > 4,4'-DDE > 4,4'-DDT > 4,4'-DDD > dieldrin. This pattern of bioaccumulation matched the distribution of OCPs in soils.
5. Regardless of species and treatment, chemical concentrations in tissues increased significantly during the first two weeks of experiment. From there on, chemical concentrations remained more or less constant until the end of the study (week 16), although with some variation due to outliers. It was concluded that animals reached steady-state after 4 weeks of exposure.
6. Overall, mosquitofish tended to bioaccumulate higher concentrations of OCPs compared to crayfish. However, when chemical data was normalized by lipid contents, these differences either disappeared or were lessened.
7. There was a significant positive relationship between the TOC-normalized soil OCP concentrations and the OCP lipid-normalized concentrations in crayfish and mosquitofish for most of the chemicals studied. The exceptions were aldrin, endosulfan sulfate, and endrin ketone for both biota species, and heptachlor epoxide and methoxychlor for mosquitofish.
8. For some of the treatments, mosquitofish and crayfish had body burdens of OCPs that fell within survival threshold values previously reported for other freshwater fish species. This is a significant finding because it would suggest that these species are less likely to die if exposed to relatively high

concentrations of OCPs. Effects on growth and reproduction, however, generally occur at lower doses compared to mortality, and thus should be kept in mind and potentially considered in future studies.

9. Overall, BSAFs were higher (1.5 to 2.6 times) in mosquitofish compared to crayfish. Exceptions to this pattern were observed with dieldrin, gamma-chlordane, and 4,4'-DDT for which no significant differences were found between the two biota types.
10. Log K_{ow} was not a driving factor in the BSAFs attained. The lack of a significant relationship could be attributed to the relatively low range of log K_{ow} in this study (from 3.7 for endosulfan sulfate to 6.8 for 4,4'-DDT) and/or to the potential effects of weathering on the K_{ow} of the compounds studied.
11. The OCPs with highest BSAF in this study were metabolites of DDT, namely 4,4'-DDE and 4,4'-DDD. The reason behind the higher BSAFs for metabolites as compared to that of parent compounds remains unknown at this time.
12. Regardless of species and type of OCP, the results obtained in the present study would suggest that soil TOC is an important factor driving the magnitude of bioaccumulation in biota. There was a significantly negative relationship between soil TOC and Accumulation Potential (AP) for the majority of the OCPs studied. A decline in AP with increasing TOC pairs well with results obtained elsewhere. The present study constitutes the first to evaluate BSAFs under very high TOC conditions (up to 40%).

13. Similar to other studies, BSAFs were variable and in many instances were high and exceeded the theoretical limit of 1 – 2. This large range in BSAFs across studies may limit the use of this model as the only method for screening the bioaccumulative potential of sediments, and strengthens the need for determination of specific BSAF values on a case by case basis. Nevertheless, the BSAFs reported in the present study, fall within ranges previously reported for other species and, despite all of the uncertainties, these values could still be used for estimating OCPs concentrations in tissues of fish-eating birds inhabiting NSRA flooded marshes.

Future Research Needs

Although the amount of data on BSAFs created during the course of this study is one of the largest ones currently available, there are still many unanswered questions. The following is a list of some suggestions that should help improve our knowledge and understanding on the bioaccumulation of OCPs in biota.

Studies on Factors Affecting Bioaccumulation of OCPs in Biota

In theory, the movement of hydrophobic compounds between the two carbon pools (lipids in tissues and organics in soil) should be independent of sediment type, biota species, or type of hydrophobic chemical, and BSAFs should approach an approximate value between 1 and 2. However, we know from this and other studies that BSAFs are highly more variable and approach much higher values than predicted. More studies should be conducted to better understand the factors responsible for this variation. So far we know that soil TOC plays an important role in bioavailability and thus bioaccumulation potential of OCPs. But what about other soil parameters such as

pore size? In addition, we found differences in accumulation amounts between two biota species. Were these differences mainly driven by the total amount of lipid present, or were other factors such as metabolic and clearance rates involved? How much of a role does biomagnification play in the BSAFs attained?

Field Studies on BSAFs

The results on BSAFs obtained using NSRA soils under microcosm conditions, should be compared with field studies conducted in flooded NSRA soils. These studies are actually ongoing, and several mesocosm ponds have been built in different areas within the NSRA. The comparison between both types of studies will further validate the use of BSAFs as a tool for assessing soil quality.

Studies on Potential Effects of OCPs in Biota

Since some of the OCP values attained by biota in this study were within the range known to affect survival on other aquatic species, it is important that future studies assess the potential effects of these chemicals on growth and reproduction. If the goal is to restore the NSRA to marshland conditions that will allow the establishment of healthy populations of fish-eating birds, then healthy populations of invertebrates and fish need to be maintained to support them.

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