# DIETARY EXPOSURE TO ORGANOCHLORINE PESTICIDES *p,p*'-DDE AND DIELDRIN AND THEIR EFFECTS ON STEROIDOGENESIS AND REPRODUCTIVE SUCCESS IN FLORIDA LARGEMOUTH BASS (*Micropterus salmoides floridanus*)

By

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Previous work has indicated that high organochlorine pesticide (OCP) concentrations in tissues of Florida largemouth bass (*Micropterus salmoides floridanus*), sampled from reclaimed agriculture lands within the St. Johns River Water Management District's Emeralda Marsh Conservation Area (EMCA) have been associated with reproductive abnormalities, including depressed hormone concentrations. Two of the OCPs found in highest concentration at this site are *p,p*'-DDE and dieldrin. For my first study, hatchery-reared Florida largemouth bass were fed for 30 days using chemically treated floating pelletted feed. Twenty largemouth bass, 10 males and 10 females per tank, were placed into each of nine treatments in replicate: Control; 1, 7, 35, and 136 µg/g *p,p*'-DDE; and 0.03, 0.1, 0.6, and 5 µg/g Dieldrin. After day 30, five males and five females per replicate were sacrificed, had their blood and plasma collected for circulating sex steroid hormone analysis, and gonads collected for contaminant analysis and

calculation of GSI. Gonads and carcasses from one male and one per replicate were also analyzed for OCPs, revealing a consistent correlation between the administered doses and the concentrations found in the gonads and carcasses for both *p,p*'-DDE and dieldrin. Final carcass concentrations for both *p,p*'-DDE and dieldrin were similar to those found in largemouth bass from the EMCA. Histological analysis of gonadal tissue indicated that all examined fish were sexually mature. GSI did not vary with dose of *p,p*'-DDE or dieldrin. Analysis of sex steroid hormones also revealed no consistent relationships between *p,p*'-DDE or dieldrin dosages and circulating concentrations of 17β-estradiol or 11-ketotestosterone.

For my second study, *p,p*'-DDE and dieldrin exposure length was extended to a 120-day period, between the months of November and March, encompassing a larger portion of the steroidogenic and gametogenic portions of the reproductive cycle. One hundred largemouth bass were placed into each of seven treatments: Control; 5, 46 and 50 µg/g *p,p*'-DDE; and 0.04, 0.4, and 0.8 µg/g Dieldrin. On day 0 of the experiment, 24 fish were sampled to collect background measurements; then approximately every 30 days, six males and six females per treatment were sampled to collect measurements on the same reproductive biomarkers. Extension of exposure length demonstrated reductions in female $E_2$ concentrations, a lack of expected seasonal increasing trend in female $E_2$ concentrations, and abnormal increases in female 11-KT concentrations, similar to sex steroid hormone abnormalities reported for largemouth bass from the EMCA. Attained OCP carcass concentrations and achieved depressions of female $E_2$ concentrations did not translate into a reduction of percent hatch of eggs produced by these largemouth bass with high *p,p*'-DDE and dieldrin tissue concentrations.

# CHAPTER 1
# INTRODUCTION

## The Situation

Sawgrass marshes surrounding a number of central Florida lakes were drained for farming (muck farms) between the mid-1900s and the 1980s. In the 1990s, the State of Florida began purchasing muck farms in order to restore the farms back to their original floodplain marsh ecosystems. Between 1991 and 1994, the St. Johns River Water Management District (SJRWMD) acquired a 2,630-hectare portion of muck farms along the north-east shore of Lake Griffin. The site was designated the Emeralda Marsh Conservation Area (EMCA) and was to be used as a tool to reduce nutrient loading into Lake Griffin (Marburger *et al*., 1999).

Once flooding of the EMCA started in 1992, the Florida Fish and Wildlife Conservation Commission (FFWCC) began stocking forage and game fish into these systems in an attempt to establish public-accessible sport fish populations. FFWCC reported limited success establishing reproducing game fish populations. Of great concern was the apparent limited reproduction by stocked adult Florida largemouth bass (*Micropterus salmoides floridanus*) or recruitment of largemouth bass to the fingerling stage. FFWCC fish population surveys, conducted on several of the flooded properties in 1995 and 1996, however, revealed excellent growth rates for adult and fingerling largemouth bass (Benton and Douglas, 1996; Marburger *et al*., 1999).

The reason or reasons for the low largemouth bass recruitment at the EMCA were unclear, but there was speculation that poor reproductive success might be related to the

presence of residual organochlorine pesticides (OCPs). OCPs were produced after World War II and were widely used throughout the United States for crop pest control on muck farms. According to standard operating practices, OCPs were selectively applied as pre-emergence soil insecticides for vegetable production. Pesticides included DDT derivatives, dieldrin, aldrin, endrin, chlordane, and heptachlor. The U.S. Environmental Protection Agency (USEPA), however, began to restrict or ban the use of many of these OCPs on agricultural lands between 1978 and 1983 because of their environmental persistence and their ability to biomagnify in food webs (USEPA, 1990). A study of OCP levels, in soil and largemouth bass tissues from the EMCA, demonstrated soil concentrations of *p,p*'-DDE, dieldrin, and toxaphene to be over 3,000, 500, and 40,000 ng/g, respectively. The same study also revealed concentrations of OCPs in largemouth bass ovaries and fat reached over 4,000 and 17,000 ng/g, respectively, for total DDT derivatives, over 100 and 700 ng/g for dieldrin, and over 4,000 and 20,000 ng/g for toxaphene (Marburger *et al*., 2002).

This evidence advanced the hypothesis that the low largemouth bass reproductive success in the EMCA could be related to OCPs. Research, therefore, became focused on determining the extent of pesticide contamination in largemouth bass found in the waters of the reclaimed muck farms adjacent to both Lakes Apopka and Griffin. Marburger *et al*. (1999) suggested that largemouth bass were bioaccumulating OCPs due to their top predator status and the persistence of OCPs in the muck farm soils. In addition to high OCP concentrations in tissues of largemouth bass sampled from the EMCA, depressed sex steroid hormone (i.e., 17β-estradiol and 11-ketotestosterone) concentrations were also reported (Marburger *et al*., 1999). In addition, monthly sex steroid hormone values of

both male and female tagged largemouth bass, captured from the EMCA, demonstrated that sex steroid hormone concentrations remained low throughout the year and showed no seasonal trends (Marburger *et al*., 1999). However, there have been no experimental studies directly demonstrating any causal relationship between low sex steroid hormone concentrations and reproductive success (Benton and Douglas, 1996).

## Largemouth Bass Reproductive Biology

Reproductive processes of teleost fishes, including the Florida largemouth bass, are well defined. Chew (1974) described the early life history traits of Florida largemouth bass and found that sexual maturity is generally achieved at a total length (TL) of 250 mm, a length that can be obtained in only 1 year. Mature largemouth bass in Florida are capable of spawning between mid-November to August, with a peak spawning period in February and March (Clugston, 1966). Largemouth bass are synchronous spawners, an act that is mainly triggered by a rise in water temperature during the spring months to a level between 20 and 24°C. It is also reported that spawning generally ceases at water temperatures below 18°C, and above 27°C (Clugston, 1966). Largemouth bass fecundity is highly variable, ranging from 2,000 to 145,000 eggs per female, but is generally accepted that females average about 4,000 eggs per pound of body weight (Tidwell *et al*., 2000). Fecundity also appears to be directly related to age, condition, size, and to some environmental factors such as water temperature (Chew, 1974).

The reproductive biology of teleost fishes follows a well-defined cycle that is regulated by exogenous environmental cues such as photoperiod and temperature, and endogenous hormonal cues (Gross *et al*., 2002). This process is dependent on the coordinated actions of hormones associated with the brain-hypothalamus-pituitary-gonad axis (Van Der Kraak *et al*., 1998). The hypothalamus controls the synthesis and release

of gonadotropin-releasing hormone (GnRH), resulting from neural stimulation of the central nervous system. This messenger hormone controls the synthesis and release of the primary teleost gonadotropin hormones GTH-I and GTH-II from the pituitary. These two gonadotropins are the regulators of reproduction and are analogous to mammalian follicle-stimulating hormone (FSH) and luteinizing hormone (LH), respectively (Redding and Patino, 1993). GTH-I is typically involved in stimulating events leading to vitellogenesis or spermatogenesis and early gonadal development, whereas GTH-II is typically involved in stimulating events leading to final oocyte maturation and ovulation in females and spermiation in males. Despite the difference with regards to the role of GTH-I and GTH-II in female and male fish, these gonadotropins are known to be responsible for stimulating steriodogenesis or the synthesis of sex steroid hormones (androgens, estrogens, and progestins), which, in turn, act on target tissues to regulate gametogenesis (Van Der Kraak *et al*., 1998).

In the majority of female and male teleosts, 17β-Estradiol and 11-Ketotestosterone are the primary sex steroid hormones responsible for regulating gametogenesis, and increases in plasma concentrations of these hormones are associated with the onset of seasonal reproductive activity (Gross *et al*., 2002). 17β-estradiol and 11-ketotestosterone are the same hormones reported to have depressed concentrations throughout the year for largemouth bass from the EMCA (Marburger *et al*., 1999).

In female fish, the development of oogenesis is controlled by GTH-I (Redding and Patino, 1993). Plasma concentrations of GTH-I increase during early oocyte development and bind to receptors on follicle cells. The cells synthesize testosterone and allow for aromatization to result in the formation of estradiol. Subsequently, estradiol is

released by the follicle cells into the blood, where it binds to estrogen receptors in the liver, initiatimg a cascade of events resulting in the production of vitellogenin (vitellogenesis), a precursor to egg yolk protein, produced by the liver (Wahli *et al*., 1981). Vitellogenin is released from the liver into the blood and binds to receptors on the oocytes which incorporate the protein as a nutrient source. As development of the oocytes continues, concentrations of GTH-I begin to decrease and are replaced by increasing concentrations of GTH-II (Van Der Kraak *et al*., 1998). Receptors for GTH-II are found predominately on the granulosa cells of the follicles and binding stimulates the synthesis and release of progestins, which play a role in final gamete maturation and stimulates ovulation (Redding and Patino, 1993; Van Der Kraak *et al*., 1998). Similarly, in male fish, GTH-I is typically elevated throughout spermatogenesis and decreases at the time of spawning, whereas GTH-II is typically low throughout the growth process and is elevated at spawning. These gonadotropins stimulate proliferation of spermagonia as well as the synthesis of androgens required for gametogenesis in male fish (Nagahama, 1994; Van Der Kraak *et al*., 1998).

Vitellogenesis in oviparous fish is the principle event contributing to the massive growth of oocytes, due to a rapid uptake of the egg yolk precursor vitellogenin (Wallace and Selman, 1981). Once vitellogenin is taken up by the vitellogenin receptors on the surface of an oocyte, it is cleaved into smaller yolk proteins. These proteins are then incorporated into yolk granules, which account for about 90 percent of the protein content of mature oocytes. The yolk granules are stored during oogenesis and serve as a nutrient source for embryonic development (Wahli *et al*., 1981).

Vitellogenesis ceases once the oocytes reach their fully developed size, and is followed by a period of maturation (Wallace and Selman, 1981). During this time, follicles increase in volume due to hydration and accumulation of other vital proteins. Protein uptake stops at the time of germinal vesicle breakdown. The follicle however, continues to increase in volume by hydration. It is during this time that the chorion, the cellular envelope which surrounds an egg in preparation for ovulation, begins to develop. The timing of ovulation is species specific and takes place when follicles reach a specific size (Nelson, 2001; Wallace and Selman, 1981). Vitellogenesis represents a critical process in the development of teleost oocytes. This process is initiated by seasonal changes in estradiol concentrations, however, monthly sex steroid hormone values of female largemouth bass from the EMCA demonstrated that sex steroid hormone concentrations remained low throughout the year and showed no seasonal trends (Marburger *et al*., 1999). A lack of expected seasonal trends in estradiol concentrations could have detrimental effects on vitellogenesis.

Gross *et al*. (2002) characterized the annual cycles of circulating sex steroid hormones, vitellogenin, and gonad development over a one-year period for pond-reared Florida largemouth bass in the state of Florida. Plasma samples for both male and female largemouth bass were analyzed for 17β-estradiol ($E_2$), 11-tetotestosterone (11-KT), testosterone (T), and vitellogenin (VTG). For males, 11-KT was the predominate androgen, and the only sex steroid observed to show a strong seasonal pattern which had a peak concentration of about 2,800 pg/mL in February. Even though T did show a seasonal pattern, the peak concentration of this steroid in March was less than one-half that of the February peak for 11-KT, supporting the idea that 11-KT is the predominate

androgen synthesized for endocrine function in male teleosts. $E_2$ was detected in male largemouth bass, but at concentrations of about one-third that of females (Gross *et al*., 2002). Females showed distinct seasonal patterns for $E_2$, T, and VTG. $E_2$ showed the strongest pattern with circulating concentrations nearly twice those of T, with a peak concentration of almost 4,000 pg/mL in February; however, T did follow a similar seasonal pattern and peaked at the same time that $E_2$ peaked. As previously mentioned, follicles must first synthesize testosterone before estradiol is formed, and may explain the similar seasonal trends demonstrated by both of these hormones. 11-KT was detected in females, but at concentrations nearly one-half that of males. Circulating VTG concentrations closely mimicked those of $E_2$, rising in November and peaking in January at about 6 mg/ml. 17β-estradiol is one of the sex steroid hormones reported to have depressed concentrations throughout the year for largemouth bass from the EMCA. Vitellogenin is synthesized by the liver in response to estradiol production; however, it is believed that changes in seasonal concentrations of this sex steroid hormone could lead to decreased vitellogenin production, causing impaired female oocyte development (Muller, 2003). Interruption of the vitellogenic process could then have detrimental effects on oogenesis, ultimately leading to developmental abnormalities, increased sac fry mortality, and even spawning inhibition (Burdick *et al*., 1964; Cross and Hose, 1988; Hose *et al*., 1989; Macek, 1968; Monod, 1985; Smith and Cole, 1973).

Gross *et al*. (2002) also calculated annual changes in gonadosomatic index (GSI), a number that reflects the percent total body weight which the gonad comprises for a fish for pond-reared Florida largemouth bass in the state of Florida. Largemouth bass GSI was found to show similar seasonal changes regardless of sex. GSI began to rise in

November, peaking between January and May at a mean of approximately 5 and 2% for female and male largemouth bass, respectively. Dramatic decreases in GSI were reported between July and October. Reproductive seasonal changes in gonad maturation, as indicated by tissue histology, were also correlated to changes in GSI and sex steroids in both sexes, and to fluctuations in VTG in females (Gross *et al*., 2002). In female largemouth bass, gonadal stage was characterized as undeveloped (stage1) between May and July, as (stage 2) previtellogenic between August and October, and as (stages 3 and 4) vitellogenic ovaries between November and April. In male largemouth bass, gonadal stage was generally characterized as having low spermatogenic activity between June and November and moderate to high spermatogenic activity between December and May (Gross *et al*., 2002).

**Endocrine Disruption and Reproductive Effects of OCP Exposure**

In both fish and wildlife, the majority of research concerning potential biological effects of OCPs has been focused on endocrine disruption (Gallagher *et al*., 2001; Gross *et al*., 1994; Guillette *et al*., 1994; Mills *et al*., 2001; Muller *et al*., 2004). Altered sex steroid concentrations in fish, environmentally and experimentally exposed to OCPs, could result from the disruptive effects of these chemicals on hypothalamic or pituitary gonadotropin hormones (Gore, 2002; Shukla and Pandey, 1986; Spies and Thomas, 1995). Gonadotropin hormones serve as stimulating hormones for the synthesis and secretion of sex steroid hormones, and any alteration in normal concentrations of these hormones could ultimately lead to decreased sex steroid production. Largemouth bass from the EMCA have demonstrated depressed sex steroid hormone (17β-estradiol and 11-ketotestosterone) concentrations, and could be the result of a disruption in the

hypothalamus-pituitary-gonad axis, responsible for stimulating synthesis and secretion of sex steroid hormones (Gross *et al*., 2003).

Competitive binding of these exogenous compounds to estrogen receptors could severely affect normal estrogen function, leading to depressed hormone concentrations, impaired gonadal development, decreased vitellogenesis, and ultimately poor egg quality (Muller, 2003). This hypothesis is based on recent studies that demonstrated a significant depression of genes responsible for vitellogenesis and egg chorion development in largemouth bass, when exposed to *p,p*'-DDE (Larkin *et al*., 2002). Cultured ovarian cells, treated with *p,p*'-DDE, exhibited an inhibition of steroid synthesis as a consequence of FSH receptor interference (Chedrese and Feyles, 2001). DDT derivatives have also demonstrated the ability to displace estrogen by competitively binding with estrogen receptors (Danzo *et al*., 2002; Larkin *et al*., 2002; Matthews *et al*., 2000; Spies and Thomas, 1995; Vonier *et al*., 1996). Other prominent OCPs, including dieldrin, methoxychlor, and endosulfan, have also demonstrated the ability to bind to estrogen receptors (Matthews *et al*., 2000; Tollefsen *et al*., 2002). Alternatively, dieldrin and DDT derivatives have demonstrated the ability to inhibit androgen binding to the androgen receptors, thereby preventing the transcription of testosterone, resulting in demasculinization (Baatrup and Junge, 2001; Bayley *et al*., 2002; Danzo *et al*., 1997; Foster *et al*., 2001; Kelce *et al*., 1995; Wells and Van Der Kraak, 2000).

The effect of OCPs on reproduction and early-life stages of development in fish has also been a focus of research. As previously mentioned, vitellogenesis, represents a critical process in the development of teleost oocytes. This process is responsible for the major source of nutrition during embryonic and early-life stage development. It is

hypothesized then, that any effect on this process by an anthropogenic compound could have detrimental effects on oogenesis, embryonic development, hatching and larval survival. Decreased egg production and survival of early-life stages could in turn affect recruitment and have significant population-level effects (Muller, 2003).

Exposure of several fish species to DDT and its derivatives have resulted in decreased fertilization and increased embryo and fry mortality (Burdick *et al*., 1964; Macek, 1968; Monod, 1985). White croaker *Genyonemus lineatus*, environmentally exposed to DDT at ovarian DDT concentrations of 4 mg/L and above, demonstrated an inability to spawn (Cross and Hose, 1988; Hose *et al*., 1989). Exposure to aqueous sublethal concentrations of DDT have also resulted in fry vertebral deformities at time of hatch. Deformities included erosion and hemorrhaging at the vertebral junctions, and were found in fry which hatched from eggs containing DDT concentrations equal to or exceeding 2.39 mg/L (Smith and Cole, 1973).

Exposure of fish and wildlife to OCPs can result in a myriad of negative endocrine effects including inhibition or suppression of GnRH and gonadotropin hormones, necrosis of gonadotroph cells responsible for the secretion of gonadotropin hormones, and estrogen and androgen receptor binding; all of which could ultimately lead to decreased sex steroid production (Baatrup and Junge, 2001; Bayley *et al*., 2002; Chedrese and Feyles, 2001; Danzo *et al*., 1997; Danzo *et al*., 2002; Foster *et al*., 2001; Gore, 2002; Kelce *et al*., 1995; Larkin *et al*., 2002; Matthews *et al*., 2000; Shukla and Pandey, 1986; Spies and Thomas, 1995; Tollefsen *et al*., 2002; Vonier *et al*., 1996; Wells and Van Der Kraak, 2000). Depressed hormone concentrations could then lead to impaired gonadal development and decreased vitellogenesis. Interruption of the vitellogenic process could

then have detrimental effects on oogenesis, ultimately leading to developmental abnormalities, increased sac fry mortality, and even spawning inhibition. Decreased survival of early-life stages could in turn affect recruitment and have significant population-level effects. Definitive causes and mechanisms of endocrine disruption and reproductive effects following OCP exposure are still under investigation (Muller *et al*., 2004); however, it is for these reasons that reproductive abnormalities reported for largemouth bass in the EMCA may be occurring.

**Research Significance**

Because largemouth bass are an important sport fish in Florida and nationally, much effort is being placed into the restoration of a viable fishery in the EMCA and Upper Ocklawaha River Basin (Benton *et al*., 1991; Benton and Douglas, 1996; Marburger *et al*., 1999). For this reason, I chose the Florida largemouth bass as an animal model for laboratory studies of OCP exposure. This research aims to determine whole carcass and gonad OCP concentrations, and to compare several health parameters and reproductive biomarkers [weight, length, condition index (K), hepatosomatic index (HSI), gonadosomatic index (GSI), circulating sex steroid hormones, and percent hatch] for Florida largemouth bass following dietary exposure to *p,p*'-DDE and dieldrin, two predominant OCPs found in the EMCA. Single chemical exposures were therefore performed to assess the potential contribution of each pesticide to overall reproductive function and steroidogenic declines found to occur in largemouth bass environmentally exposed to these contaminants in the EMCA (Marburger *et al*., 1999).

Because these pesticides have been shown to act as endocrine system modulators in other animals (Gallagher *et al*., 2001; Gross *et al*., 1994; Guillette *et al*., 1994; Mills *et al*., 2001; Muller *et al*., 2004), it is hypothesized that dietary exposure, of largemouth

bass to either *p,p*'-DDE or dieldrin at increasing concentrations, will cause a dose-response decrease in circulating sex steroid hormones, for both male and female largemouth bass. This, in turn, should alter gonad development and cause a dose-response decrease in GSI. Altered female largemouth bass oocyte gonad development, in turn, should cause a dose-response decrease in the number of fry that hatch from clutches produced by these treated largemouth bass administered pesticide doses at sub-lethal concentrations, similar to wild caught adult largemouth bass, at contaminated sites (Marburger *et al*., 1999). Therefore, no dose-response decreases on weight, length, and condition index (K) of the largemouth bass should be seen for either sex.

# CHAPTER 2
# DIETARY SUBACUTE EXPOSURE TO *P,P*'-DDE AND DIELDRIN AND THEIR EFFECTS ON REPRODUCTIVE AND HEALTH BIOMARKERS IN FLORIDA LARGEMOUTH BASS

## Introduction

Levels of organochlorine pesticide (OCP) concentrations in tissues of Florida largemouth bass (*Micropterus salmoides floridanus*) and American alligators (*Alligator mississippiensis*) sampled from reclaimed agriculture lands within the Ocklawaha River Basin and St. Johns River Water Management District's Emeralda Marsh Conservation Area (EMCA) have been associated with reproductive abnormalities, including depressed and/or reversed sex steroid hormone concentrations (Gross *et al*., 1994; Marburger *et al*. 1999). Two of the OCPs found in highest concentration at these sites are *p,p*'-DDE and dieldrin (Marburger *et al*. 2002).

The objectives of this study were to determine whole carcass and gonad OCP concentrations for Florida largemouth bass, and to compare several health parameters and reproductive biomarkers [weight, length, condition index (K), hepatosomatic index (HSI), gonadosomatic index (GSI), and circulating sex steroid hormones] following a 30-day dietary exposure period to *p,p*'-DDE and dieldrin. *p,p*'-DDE and dieldrin, were chosen to evaluate single chemical dose-response relationships for the health and reproductive biomarkers listed above. Doses for both pesticides were chosen to create whole carcass concentrations similar to those reported for wild largemouth bass in reclaimed agricultural areas in central-Florida (Marburger *et al*. 1999 and 2002), and were based on a feeding rate and percent accumulation for a 30-day largemouth bass

*p,p*'-DDE and dieldrin dietary exposure study described in Muller *et al*. (2004). Characterization of the possible effects that these two pesticides have on these biomarkers will assist in determining if these chemicals act alone as a causative agent to impair the endocrine system of largemouth bass.

## Materials and Methods

### Largemouth Bass

Hatchery-reared two-year-old Florida largemouth bass, with a mean body weight of 150 g, were obtained from American Sportfish Hatchery, Montgomery, AL on March 10, 2003.

### Feed Preparation

Chemically treated floating pelletted feed was developed using methods modified from those described by Muller *et al*. (2004). Organochlorine pesticides *p,p*'-DDE (2,2-bis(4-chlorophenyl)-1,1-dichloroethylene, Lot # 09020KU, 99% purity) and dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-dimethanonaphthalene, Lot # 77H3578, 90% purity) were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO). The pesticides were mixed into menhaden fish oil supplied by Zeigler Brothers, Inc. (Gardners, PA) to form two separate concentrated stock solutions (2.5 g *p,p*'-DDE/50 mL fish oil and 1.6 g dieldrin/100 mL fish oil) The stock solutions were then shipped to Zeigler Brothers, Inc., where they were used as a top-dressing to coat Silver Finfish floating pelletted feed. The pesticide-laden floating feed was manufactured by first diluting a measured amount of the stock solution into menhaden fish oil. This fish oil/stock solution mixture was then added into a mixer containing the pelleted feed to achieve a consistent coating of all pellets. Continued dilutions of both the *p,p*'-DDE and dieldrin stock solutions allowed for pesticide-laden

feed of both chemicals to be manufactured at different concentrations. Control feed was also manufactured by combining the floating pelletted feed with a top dressing of pure menhaden fish oil.

All pesticide-laden feeds and one sample of the control feed were sent to New Jersey Feed Laboratory, Inc. (Trenton, NJ) by Zeigler Brothers, Inc. for a chlorinated pesticide OCP screen analysis. The control feeds had no detectable levels of organochlorine pesticides. Target feed doses of 1, 7, 37, and 185 µg/g *p,p*'-DDE had actual concentrations of 1, 7, 35, and 136 µg/g *p,p*'-DDE, respectively. Target feed doses of 0.02, 0.1, 0.6, and 3 µg/g dieldrin had actual concentrations of 0.03, 0.1, 0.6, and 5 µg/g dieldrin, respectively.

**Experimental Design**

The largemouth bass were housed in groups of 20 fish (10 males and 10 females) in 18 separate 700-liter round plastic tanks, equipped with a flow-though water system supplied by on-site well water and aeration. Fish sex was determined by external examination of the urogenital pore or by palpating to evaluate the release of eggs or milt. Water quality parameters: temperature, dissolved oxygen, pH, and ammonia were measured twice a week for every tank. Temperature ranged from 20.6 to 22.7 °C, dissolved oxygen ranged from 6.74 to 8.60 mg/L, percent saturation ranged from 77 to 102%, pH ranged from 7.6 to 8.0, and ammonia content remained below 1 mg/L.

Largemouth bass were fed for 30 days using the chemically treated floating pelletted feed beginning March 21, 2003. Largemouth bass were randomly placed into each of nine feed treatments in replicate: Control; 1, 7, 35, and 136 µg/g *p,p*'-DDE; and 0.03, 0.1, 0.6, and 5 µg/g Dieldrin. After day 30, subsets of 10 fish (5 males and 5 females) per replicate were sampled to collect measurements of health parameters

(weight, length, condition index, and HSI) and reproductive biomarkers (GSI and circulating sex steroid hormones).

**Feeding Rate**

The mean fish body weight of 150 g, at the beginning of the study, was used to determine the feeding rate. Feed was administered to each tank for 30 days at 1% mean body weight per day. Target feed doses of 1, 7, 37, and 185 µg/g *p,p*'-DDE were chosen to create final theoretical carcass concentrations of 0.1, 0.63, 3, and 17 µg/g *p,p*'-DDE, respectively. Target feed doses of 0.02, 0.1, 0.6, and 3 µg/g dieldrin were chosen to create final theoretical carcass concentrations of 0.003, 0.015, 0.09, and 0.45 µg/g dieldrin, respectively. Estimations of final carcass concentrations were based on a *p,p*'-DDE and dieldrin feeding study described in Muller *et al*. (2004), which characterized percent accumulation of both pesticides after 30 days of dietary exposure for pond-reared Florida largemouth bass.

**Fish Collection and Bleeding**

On April 20, 2003, ten largemouth bass (5 males & 5 females) per replicate were weighed to the nearest gram using a portable digital scale and measured (total length) to the nearest millimeter for determination of condition index (K) (Anderson and Neumann, 1996). Fish were then bled from the caudal vein with a heparinized 20-gauge 3.81-cm needle and a 3-mL syringe to collect approximately 1 mL of blood. Blood samples were then dispensed into 3-mL heparinized vacutainers and kept on ice until centrifuged at 1,000 *g* at 4 °C for 15 minutes to separate red blood cells from the plasma. Plasma was removed with transfer pipettes, placed into cryovials, and stored in a –80 °C freezer for later analysis of circulating sex steroid hormones. After bleeding, fish were dissected and sex was determined by gonad morphology. The gonads and livers were excised from all

fish and weighed on a portable scale to the nearest 0.01 g for determination of gonadosomatic index (GSI) and hepatosomatic index (HSI) (Anderson and Neumann, 1996). After removal, a cross section of one lobe of each gonad was collected, placed into a histological cassette and fixed in 10% buffered formalin for later histological analysis. Remaining gonad tissue and the fish carcass was then wrapped in aluminum foil, placed into a labeled whorl pack, and set into a freezer for later GC-MS analysis of *p,p*'-DDE and dieldrin. However, only one male and one female carcass and gonad composite from each replicate was used for analyses. Data for the males and females from identical replicates were pooled for graphical representation to form an n = 2 for every treatment.

**Gonad Histology**

Gonad tissue samples were embedded in paraffin, sectioned at 5 μm, mounted on glass slides, air dried, and stained with Mayer's hematoxylin and eosin (H&E) by Histology Tech Services (Gainesville, FL). Slides were observed under a light microscope at 40X and stages of sexual maturation were assigned according to Gross *et al*. (2002). Ovaries were classified into four stages of sexual maturation: (stage1), ovaries were undeveloped with mostly perinucleolar oocytes at various stages of previtellogenic growth; (stage 2), ovaries were previtellogenic with perinucleolar oocytes and cortical alveoli oocytes; (stage 3), ovaries were early vitellogenic with some vitellogenic oocytes of different sizes, with low to moderate amounts of vitelline granules, and few to no fully developed eggs; and (stage 4), ovaries were late vitellogenic with the majority of the oocytes fully developed and containing numerous vitelline granules. Similarly, testes were classified into four stages of sexual maturation: (stage 1), no sperm present with an extremely thin germinal epithelium; (stage 2), presence of

scattered spermatogenic activity with a thin germinal epithelium; (stage 3), presence of moderate spermatogenic activity (diffuse to moderate presence of mature sperm) with a reasonably thick germinal epithelium; and (stage 4), presence of high spermatogenic activity (heavy presence of mature sperm) with a thick germinal epithelium.

**Determination of Circulating Sex Steroid Hormones**

Plasma samples from largemouth bass were analyzed for sex steroid hormones 17β-Estradiol ($E_2$) and 11-Ketotestosterone (11-KT) with a validated $^3$H radioimmunoassay (RIA) procedure, using methods modified from those described by Gross *et al*. (2002) and Muller *et al*. (2004). All plasma samples were assayed in duplicate, and values were reported as pg/mL of plasma. Plasma samples (50 mL) were extracted twice with diethyl ether prior to RIA analysis. Standard curves (1, 5, 10, 25, 50, 100, 250, 500, and 1000 pg/mL) were prepared in phosphate buffered saline plus gelatin and sodium azide (PBSGA) with known amounts of radioinert $E_2$ (ICN Biomedicals, Costa Mesa, CA) or 11-KT (Sigma Chemicals, St. Louis, MO) and $^3$H-$E_2$ or $^3$H-11-KT. PBSGA buffer and antibodies specific to each sex steroid hormone were also added to each sample tube and incubated overnight at 4 °C. Antibodies were purchased from ICN Biomedicals ($E_2$) or Helix Biotech, Richmond, BC, Canada (11-KT). After incubation, unbound sex steriod was removed by the addition of dextran-coated charcoal and centrifugation for 10 minutes at 1,000 *g*. Four hundred μL of sample supernatant was removed and added to a scintillation vial with 4 mL of Scintiverse scintillation cocktail (Fisher Scientific, Pittsburg, PA). Each sample vial was then placed into a liquid scintillation counter (Packard Tricarb, Model 1600) and counted for two minutes. Cross-reactivities of the $E_2$ antiserum with other steroids were: 11.2% for estrone, 1.7% for estriol, and $< 1.0\%$ for 17α-estradiol and androstenedione. Cross reactivity of the 11-KT antiserum

with other steroids were: 9.7% for testosterone, 3.7% for α-dihydrotestosterone, and < 1.0% for androstenedione. The minimum concentration distinguishable from zero for all assays were (mean ± SD) 66.8 ± 15.2 pg/mL for $E_2$ and 45.8 ± 19.5 pg/mL for 11-KT.

**OCP Analysis**

One male and one female carcass and gonad composite from each replicate was analyzed for *p,p*'-DDE and dieldrin at the Center for Environmental and Human Toxicology, University of Florida, using methods described by Rauschenberger *et al*. (2004). First, the largemouth bass carcass/gonad tissue was homogenized to eliminate any variability within the sample. Then, a 2-g portion of each sample was extracted into ethyl acetate. The sample was then prepared for analysis by solid phase extraction on C18 and NH2 SPE cartridges. Total OCP content was determined using gas chromatography-mass spectrometry (GC-MS). Percent recovery for *p,p*'-DDE ranged between 90-98%, with a limit of detection of 0.11-1.5 ng/g. Percent recovery for dieldrin ranged between 70-89%, with a limit of detection of 0.46-1.5 ng/g.

**Statistical Analysis**

Parameters were analyzed using the Statistical Analysis System (SAS), version 9. Data were analyzed using the univariate procedure to determine if the data were normally distributed. ANOVAs were then performed and significance was declared at a $p$ value equal to or lower than 0.05. Duncan's Multiple Range test followed as a multiple comparison procedure to determine which treatments differed. Results are presented as means ± SD.

**Area 7 Largemouth Bass**

Five female largemouth bass were collected from Emeralda Marsh Conservation Area 7 using electofishing on February 26, 2003 for whole carcass and gonad GC-MS

contaminant analysis of *p,p*'-DDE and dieldrin. These fish, which had a mean weight of 1470 g, were collected to compare current wild largemouth bass OCP concentrations to the pesticide concentrations achieved in this study.

## Results and Discussion

The results of this study demonstrated that a 30-day exposure to diets containing the OCPs *p,p*'-DDE and dieldrin at varying doses can result in internal carcass and gonad concentrations of these two pesticides (Table 2-1), at levels similar to those found in largemouth bass from the EMCA. The five female largemouth bass from Area 7 had mean carcass *p,p*'-DDE and dieldrin concentrations of 270 ± 120 ng/g and 8.5 ± 5.8 ng/g respectively. These fish had mean gonad *p,p*'-DDE and dieldrin concentrations of 4900 ± 1000 ng/g and 11.4 ± 2.6 ng/g, respectively. Graphical analysis of these data demonstrates a consistent dose accumulation in the whole carcass and gonads for both males and females, across all treatment levels, for both *p,p*'-DDE and dieldrin (Figures 2-1, 2-2, 2-3, and 2-4). Carcass concentrations for all *p,p*'-DDE and dieldrin treatments were greater than predicted target concentrations (Figures 2-1 and 2-2). Achieved *p,p*'-DDE and dieldrin female carcass and gonad concentrations for the treatments fell within mean *p,p*'-DDE and dieldrin concentrations of the five female largemouth bass from Area 7 (Figures 2-1 and 2-3), thereby achieving eco-relevant loads of both OCPs.

Achieved concentrations of both pesticides did not induce biologically significant dose-response decreases in weight, length, K, HSI, GSI (Tables 2-2 and 2-3), or circulating sex steroid hormone concentrations, $E_2$ and 11-KT (Figures 2-5, 2-6, 2-7, and 2-8), for either female or male largemouth bass. Histological analysis demonstrated that ≥ 72% of females and 100% of male largemouth bass at the end of the study were at their fully developed stage of gonad maturation. The presence of fully developed gonads prior

to this study may have been the reason why the pesticides were not able to induce dose-response decreases in GSI or circulating sex steroids. Fully developed gonads indicate that the endocrine system processes of largemouth bass that initiate biological changes leading to gonad maturation, including surges in $E_2$ and 11-KT sex steroid production, had already taken place and were already on a seasonal decline by the time pesticide exposure in this study began.

Endocrine events of a largemouth bass follow a well-established cycle beginning with the secretion of gonadotropins in the pituitary, which bind estrogen or androgen receptors, stimulating gonad sex steroid hormone production in preparation for testicular maturation (spermatogenesis) in males and oocyte maturation and vitellogenesis in females (Van Der Kraak *et al*., 1998). Increasing $E_2$ concentrations in females stimulate the liver to produce vitellogenin, a protein that serves as a yolk precursor in oviparous vertebrates (Wahli *et al*., 1981). Vitellogenin produced in the liver is released into circulation to travel to the gonad where it is sequestered as a nutrient source in developing oocytes.

Gross *et al*. (2002), who characterized the annual cycles of circulating sex steroid hormones for pond-reared Florida largemouth bass, found that female $E_2$ concentrations began to increase in September and peaked in February at a concentration of 4,000 pg/mL. $E_2$ then declined sharply to a concentration of approximately 2,600 pg/mL in March and then to a concentration of approximately 2,000 pg/mL in April. For male largemouth bass, 11-KT concentrations began to increase in October, with a peak concentration of 2,800 pg/mL in February. 11-KT then declined to a concentration of approximately 2,300 pg/mL in March and then to a concentration of approximately 1,900

pg/mL in April. Gross *et al*. (2002) also found that GSI, which is an index correlated with an increase in gonad maturation, began to rise in October for both males and females, peaking in February to March, respectively.

Pesticide exposure for my study did not begin until March 21 because of delays in the manufacturing of the feed. By that date, sex steroids used to trigger the endocrine processes that lead to gonad maturation for both sexes, had probably peaked and were on the decline by the time pesticide dietary exposure for this experiment began. Histological analyses demonstrated that a majority of the largemouth bass had gonads that were fully developed. Pesticide exposure, beginning this late in the reproductive cycle of a largemouth bass therefore, led this study to miss critical events in the endocrine processes, such as estrogen or androgen receptor binding (Garcia *et al*., 1997; Larkin *et al*., 2002). The ability of any exogenous compound to bind a sex steroid hormone receptor and agonize and/or antagonize the action of an endogenous hormone can severely affect normal endocrine function. This is because normal estrogen or testosterone concentrations and actions are critical for development of both male and female gonads. Largemouth bass, from reclaimed agriculture areas reported to have impaired endocrine function, are exposed to these pesticides year round, thus spanning the entire duration of their annual reproductive cycle.

Circulating concentrations of both $E_2$ for females and 11-KT for males were on average 1,500 pg/mL less than what was reported for pond-reared largemouth bass in the Gross *et al*. (2002) study, sampled during the same time of the calendar year. Largemouth bass used in the Gross *et al*. (2002) study were on average 3-4 years old and were from a different population of largemouth bass. This may have attributed to the

differences in hormone concentrations between these two studies because the fish used in this study were 2 years of age. These fish may have been going through their first reproductive season, which could have also increased the variability in hormone concentrations.

In my study, achieved OCP concentrations were not able to induce dose-response decreases in GSI or circulating sex steroids $E_2$ and 11-KT for both female and male largemouth bass. This is likely attributable to OCP exposure which took place during a portion of the annual reproductive cycle of the largemouth bass, after their reproductive organs were already fully developed, causing this experiment to miss critical events in the reproductive system that could have facilitated endocrine disruption. Endocrine system changes that initiate gonad maturation, including surges in $E_2$ and 11-KT sex steroid production, had already taken place and were already on a seasonal decline by the time pesticide exposure in this study began. Despite a lack of response in measured reproductive biomarkers, exposing largemouth bass to floating pelletted feed coated with OCP contaminated fish oil served as an effective and accurate dosing method, achieving OCP concentrations in measured tissues consistent with those found in largemouth bass from reclaimed agriculture lands. Future research needs to focus on *p,p*'-DDE and dieldrin dietary exposure over a larger portion of the reproductive cycle of the largemouth bass to see if these pesticides can induce endocrine system and reproductive function changes similar to those reported for largemouth bass, exposed to these OCPs in the wild.

Table 2-1. Day-30 GC-MS mean ± SD results of both female and male largemouth bass *p,p*'-DDE and dieldrin concentrations (ng/g) in both the carcass and gonads per treatment (n = 2 samples per treatment, for each sex).

| | Treatments (µg/g) | | | | |
|---|---|---|---|---|---|
| *p,p*'-DDE | Control | 1 | 7 | 35 | 136 |
| Female Carcass | 8 ± 2 | 239 ± 21 | 1276 ± 331 | 9226 ± 3065 | 44502 ± 20176 |
| Male Carcass | 10 ± 1 | 251 ± 39 | 1436 ± 83 | 7231 ± 1445 | 39093 ± 12840 |
| Female Gonad | 10 ± 1 | 397 ± 29 | 1474 ± 493 | 8913 ± 33 | 57208 ± 724 |
| Male Gonad | 10 ± 2 | 476 ± 270 | 953 ± 395 | 19721 ± 5093 | 83701 ± 15659 |

| | Treatments (µg/g) | | | | |
|---|---|---|---|---|---|
| Dieldrin | Control | 0.03 | 0.1 | 0.6 | 5 |
| Female Carcass | 0.5 ± 0 | 7.3 ± 1.9 | 28.7 ± 3.4 | 108.5 ± 6.7 | 876.6 ± 463.3 |
| Male Carcass | 0.5 ± 0 | 6.5 ± 1 | 24.6 ± 2.6 | 103.3 ± 46.3 | 1155 ± 221.3 |
| Female Gonad | 2 ± 1.3 | 5 ± 0.9 | 28.9 ± 9.6 | 84.3 ± 12.6 | 793.6 ± 289.4 |
| Male Gonad | 2.1 ± 2 | 5.9 ± 4.2 | 10.1 ± 3.6 | 169.6 ± 70.6 | 1488.1 ± 421.3 |

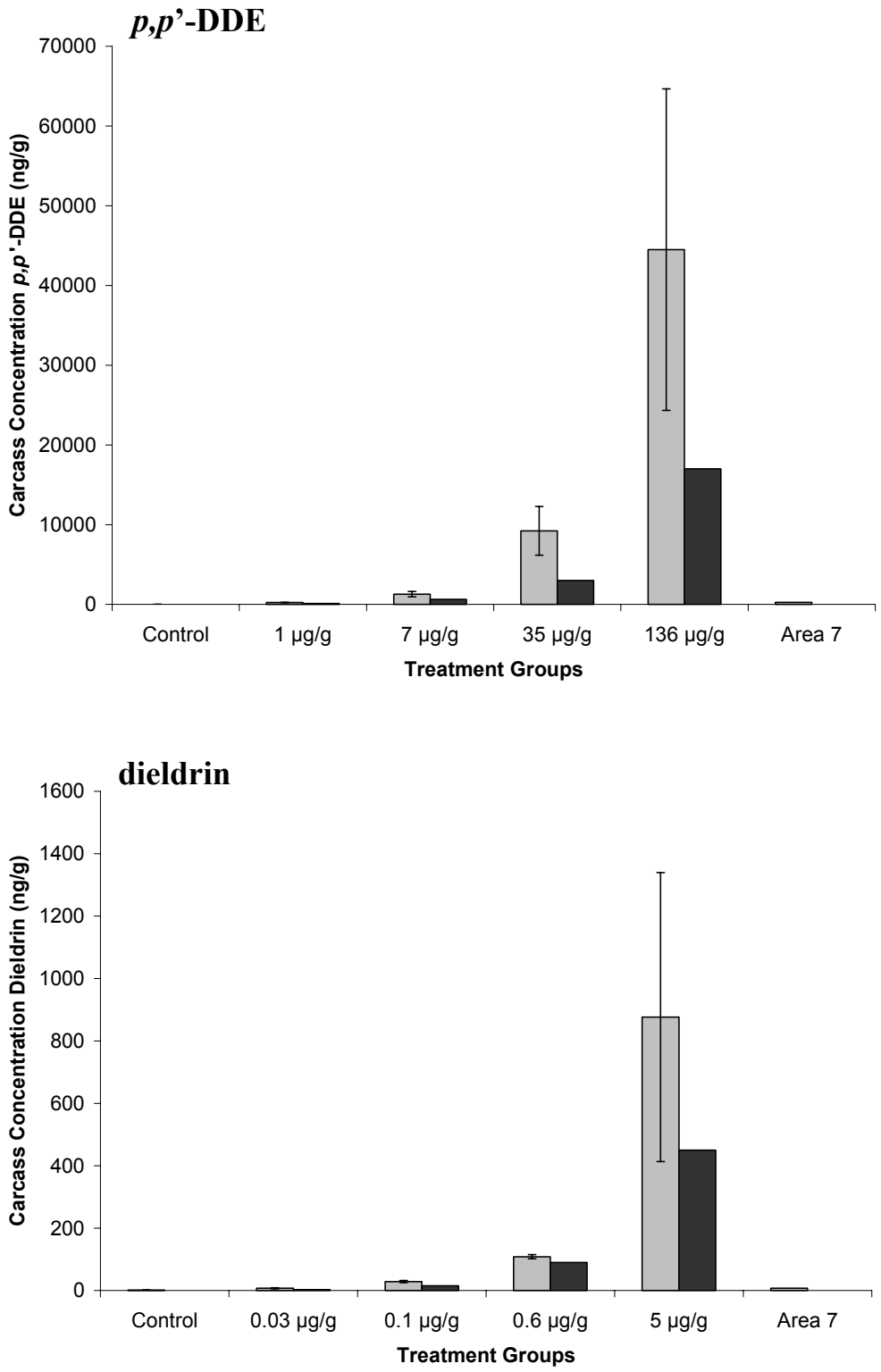


Figure 2-1. Female largemouth bass mean ± SD carcass concentrations (shaded bars) of *p,p*'-DDE and dieldrin treatments (n = 2 largemouth bass per treatment), as compared to the target carcass concentrations (black bars). Included is the mean carcass concentration of *p,p*'-DDE and dieldrin for the five female largemouth bass sampled from Area 7 (shaded bar) on February 26, 2003.

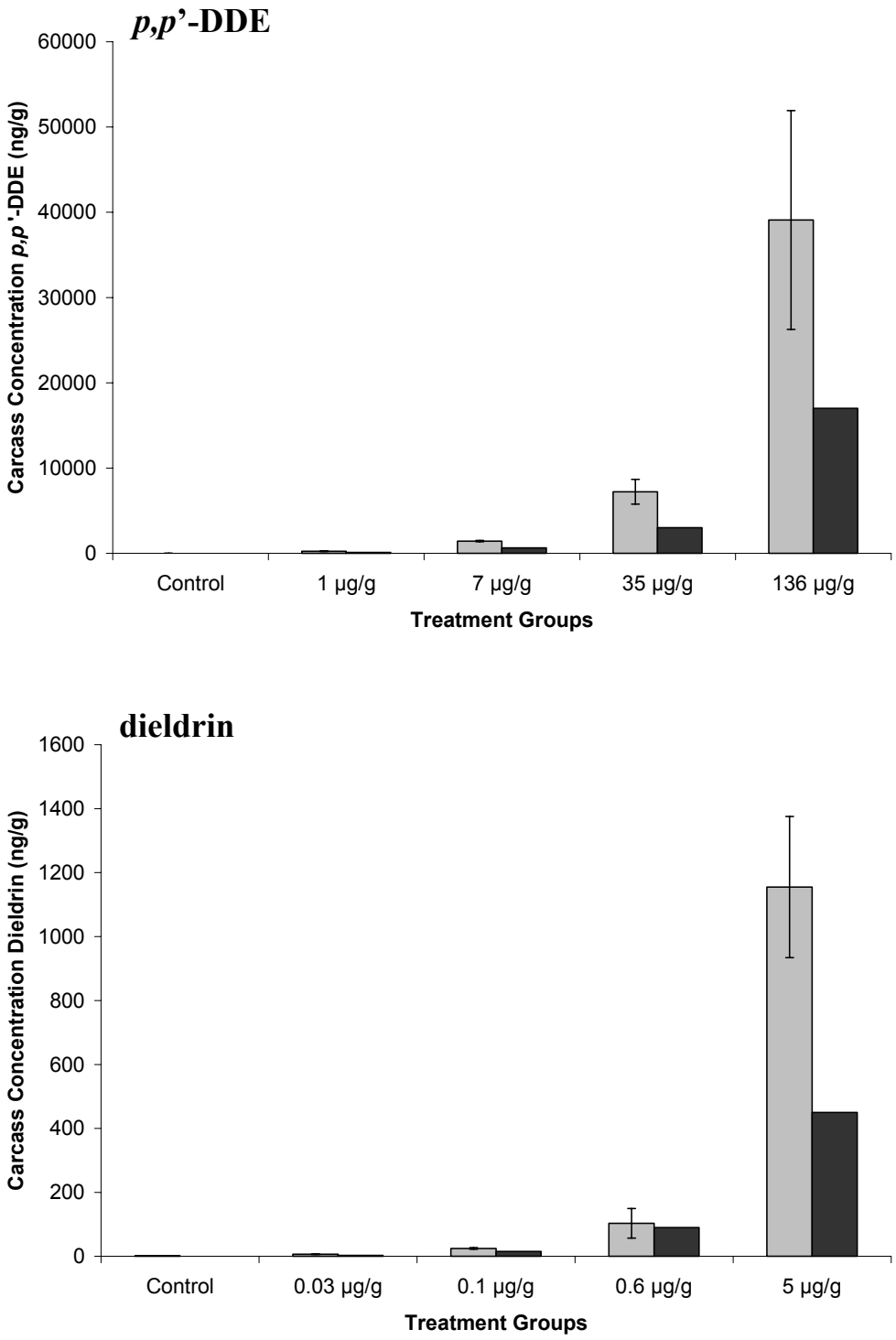


Figure 2-2. Male largemouth bass mean ± SD carcass concentrations (shaded bars) of *p,p*'-DDE and dieldrin treatments (n = 2 largemouth bass per treatment), as compared to the target carcass concentrations (black bars).

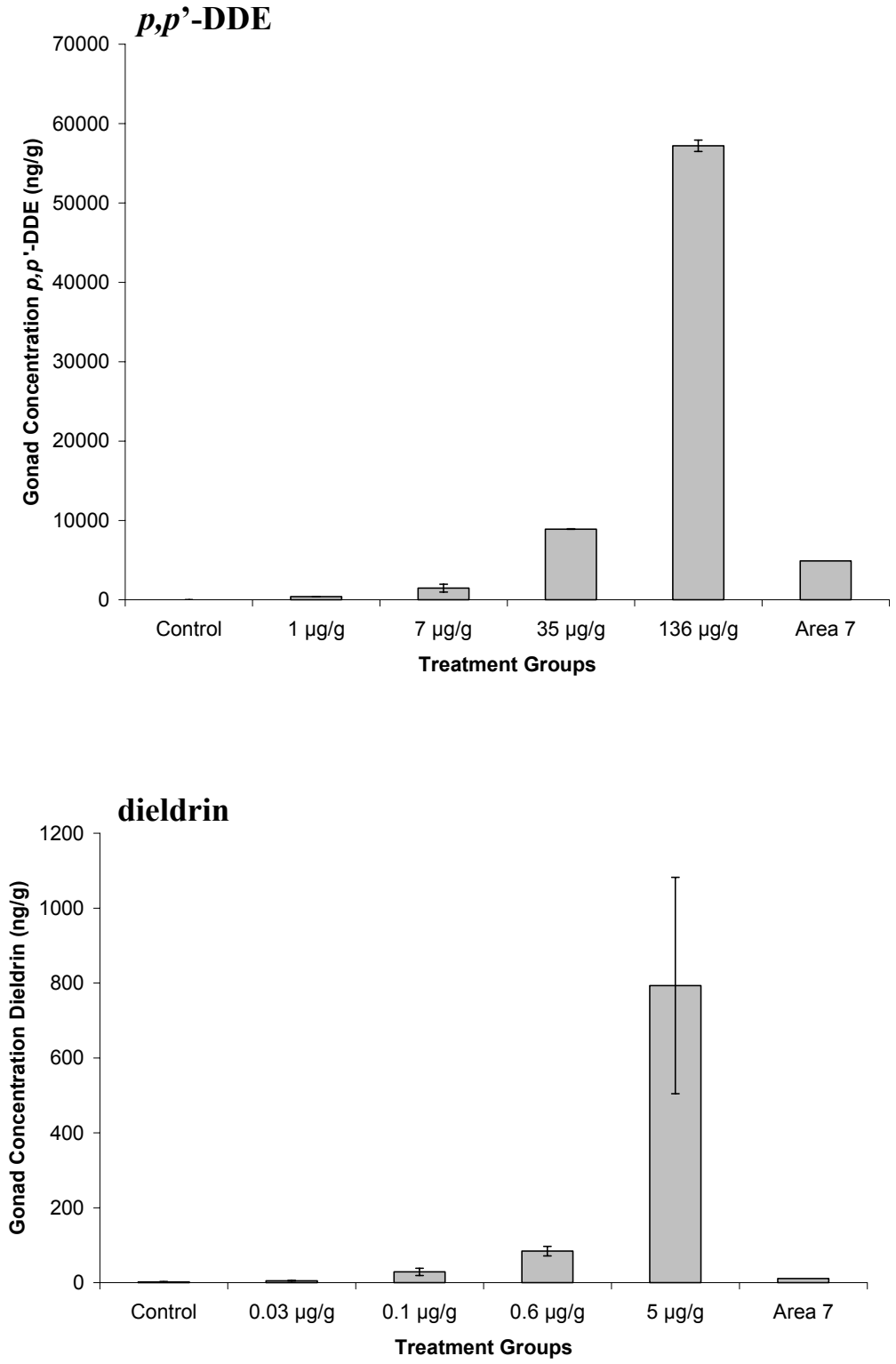


Figure 2-3. Female largemouth bass mean ± SD gonad concentrations of *p,p*'-DDE and dieldrin treatments (n = 2 largemouth bass per treatment). Included is the mean gonad concentration of *p,p*'-DDE and dieldrin for the five female largemouth bass sampled from Area 7 on February 26, 2003.

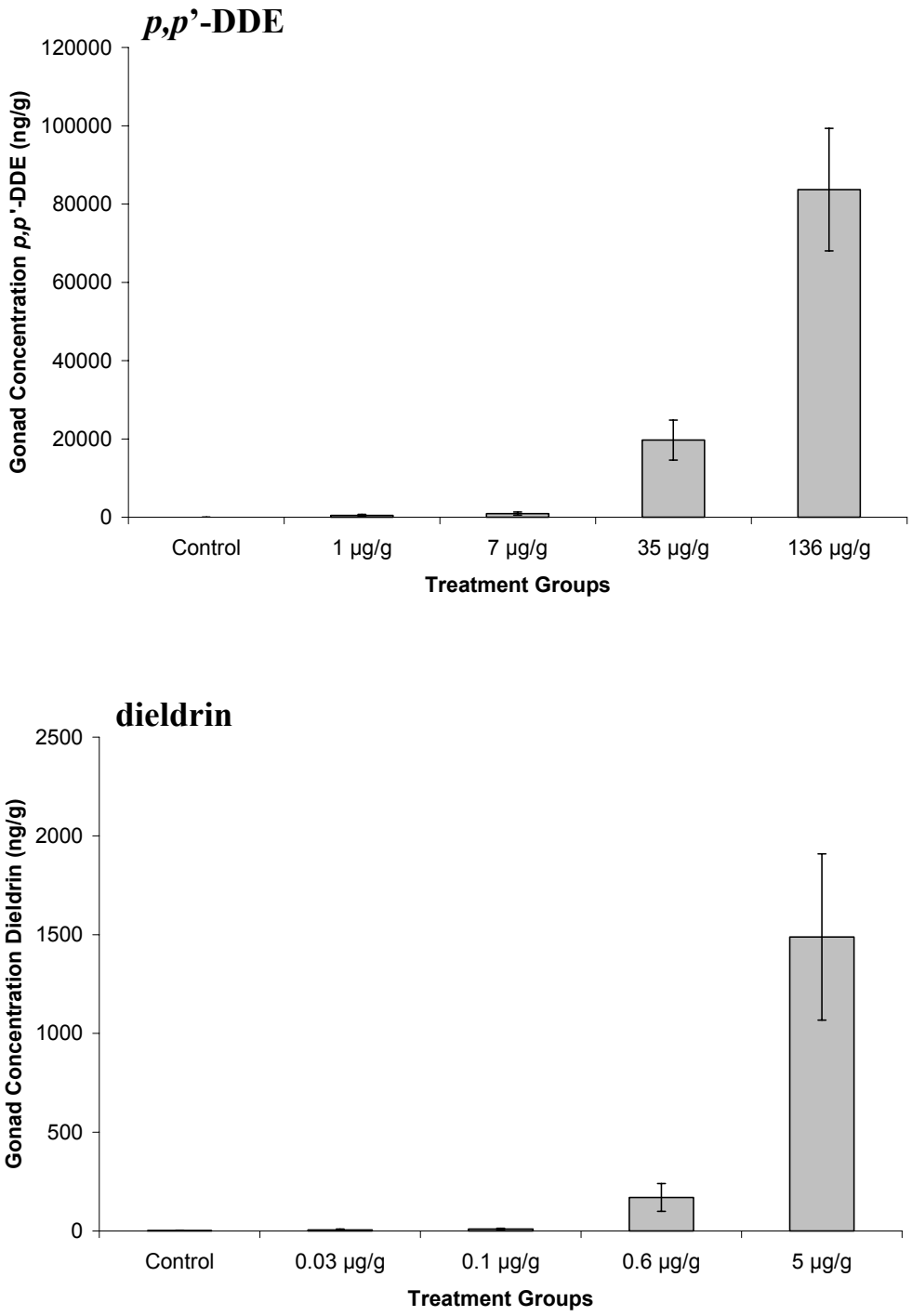


Figure 2-4. Male largemouth bass mean ± SD gonad concentrations of *p,p*'-DDE and dieldrin treatments (n = 2 largemouth bass per treatment).

Table 2-2. Day-30 mean ± SD results of female and male weight, total length, condition index (K), GSI, and HSI for each treatment, for largemouth bass fed *p,p*'-DDE diets. Treatments with the same lower case letter were not significantly different ($p > 0.05$), with a sample size of 10 largemouth bass per treatment.

| | *p,p*'-DDE Treatments (µg/g) | | | | |
|---|---|---|---|---|---|
| Female | Control | 1 | 7 | 35 | 136 |
| Weight (g) | 183 ± 32[a] | 176 ± 21[a] | 193 ± 23[a] | 188 ± 27[a] | 180 ± 12[a] |
| Length (mm) | 234 ± 12[a] | 231 ± 9[a] | 234 ± 9[a] | 234 ± 10[a] | 228 ± 5[a] |
| K | 1.41 ± 0.11[c] | 1.42 ± 0.08[b,c] | 1.50 ± 0.08[a,b] | 1.47 ± 0.08[a,b,c] | 1.51 ± 0.08[a] |
| GSI (%) | 2.05 ± 0.96[b] | 4.26 ± 1.87[a] | 2.80 ± 1.39[a,b] | 3.06 ± 1.96[a,b] | 3.87 ± 1.58[a] |
| HSI (%) | 3.68 ± 0.74[a] | 4.19 ± 0.43[a] | 3.83 ± 0.84[a] | 3.79 ± 0.80[a] | 3.74 ± 0.52[a] |
| | *p,p*'-DDE Treatments (µg/g) | | | | |
| Male | Control | 1 | 7 | 35 | 136 |
| Weight (g) | 184 ± 28[a] | 178 ± 23[a] | 194 ± 22[a] | 183 ± 26[a] | 190 ± 25[a] |
| Length (mm) | 237 ± 9[a] | 234 ± 8[a] | 238 ± 8[a] | 234 ± 9[a] | 237 ± 9[a] |
| K | 1.38 ± 0.07[a] | 1.39 ± 0.09[a] | 1.43 ± 0.06[a] | 1.42 ± 0.07[a] | 1.43 ± 0.06[a] |
| GSI (%) | 0.68 ± 0.40[a] | 0.65 ± 0.16[a] | 0.64 ± 0.20[a] | 0.66 ± 0.12[a] | 0.58 ± 0.16[a] |
| HSI (%) | 3.46 ± 0.61[a] | 3.44 ± 0.58[a] | 3.46 ± 0.78[a] | 3.17 ± 1.08[a] | 3.16 ± 0.66[a] |

Table 2-3. Day-30 mean ± SD results of female and male weight, total length, condition index (K), GSI, and HSI for each treatment, for largemouth bass fed dieldrin diets. Treatments with the same lower case letter were not significantly different ($p > 0.05$), with a sample size of 10 largemouth bass per treatment.

| | Dieldrin Treatments (µg/g) | | | | |
|---|---|---|---|---|---|
| Female | Control | 0.03 | 0.1 | 0.6 | 5 |
| Weight (g) | 186 ± 21$^{a,b}$ | 199 ± 23$^{a}$ | 188 ± 24$^{a,b}$ | 196 ± 16$^{a,b}$ | 179 ± 28$^{b}$ |
| Length (mm) | 228 ± 17$^{a,b}$ | 237 ± 10$^{a}$ | 233 ± 8$^{a,b}$ | 232 ± 8$^{a,b}$ | 227 ± 11$^{b}$ |
| K | 1.66 ± 0.73$^{a}$ | 1.49 ± 0.11$^{a}$ | 1.48 ± 0.13$^{a}$ | 1.58 ± 0.12$^{a}$ | 1.52 ± 0.13$^{a}$ |
| GSI (%) | 3.00 ± 2.06$^{a,b}$ | 4.53 ± 1.73$^{a}$ | 2.82 ± 1.26$^{b}$ | 4.19 ± 2.34$^{a,b}$ | 3.90 ± 1.88$^{a,b}$ |
| HSI (%) | 3.55 ± 1.02$^{b}$ | 4.17 ± 0.64$^{a,b}$ | 3.76 ± 0.46$^{a,b}$ | 2.89 ± 0.90$^{c}$ | 4.20 ± 0.80$^{a}$ |
| | Dieldrin Treatments (µg/g) | | | | |
| Male | Control | 0.03 | 0.1 | 0.6 | 5 |
| Weight (g) | 193 ± 42$^{a}$ | 190 ± 30$^{a}$ | 189 ± 23$^{a}$ | 201 ± 39$^{a}$ | 203 ± 27$^{a}$ |
| Length (mm) | 239 ± 10$^{a}$ | 236 ± 10$^{a}$ | 236 ± 8$^{a}$ | 233 ± 10$^{a}$ | 237 ± 11$^{a}$ |
| K | 1.39 ± 0.19$^{c}$ | 1.43 ± 0.09$^{b,c}$ | 1.43 ± 0.08$^{b,c}$ | 1.58 ± 0.12$^{a}$ | 1.52 ± 0.11$^{a,b}$ |
| GSI (%) | 0.71 ± 0.30$^{a}$ | 0.67 ± 0.16$^{a}$ | 0.68 ± 0.24$^{a}$ | 0.65 ± 0.18$^{a}$ | 0.60 ± 0.12$^{a}$ |
| HSI (%) | 4.07 ± 1.29$^{a}$ | 3.37 ± 0.56$^{a,b}$ | 3.10 ± 0.74$^{b}$ | 3.81 ± 1.14$^{a,b}$ | 3.91 ± 0.57$^{a}$ |

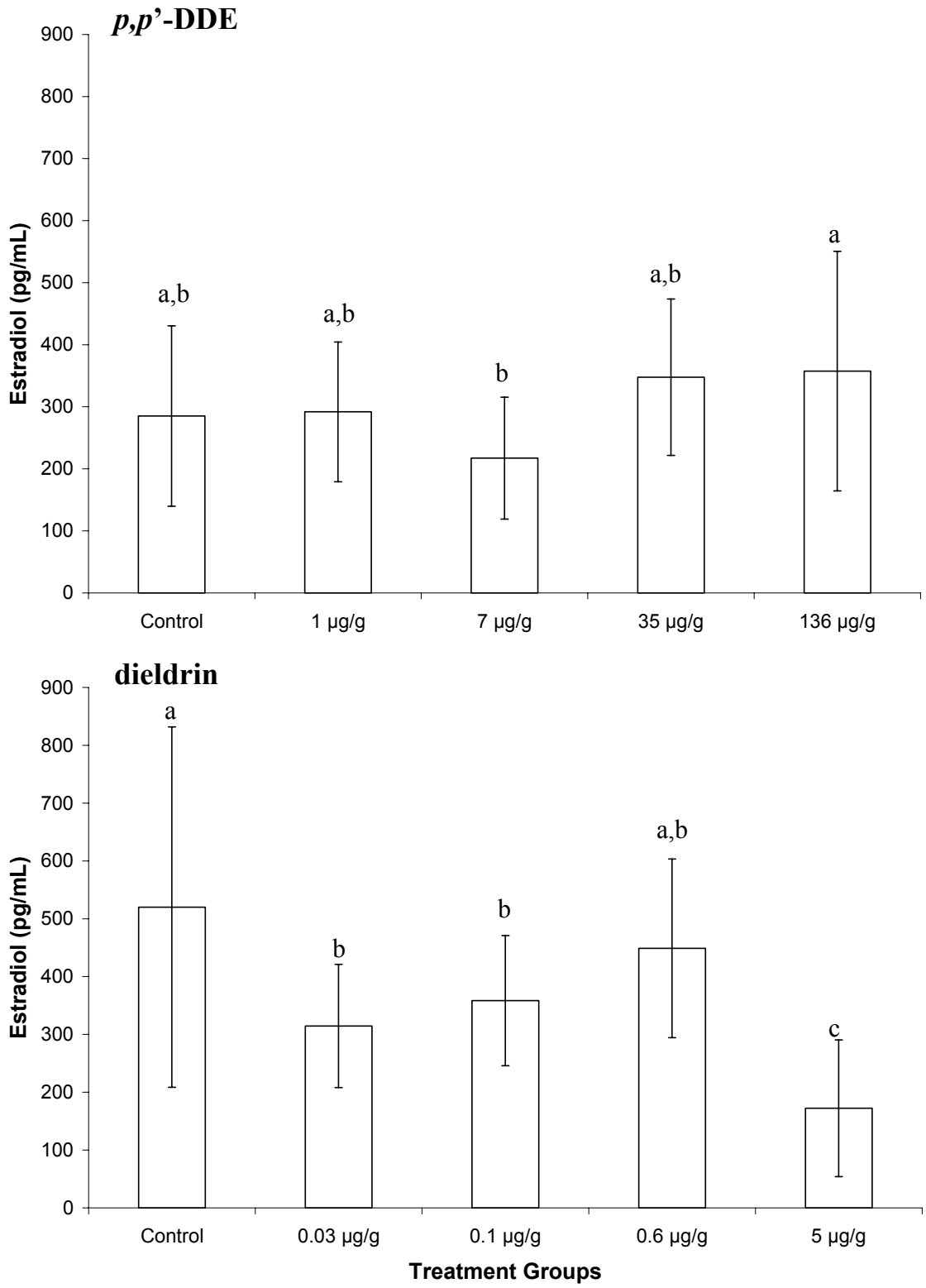


Figure 2-5. Mean female estradiol concentrations at day 30 for *p,p*'-DDE and dieldrin, with a sample size of 10 largemouth bass per treatment. Treatments with the same lower case letter were not significantly different ($p > 0.05$).

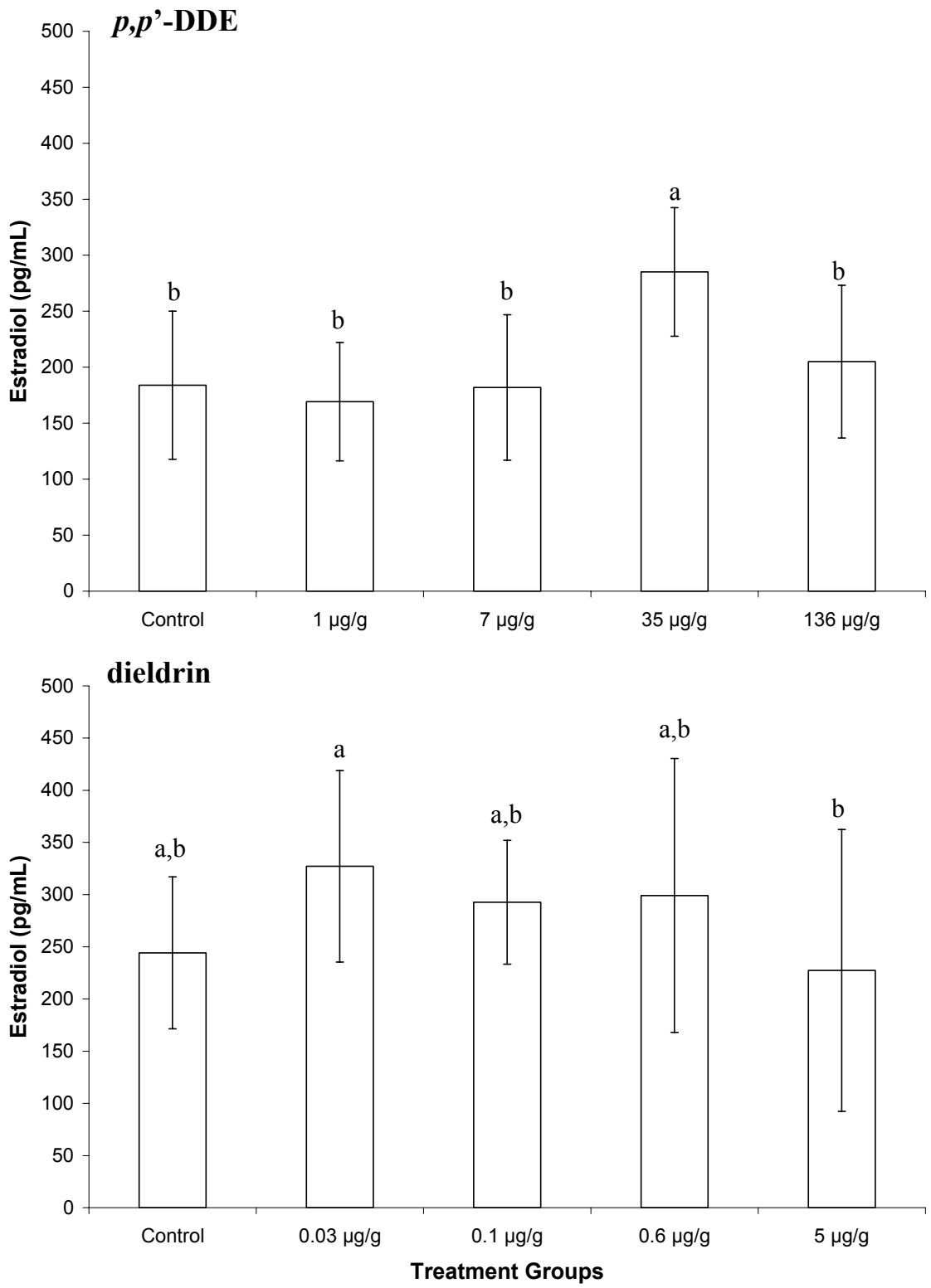


Figure 2-6. Mean male estradiol concentrations at day 30 for *p,p*'-DDE and dieldrin, with a sample size of 10 largemouth bass per treatment. Treatments with the same lower case letter were not significantly different ($p > 0.05$).

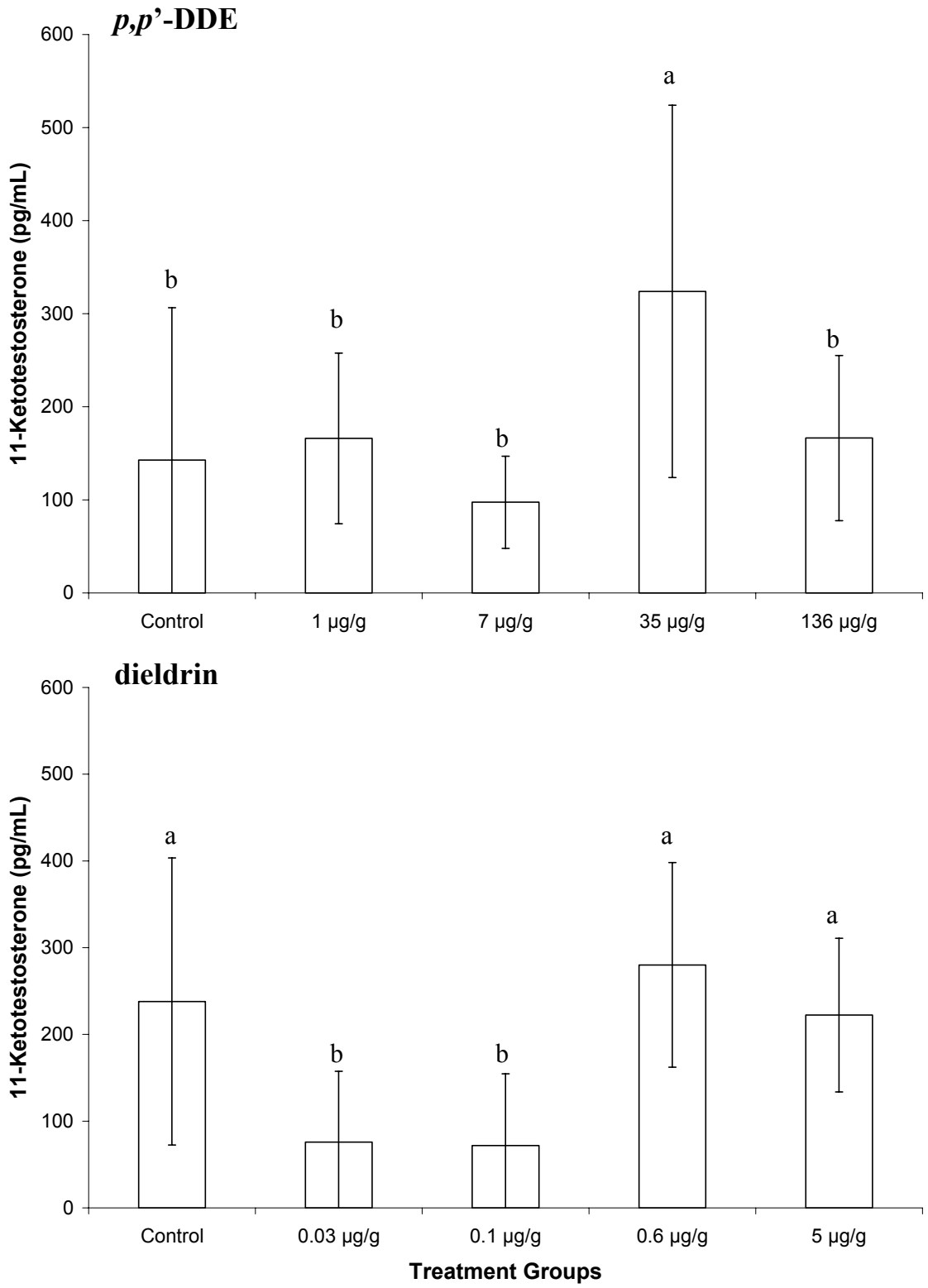


Figure 2-7. Mean female 11-ketotestosterone concentrations at day 30 for *p,p*'-DDE and dieldrin, with a sample size of 10 largemouth bass per treatment. Treatments with the same lower case letter were not significantly different ($p > 0.05$).

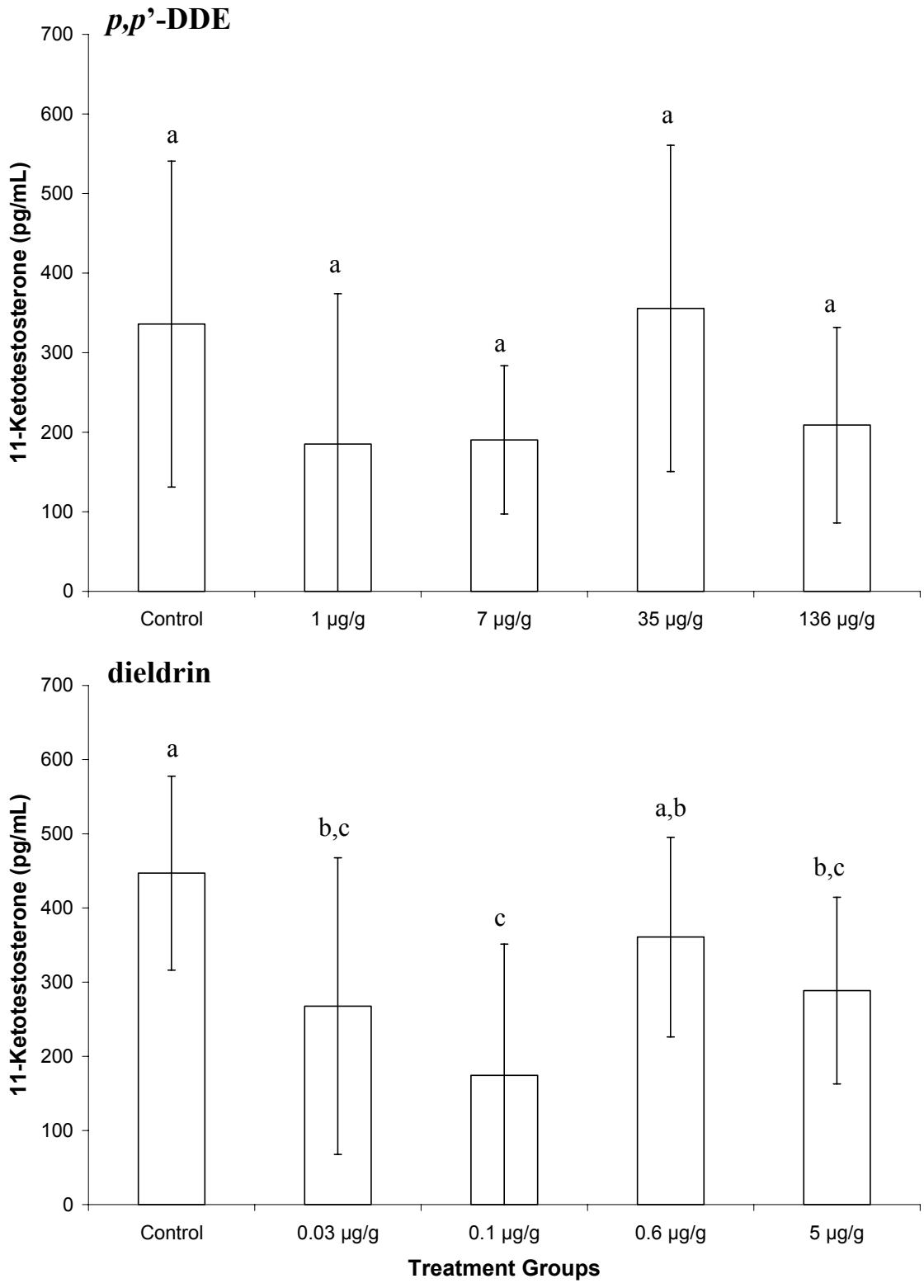


Figure 2-8. Mean male 11-ketotestosterone concentrations at day 30 for *p,p*'-DDE and dieldrin, with a sample size of 10 largemouth bass per treatment. Treatments with the same lower case letter were not significantly different ($p > 0.05$).

# CHAPTER 3
# DIETARY CHRONIC EXPOSURE TO *P,P*'-DDE AND DIELDRIN AND THEIR EFFECTS ON REPRODUCTIVE SUCCESS IN LARGEMOUTH BASS

## Introduction

A lack of dose-response effects from dietary exposure to *p,p*'-DDE and dieldrin on measured reproductive biomarkers (GSI and circulating sex steroid hormones) in the 30-day study (see Chapter 2) led to the need for extending the exposure period. Exposure length for this study was extended to a 120-day period, between the months of November and March, encompassing a larger part of the steroidogenic and gametogenic portion of the reproductive cycle of the largemouth bass. Extending exposure length for this study aimed to reevaluate single chemical dose-response effects of dietary exposure to *p,p*'-DDE and dieldrin on health parameters and reproductive biomarkers [weight, length, condition index (K), hepatosomatic index (HSI), gonadosomatic index (GSI), and circulating sex steroid hormones] for Florida largemouth bass (*Micropterus salmoides floridanus*) and to determine if *p,p*'-DDE or dieldrin doses affect clutch hatchability of eggs produced by the spawning of the treated fish.

## Materials and Methods

### Largemouth Bass

Hatchery-reared two-year-old Florida largemouth bass, with a mean body weight of 159 g, were obtained from American Sportfish Hatchery, Montgomery, AL on October 20, 2003.

**Feed Preparation**

Chemically treated floating pelletted feed was developed using methods described in Chapter 2. Stock solutions contained 20 g *p,p*'-DDE/400 mL fish oil and 6.4 g dieldrin/400 mL fish oil. Control feed had no detectable levels of organochlorine pesticides. Target feed doses of 7, 37, and 185 µg/g *p,p*'-DDE had actual concentrations of 5, 46, and 50 µg/g *p,p*'-DDE, respectively. Target doses of 0.1, 0.6, and 3 µg/g dieldrin had actual concentrations of 0.04, 0.4, and 0.8 µg/g dieldrin, respectively.

**Experimental Design**

Largemouth bass were housed in groups of 100 fish in seven separate 6,000-liter outdoor concrete raceways (366 cm x 183 cm x 91 cm) with flow-through pond water and aeration. Water temperature and dissolved oxygen were measured twice a week for every tank. Temperature ranged from 12.2 to 22.4 °C, dissolved oxygen from 7.06 to 11.75 mg/L, and percent saturation from 67 to 140%. One hundred largemouth bass were randomly placed into each of seven feed treatments: Control; 5, 46 and 50 µg/g *p,p*'-DDE; and 0.04, 0.4, and 0.8 µg/g Dieldrin. Largemouth bass were fed the seven diets, five days a week beginning on November 4, 2003, represented as day 0. On day 0 of the experiment, 24 largemouth bass were sampled from the Control to collect measurements of health parameters (weight, length, condition index, and HSI) and reproductive biomarkers (GSI and circulating sex steroid hormones). Approximately every 30 days, six males and six females per treatment were sampled to collect measurements on the same health parameters and reproductive biomarkers. Fish sex was determined by external examination of the urogenital pore or by palpating to evaluate the release of eggs or milt. Sample days took place on December 1, 2003 and January 6 and February 3, 2004 for all treatments. The last sample was on March 10, 2004 for the Control and *p,p*'-

DDE treatments, and on March 26, 2004 for the dieldrin treatments and a second set of Control fish. Largemouth bass in the *p,p*'-DDE treatments were housed in their corresponding raceways for a total of 128 days, and were fed their corresponding diets for a total of 86 days. Largemouth bass in the Control and dieldrin treatments were housed in their corresponding raceways for a total of 144 days, and were fed their corresponding diets for a total of 98 days. There was only one mortality throughout the entire duration of this study, which occurred in the 0.8 µg/g Dieldrin treatment raceway.

**Feeding Rate**

All feed was administered to each tank at 1% mean body weight for (100) largemouth bass. Feeding rate was adjusted every thirty days according to changes in mean body weight and the number of largemouth bass remaining in each raceway.

**Fish Collection and Bleeding**

Twenty four largemouth bass (12 males & 12 females) on day 0, and a subset of 12 largemouth bass (6 males and 6 females) per treatment were sampled on days 30, 60, 90, and 120 to collect measurements of the same health parameters and reproductive biomarkers as described in Chapter 2. On the last sample day (day 120), carcasses of sampled largemouth bass from every treatment were wrapped in aluminum foil, placed into a labeled whorl pack, and set into a freezer for later GC-MS analysis of *p,p*'-DDE and dieldrin. However, carcasses of only three males and three females from every treatment were used for contaminant analysis. The final concentrations from the contaminant analysis were then pooled by sex for graphical representation to form an n = 3 for every treatment.

**Day 120 Spawning**

After the last sample days in March for both *p,p*'-DDE and dieldrin, one group of eight males and eight females from each treatment and a set of control diet largemouth bass were placed into four separate 0.10-acre experimental ponds, which contained spawning mats. Daily snorkeling was used to locate largemouth bass nests along the bottom of each pond. If a fertilized nest was found on the spawning mat, that portion of the mat was cut out, folded over, gently raised to the surface, and placed into a cooler of pond water for transport to the lab. The first six clutches collected per pond were used to characterize differences in percent hatch of eggs produced by the spawning of these treated largemouth bass.

Spawning mat sections containing egg clutches were removed from the cooler and placed into a 1.5% sodium sulfite solution for 5-7 minutes to loosen the eggs from the spawning mat. The mat was then carefully removed from the solution and placed into a nalgene container of pond water. The sodium sulfite solution was then poured through a series of sieves to collect any eggs that may have fallen off of the mat. If any eggs were collected, they were placed into a pyrex dish containing pond water. To continue the egg removal process, the mat sections placed into the nalgene container, were sprayed with water to remove the remaining eggs. Water from the nalgene container was then poured through the same series of sieves to collect the remaining eggs. These eggs were added to the pyrex dish to keep an entire clutch together.

Once an entire clutch was separated from the spawning mat, three separate live 100-embryo sets from each clutch were counted and placed randomly into three separate McDonald jars to allow for hatching of the eggs. Progress of the embryos was recorded once daily. Temperature and dissolved oxygen of the head tank, that supplied on-site

well water to the jars, was recorded once a day. Temperature ranged from 20.1 to 21.3 °C, with a mean of 20.7 °C and dissolved oxygen from 7.13 to 8.49 mg/L, with a mean of 7.76 mg/L. The eggs were treated daily with a hydrogen peroxide (500 mg/L of 35% active ingredient) static bath for 30 min to prevent fungal growth. Once hatching of the embryos in each jar was complete, the fry were removed and set in 10% buffer formalin. The number of fry produced by the three separate 100-embryo sets for each clutch were counted, added together, and divided by 300. The resulting value represented the percent hatch for each clutch. The mean percent hatch values of the six clutches, collected for each treatment, were used to characterize differences in percent hatch among the treatments.

**Determination of Circulating Sex Steroid Hormones**

Plasma samples from the largemouth bass were again analyzed for sex steroid hormones 17β-Estradiol ($E_2$) and 11-Ketotestosterone (11-KT) with a validated $^3H$ radioimmunoassay (RIA) procedure, using methods described in Chapter 2. The minimum concentration distinguishable from zero for all assays were (mean ± SD) 89 ± 32.9 pg/mL for $E_2$ and 72.3 ± 17.6 pg/mL for 11-KT.

**OCP Analysis**

Carcasses of three males and three females from every treatment were analyzed for *p,p*'-DDE and dieldrin content at the Center for Environmental and Human Toxicology, University of Florida, using methods described in Chapter 2. Percent recovery for *p,p*'-DDE was 87%, with a limit of detection of 0.1-1.5 ng/g. Percent recovery for dieldrin was 95%, with a limit of detection of 0.6-1.5 ng/g.

**Statistical Analysis**

Parameters were again analyzed using the Statistical Analysis System (SAS), version 9. Data were analyzed using the univariate procedure to determine if the data were normally distributed. ANOVAs were then performed and significance was declared at a *p* value equal to or lower than 0.05. Duncan's Multiple Range test followed as a multiple comparison procedure to determine which treatments differed. Results are presented as means ± SD.

**Area 7 Largemouth Bass**

Five female largemouth bass were collected from Emeralda Marsh Conservation Area 7 using electofishing on February 23, 2004 for whole carcass and gonad GC-MS contaminant analysis of *p,p*'-DDE and dieldrin. These fish, with a mean weight of 1020 g, were collected to compare current wild largemouth bass OCP concentrations to the pesticide concentrations achieved in this study.

## Results and Discussion

The outcome of this study demonstrated that attained carcass concentrations following 120 days of dietary exposure to *p,p*'-DDE and dieldrin did not result in any meaningful dose-response decreases across all treatment levels, for both female and male weight, length, condition index (K), and HSI, at the four different sampling days (Tables 3-1, 3-2, 3-3, and 3-4). Exposure to *p,p*'-DDE and dieldrin led to depressed concentrations of plasma $E_2$ for female largemouth bass (Figures 3-1 and 3-2), increases in 11-KT concentrations for female largemouth bass (Figures 3-3 and 3-4), and a lack of consistent increases and/or depressions of male largemouth bass $E_2$ and 11-KT concentrations (Figures 3-5, 3-6, 3-7, and 3-8) over the 120-day period.

Changes in female plasma $E_2$ concentrations over the sampling period followed a similar pattern regardless of pesticide or dose, and demonstrated a lack of an expected seasonal increasing trend in $E_2$ concentrations demonstrated by the Control. Not only were their significant reductions in treated group $E_2$ concentrations from the Control concentrations on at least two of the three sample days (Figures 3-1 and 3-2), including a day-30 reduction shown by all treatments, but a day-60 recovery in $E_2$ concentrations back to a concentration not statistically different from the Control was also demonstrated by all treatments (Figures 3-1 and 3-2). The 5 μg/g *p,p*'-DDE treatment demonstrated a recovery in $E_2$ concentrations back to a concentration not statistically different from the Control on day 90 (Figure 3-1). The recovery shown by all treatments was also immediately followed by a significant reduction in $E_2$ concentrations on day 120 (Figures 3-1 and 3-2). Reductions in plasma $E_2$ concentrations demonstrated by the treatments also averaged 2 to 3 times less than the Control, indicating that both OCPs induced not only statistically significant changes, but that biologically significant reduction in $E_2$ concentrations were shown by the treatments.

Even though 11-KT is believed to be a male specific androgen, one that is responsible for spermatogenesis, it was detected on every sample day in female largemouth bass plasma. Gross *et al*. (2002) also found 11-KT present in the plasma of hatchery reared female largemouth bass, similar in concentrations and depicting a lack of seasonal pattern, comparable to the Control in this study. Dietary exposure to either *p,p*'-DDE or dieldrin manifested as an increase in female 11-KT concentrations on at least two of the four sample days (Figures 3-3 and 3-4). Most notably was the day-90 peak in 11-

KT concentrations demonstrated by all three dieldrin treatments, to a concentration 4-fold greater than the Control (Figures 3-4).

Male largemouth bass 11-KT concentrations did not follow a similar pattern of reduction over the 120-day sampling period (Figures 3-7 and 3-8), as was demonstrated by female largemouth bass $E_2$ concentrations (Figures 3-1 and 3-2). An expected, seasonal increasing pattern in 11-KT concentrations was shown by all treatments, comparable to that of the Control (Figures 3-7 and 3-8). Day 120, on which a significant reduction by all three dieldrin treatments occurred, was the only sample day when 11-KT concentrations departed from the seasonal increasing pattern shown by all six treatments (Figures 3-7 and 3-8). This may have been attributed to a threshold whole-body concentration of dieldrin, reached by these three treatments prior to the last sample day, enough to initiate a mechanism responsible for endocrine disruption.

The purpose of this study was not to test for reported mechanisms of endocrine disruption in fish and other wildlife exposed to OCPs, but was intended to replicate in a laboratory setting reproductive abnormalities reported for largemouth bass sampled from Emeralda Marsh Conservation Area. Similar to this study, female largemouth bass sampled monthly from these flooded muck farms had depressed 17β-estradiol concentrations, elevated 11-KT concentrations, and demonstrated a lack of seasonal trend in $E_2$ concentrations (Marburger *et al*., 1999). Despite non-detectable levels of dieldrin in the five female largemouth bass sampled from Area 7 in 2004 for my study, achieved *p,p*'-DDE and dieldrin carcass concentrations (Table 3-5) were within carcass concentrations reported for largemouth bass sampled from the EMCA by Marburger *et al*. (1999). The five female largemouth bass, sampled from Area 7, had a mean carcass

*p,p*'-DDE concentration of 2300 ± 510 ng/g. Graphical analysis of *p,p*'-DDE carcass concentrations demonstrated that both female and male largemouth bass accumulated a consistent dose from dietary exposure for the 46 and 50 µg/g *p,p*'-DDE treatments, while both female and male carcasses in the 5 µg/g *p,p*'-DDE treatment averaged about 8,000 ng/g less *p,p*'-DDE (Figure 3-9). Achieved *p,p*'-DDE female and male carcass concentrations, for 46 and 50 µg/g *p,p*'-DDE treatments, were above the mean *p,p*'-DDE concentration of the five female largemouth bass sampled from Area 7, while fish in the 5 µg/g *p,p*'-DDE treatment fell below this concentration (Figures 3-9). Graphical analysis of dieldrin carcass concentrations demonstrated that both female and male largemouth bass accumulated a consistent dose from dietary exposure for the 0.4 and 0.8 µg/g Dieldrin treatments, while both female and male carcasses in the 0.04 µg/g Dieldrin treatment were just above detectable limits of dieldrin (Figure 3-9b).

My study also sought to address the issue that poor recruitment that has limited the development of the EMCA into a quality largemouth bass fishery might be related to reported reproductive abnormalities (Benton and Douglas, 1996). The ability of these OCPs to induce reductions in female largemouth bass seasonal $E_2$ concentrations could lead to decreased vitellogenesis, or egg yolk synthesis. Vitellogenins are proteins synthesized liver in response to changes in estradiol concentrations, and serve as a major source of nutrition during embryonic and early-life stage development in all oviparous vertebrates (Wahli *et al*., 1981). It is hypothesized that reduced estrogen function could lead to decreased vitellogenesis and impaired gonad development; ending in the production of poor quality eggs and decreased reproductive success (Muller, 2003). Decreased egg production and survival of early-life stages could in turn affect

recruitment and have significant population-level effects. Reductions in female largemouth bass $E_2$ concentrations for all treatments did not translate into dose-response reductions in female GSI (Tables 3-1, 3-2, 3-3, and 3-4), indicating that alterations in female $E_2$ concentrations did not have an effect on ovarian development. Female GSI values demonstrated an expected seasonal increasing trend between the months of November and March regardless of pesticide or dose (Figures 3-10 and 3-11). The *p,p*'-DDE or dieldrin treatments did not translate into a dose-response reduction in percent hatch values, as compared to the control treatment (Table 3-6). The 50 *p,p*'-DDE and 0.04 µg/g Dieldrin treatments demonstrated the highest percent hatch values (Table 3-6), compared to all other treatments. Hatch values of all treatments were within reported ranges for controlled spawning and hatching of largemouth bass eggs under laboratory conditions (Carlson, 1973; Jackson, 1979). Numerous field and laboratory studies have linked DDT egg concentrations to decreases in fecundity and fertility, early oocyte loss, sac fry mortality, and developmental alterations (Burdick *et al*.,1964; Macek, 1968; Smith and Cole, 1973; Hose *et al*., 1989). White croaker *Genyonemus lineatus*, environmentally exposed to DDT residues with ovarian concentrations of 4,000 ng/g or greater demonstrated the inability to spawn (Cross and Hose, 1988). The ovarian DDT concentrations reported for that study are comparable to measured ovarian DDT concentrations from largemouth bass sampled from the EMCA, where poor recruitment has been reported (Marburger *et al*., 1999; 2002). The lack of data concerning concentrations in the ovaries of female largemouth bass, treated with *p,p*'-DDE in this study, does not allow for a comparison to be made.

Despite a lack of research into the possible mechanisms of endocrine disruption in largemouth bass and other teleosts following exposure to either *p,p*'-DDE or dieldrin, the results of endocrine disruption in other animal models may help to provide insight into what occurred in this study. The dose-response decreases in female $E_2$ plasma concentrations, coupled with a lack of dose-response decreases in male 11-KT concentrations, points toward a mechanism of reduced aromatase activity found in alligators and human cells exposed to OCPs. Crain *et al*. (1997) found significantly decreased aromatase activity in female juvenile alligators sampled from OCP contaminated aquatic systems in central Florida. Toxaphene, another prominent OCP found in the soil and tissues of largemouth bass sample from Emeralda marsh, was found to decrease aromatase activity in human female breast tissue (Chen *et al*., 2001). Aromatase is an enzyme essential for the conversion of testosterone to $E_2$, and if the activity of aromatase is decreased or inhibited in any manner, the end product would manifest as a reduction in the amount of $E_2$ produced. Since male largemouth bass did not show a similar pattern of reduction in plasma 11-KT concentrations, the female-specific reductions, following dietary exposure to *p,p*'-DDE or dieldrin, indicates that endocrine disruption in this study was specific to the production of $E_2$ in female largemouth bass.

The recovery of plasma $E_2$ concentrations on day 60 and the build up of 11-KT in female largemouth bass may have been the result of positive and negative feedback actions, two mechanisms that help to regulate the release of hormones in the endocrine system of teleosts. Estradiol and testosterones levels exert positive and negative feedback on GTH release mediated by indirect effects on GnRH release (Van Der Kraak

*et al*., 1998). Low or high sex steroid concentrations either stimulate or cease the production of steroids by agonizing (positive feedback) or antagonizing (negative feedback) the release of GnRH. The seasonably low $E_2$ concentrations demonstrated by the treatments on day 30 may have exerted a "positive feedback" mechanism, stimulating the synthesis of more $E_2$, so much so that $E_2$ concentrations were able to attain a concentration comparable to that of the Control. As dietary exposure to *p,p*'-DDE or dieldrin continued between days 60 and 90, increasing whole-body OCP concentrations may have mediated an increase in aromatase inhibition, to a point that $E_2$ production was unable to recover, causing reductions in $E_2$ concentrations on days 90 and 120. The increase in 11-KT concentrations, in *p,p*'-DDE and dieldrin treated female largemouth bass, could have been the result of aromatase inhibition not allowing for the conversion of testosterone to $E_2$. A lack of $E_2$ synthesis resulted in a continued "positive feedback" for the production of more $E_2$, but since the OCPs were acting to inhibit the aromataztion of testosterone to $E_2$, this mechanism of endocrine disruption manifested as a build up of testosterone and then 11-ketotestosterone.

My study was successful in replicating reproductive abnormalities found in largemouth bass sampled from the EMCA. Dietary exposure, to *p,p*'-DDE and dieldrin during the reproductive season, manifested in reductions of female largemouth bass $E_2$ concentrations, abnormal increases in 11-KT concentrations, and demonstrated a lack of seasonal increasing trend in $E_2$ concentrations. Attained *p,p*'-DDE and dieldrin carcass concentrations and achieved depressions of female $E_2$ concentrations did not translate into a reduction of percent hatch. My study only sought to characterize single chemical dose-response effects for two of the predominate OCPs, *p,p*'-DDE and dieldrin, found in

the soils and various tissues of largemouth bass from the EMCA. Fish in this system are environmentally exposed to multiple pesticides that may not only contribute to reductions in hormone concentrations, but also to decreased reproductive success. Future research may be the study of pesticide mixtures and the effects that multiple pesticide exposure may attribute to hormone depression and reproductive success.

Table 3-1. Day-30 mean ± SD results of female and male weight, total length, condition factor (K), GSI, and HSI per treatment (n = 6 largemouth bass per treatment for each sex), for the *p,p*'-DDE and dieldrin diets. Means for treatments with the same lower case letter were not significantly different ($p > 0.05$) within each sex and pesticide.

| | *p,p*'-DDE Treatments (µg/g) | | | |
|---|---|---|---|---|
| Female | Control | 5 | 46 | 50 |
| Weight (g) | 176 ± 18[a] | 183 ± 41[a] | 176 ± 41[a] | 193 ± 20[a] |
| Length (mm) | 227 ± 6[a] | 229 ± 14[a] | 230 ± 18[a] | 239 ± 18[a] |
| K | 1.50 ± 0.06[a] | 1.50 ± 0.26[a] | 1.43 ± 0.06[a] | 1.45 ± 0.36[a] |
| GSI (%) | 1.26 ± 0.25[a] | 1.15 ± 0.14[a] | 1.29 ± 0.42[a] | 1.14 ± 0.22[a] |
| HSI (%) | 4.04 ± 0.60[a] | 3.27 ± 0.68[a] | 3.51 ± 0.69[a] | 3.77 ± 0.67[a] |
| | *p,p*'-DDE Treatments (µg/g) | | | |
| Male | Control | 5 | 46 | 50 |
| Weight (g) | 200 ± 33[a] | 224 ± 18[a] | 212 ± 36[a] | 193 ± 54[a] |
| Length (mm) | 235 ± 11[a] | 245 ± 8[a] | 238 ± 9[a] | 233 ± 19[a] |
| K | 1.53 ± 0.06[a] | 1.53 ± 0.03[a] | 1.56 ± 0.15[a] | 1.48 ± 0.11[a] |
| GSI (%) | 0.53 ± 0.18[a] | 0.59 ± 0.16[a] | 0.56 ± 0.18[a] | 0.55 ± 0.08[a] |
| HSI (%) | 4.29 ± 0.63[a] | 3.46 ± 0.83[a] | 3.86 ± 0.96[a] | 4.09 ± 0.81[a] |
| | Dieldrin Treatments (µg/g) | | | |
| Female | Control | 0.04 | 0.4 | 0.8 |
| Weight (g) | 176 ± 18[a] | 205 ± 12[a] | 188 ± 25[a] | 193 ± 46[a] |
| Length (mm) | 227 ± 6[a] | 237 ± 5[a] | 225 ± 20[a] | 232 ± 11[a] |
| K | 1.50 ± 0.06[a] | 1.53 ± 0.11[a] | 1.70 ± 0.45[a] | 1.53 ± 0.17[a] |
| GSI (%) | 1.26 ± 0.25[a,b] | 1.35 ± 0.17[a] | 1.00 ± 0.28[b] | 0.95 ± 0.30[b] |
| HSI (%) | 4.04 ± 0.60[a] | 3.01 ± 0.50[b] | 3.37 ± 0.80[a,b] | 3.27 ± 0.85[a,b] |
| | Dieldrin Treatments (µg/g) | | | |
| Male | Control | 0.04 | 0.4 | 0.8 |
| Weight (g) | 200 ± 33[a] | 191 ± 37[a] | 199 ± 31[a] | 167 ± 23[a] |
| Length (mm) | 235 ± 11[a] | 229 ± 12[a,b] | 234 ± 12[a] | 221 ± 9[b] |
| K | 1.53 ± 0.06[a] | 1.57 ± 0.09[a] | 1.56 ± 0.19[a] | 1.54 ± 0.04[a] |
| GSI (%) | 0.53 ± 0.18[a] | 0.66 ± 0.19[a] | 0.67 ± 0.06[a] | 0.56 ± 0.14[a] |
| HSI (%) | 4.29 ± 0.63[a] | 3.64 ± 0.72[a,b] | 3.45 ± 0.48[b] | 3.67 ± 0.65[a,b] |

Table 3-2. Day-60 mean ± SD results of female and male weight, total length, condition factor (K), GSI, and HSI per treatment (n = 6 largemouth bass per treatment for each sex), for the *p,p*'-DDE and dieldrin diets. Means for treatments with the same lower case letter were not significantly different ($p > 0.05$) within each sex and pesticide.

| | *p,p*'-DDE Treatments (µg/g) | | | |
|---|---|---|---|---|
| Female | Control | 5 | 46 | 50 |
| Weight (g) | 207 ± 40[a] | 222 ± 39[a] | 188 ± 56[a] | 208 ± 38[a] |
| Length (mm) | 238 ± 11[a] | 244 ± 10[a] | 232 ± 20[a] | 237 ± 14[a] |
| K | 1.52 ± 0.14[a] | 1.51 ± 0.10[a] | 1.47 ± 0.09[a] | 1.55 ± 0.10[a] |
| GSI (%) | 1.64 ± 0.57[a] | 1.65 ± 0.62[a] | 1.68 ± 0.29[a] | 2.50 ± 1.63[a] |
| HSI (%) | 4.79 ± 0.95[a] | 4.16 ± 0.88[a] | 4.24 ± 0.94[a] | 4.71 ± 0.89[a] |
| | *p,p*'-DDE Treatments (µg/g) | | | |
| Male | Control | 5 | 46 | 50 |
| Weight (g) | 209 ± 53[a] | 202 ± 24[a] | 204 ± 19[a] | 215 ± 38[a] |
| Length (mm) | 239 ± 15[a] | 243 ± 13[a] | 243 ± 10[a] | 245 ± 10[a] |
| K | 1.50 ± 0.13[a] | 1.42 ± 0.21[a] | 1.42 ± 0.09[a] | 1.46 ± 0.06[a] |
| GSI (%) | 0.53 ± 0.12[a] | 0.52 ± 0.07[a] | 0.50 ± 0.08[a] | 0.64 ± 0.17[a] |
| HSI (%) | 4.83 ± 1.34[a] | 5.16 ± 1.10[a] | 3.98 ± 0.91[a] | 4.72 ± 0.63[a] |
| | Dieldrin Treatments (µg/g) | | | |
| Female | Control | 0.04 | 0.4 | 0.8 |
| Weight (g) | 207 ± 40[a] | 224 ± 40[a] | 231 ± 30[a] | 189 ± 29[a] |
| Length (mm) | 238 ± 11[a] | 240 ± 10[a] | 245 ± 10[a] | 233 ± 11[a] |
| K | 1.52 ± 0.14[a] | 1.61 ± 0.14[a] | 1.57 ± 0.06[a] | 1.49 ± 0.10[a] |
| GSI (%) | 1.64 ± 0.57[a] | 1.89 ± 0.37[a] | 1.78 ± 0.18[a] | 1.49 ± 0.80[a] |
| HSI (%) | 4.79 ± 0.95[a] | 4.08 ± 0.92[a] | 3.88 ± 1.27[a] | 4.42 ± 0.85[a] |
| | Dieldrin Treatments (µg/g) | | | |
| Male | Control | 0.04 | 0.4 | 0.8 |
| Weight (g) | 209 ± 53[a] | 227 ± 48[a] | 209 ± 47[a] | 205 ± 49[a] |
| Length (mm) | 239 ± 15[a] | 246 ± 12[a] | 240 ± 14[a] | 238 ± 16[a] |
| K | 1.50 ± 0.13[a] | 1.51 ± 0.13[a] | 1.50 ± 0.11[a] | 1.50 ± 0.09[a] |
| GSI (%) | 0.53 ± 0.12[a] | 0.44 ± 0.05[a] | 0.48 ± 0.11[a] | 0.50 ± 0.04[a] |
| HSI (%) | 4.83 ± 1.34[a] | 4.54 ± 1.13[a] | 4.94 ± 1.48[a] | 4.94 ± 0.67[a] |

Table 3-3. Day-90 mean ± SD results of female and male weight, total length, condition factor (K), GSI, and HSI per treatment (n = 6 largemouth bass per treatment for each sex), for the *p,p*'-DDE and dieldrin diets. Means for treatments with the same lower case letter were not significantly different ($p > 0.05$) within each sex and pesticide.

| | *p,p*'-DDE Treatments (µg/g) | | | |
|---|---|---|---|---|
| Female | Control | 5 | 46 | 50 |
| Weight (g) | 227 ± 33[a] | 243 ± 65[a] | 267 ± 39[a] | 242 ± 36[a] |
| Length (mm) | 245 ± 9[a] | 250 ± 20[a] | 257 ± 11[a] | 246 ± 11[a] |
| K | 1.53 ± 0.07[a] | 1.53 ± 0.09[a] | 1.56 ± 0.06[a] | 1.61 ± 0.06[a] |
| GSI (%) | 2.05 ± 0.20[a] | 2.11 ± 0.50[a] | 1.93 ± 0.32[a] | 1.91 ± 0.55[a] |
| HSI (%) | 5.13 ± 0.68[a] | 4.57 ± 1.14[a] | 4.47 ± 1.90[a] | 5.55 ± 0.83[a] |
| | *p,p*'-DDE Treatments (µg/g) | | | |
| Male | Control | 5 | 46 | 50 |
| Weight (g) | 221 ± 22[a] | 217 ± 25[a] | 221 ± 23[a] | 220 ± 30[a] |
| Length (mm) | 241 ± 7[a] | 240 ± 6[a] | 242 ± 7[a] | 242 ± 8[a] |
| K | 1.57 ± 0.03[a] | 1.57 ± 0.11[a] | 1.56 ± 0.09[a] | 1.54 ± 0.05[a] |
| GSI (%) | 0.61 ± 0.09[a] | 0.46 ± 0.15[a] | 0.52 ± 0.06[a] | 0.54 ± 0.14[a] |
| HSI (%) | 4.95 ± 1.00[a] | 5.06 ± 1.28[a] | 4.84 ± 0.80[a] | 4.98 ± 1.09[a] |
| | Dieldrin Treatments (µg/g) | | | |
| Female | Control | 0.04 | 0.4 | 0.8 |
| Weight (g) | 227 ± 33[a] | 224 ± 35[a] | 210 ± 26[a] | 234 ± 49[a] |
| Length (mm) | 245 ± 9[a] | 245 ± 11[a] | 239 ± 9[a] | 244 ± 15[a] |
| K | 1.53 ± 0.07[a] | 1.51 ± 0.11[a] | 1.54 ± 0.04[a] | 1.59 ± 0.10[a] |
| GSI (%) | 2.05 ± 0.20[a] | 1.99 ± 0.32[a] | 2.02 ± 0.31[a] | 2.04 ± 0.30[a] |
| HSI (%) | 5.13 ± 0.68[a] | 4.64 ± 0.63[a] | 5.12 ± 1.01[a] | 5.09 ± 1.05[a] |
| | Dieldrin Treatments (µg/g) | | | |
| Male | Control | 0.04 | 0.4 | 0.8 |
| Weight (g) | 221 ± 22[a] | 217 ± 23[a] | 213 ± 62[a] | 214 ± 49[a] |
| Length (mm) | 241 ± 7[a] | 245 ± 9[a] | 237 ± 16[a] | 241 ± 15[a] |
| K | 1.57 ± 0.03[a] | 1.47 ± 0.07[a] | 1.56 ± 0.16[a] | 1.50 ± 0.11[a] |
| GSI (%) | 0.61 ± 0.09[a] | 0.49 ± 0.08[b] | 0.51 ± 0.11[a,b] | 0.58 ± 0.05[a,b] |
| HSI (%) | 4.95 ± 1.00[a] | 4.70 ± 0.72[a] | 4.45 ± 0.81[a] | 5.36 ± 0.52[a] |

Table 3-4. Day-120 mean ± SD results of female and male weight, total length, condition factor (K), GSI, and HSI per treatment (n = 6 largemouth bass per treatment for each sex), for the *p,p*'-DDE and dieldrin diets. Means for treatments with the same lower case letter were not significantly different ($p > 0.05$) within each sex and pesticide.

| | *p,p*'-DDE Treatments (µg/g) | | | |
|---|---|---|---|---|
| Female | Control | 5 | 46 | 50 |
| Weight (g) | 204 ± 24[a] | 222 ± 52[a] | 252 ± 40[a] | 254 ± 49[a] |
| Length (mm) | 243 ± 12[a] | 244 ± 16[a] | 250 ± 8[a] | 254 ± 13[a] |
| K | 1.42 ± 0.13[b] | 1.51 ± 0.10[a,b] | 1.61 ± 0.15[a] | 1.53 ± 0.12[a,b] |
| GSI (%) | 2.27 ± 1.09[a] | 2.49 ± 0.68[a] | 3.04 ± 0.61[a] | 2.99 ± 0.62[a] |
| HSI (%) | 2.71 ± 1.21[b,c] | 3.73 ± 0.90[a,b] | 2.51 ± 0.29[c] | 3.96 ± 0.95[a] |
| | *p,p*'-DDE Treatments (µg/g) | | | |
| Male | Control | 5 | 46 | 50 |
| Weight (g) | 220 ± 34[a] | 246 ± 37[a] | 243 ± 53[a] | 230 ± 52[a] |
| Length (mm) | 248 ± 7[a] | 253 ± 9[a] | 251 ± 15[a] | 246 ± 18[a] |
| K | 1.43 ± 0.13[a] | 1.51 ± 0.08[a] | 1.51 ± 0.10[a] | 1.53 ± 0.07[a] |
| GSI (%) | 0.67 ± 0.06[a] | 0.70 ± 0.20[a] | 0.63 ± 0.19[a] | 0.61 ± 0.12[a] |
| HSI (%) | 2.95 ± 0.84[a] | 3.39 ± 1.13[a] | 2.62 ± 0.74[a] | 3.80 ± 0.92[a] |
| | Dieldrin Treatments (µg/g) | | | |
| Female | Control | 0.04 | 0.4 | 0.8 |
| Weight (g) | 204 ± 24[a] | 251 ± 56[a] | 233 ± 48[a] | 238 ± 43[a] |
| Length (mm) | 243 ± 12[a] | 256 ± 13[a] | 251 ± 18[a] | 257 ± 14[a] |
| K | 1.42 ± 0.13[a] | 1.47 ± 0.11[a] | 1.46 ± 0.05[a] | 1.39 ± 0.06[a] |
| GSI (%) | 2.27 ± 1.09[a] | 3.07 ± 1.22[a] | 3.19 ± 1.78[a] | 3.14 ± 0.62[a] |
| HSI (%) | 2.71 ± 1.21[a] | 3.32 ± 1.10[a] | 2.76 ± 0.52[a] | 2.90 ± 0.73[a] |
| | Dieldrin Treatments (µg/g) | | | |
| Male | Control | 0.04 | 0.4 | 0.8 |
| Weight (g) | 220 ± 34[a] | 258 ± 38[a] | 253 ± 25[a] | 236 ± 37[a] |
| Length (mm) | 248 ± 7[a] | 262 ± 12[a] | 256 ± 16[a] | 253 ± 10[a] |
| K | 1.43 ± 0.13[a] | 1.43 ± 0.07[a] | 1.53 ± 0.33[a] | 1.45 ± 0.07[a] |
| GSI (%) | 0.67 ± 0.06[a] | 0.71 ± 0.23[a] | 0.67 ± 0.22[a] | 0.66 ± 0.11[a] |
| HSI (%) | 2.95 ± 0.84[a] | 3.06 ± 0.78[a] | 2.31 ± 0.50[a] | 2.98 ± 0.68[a] |

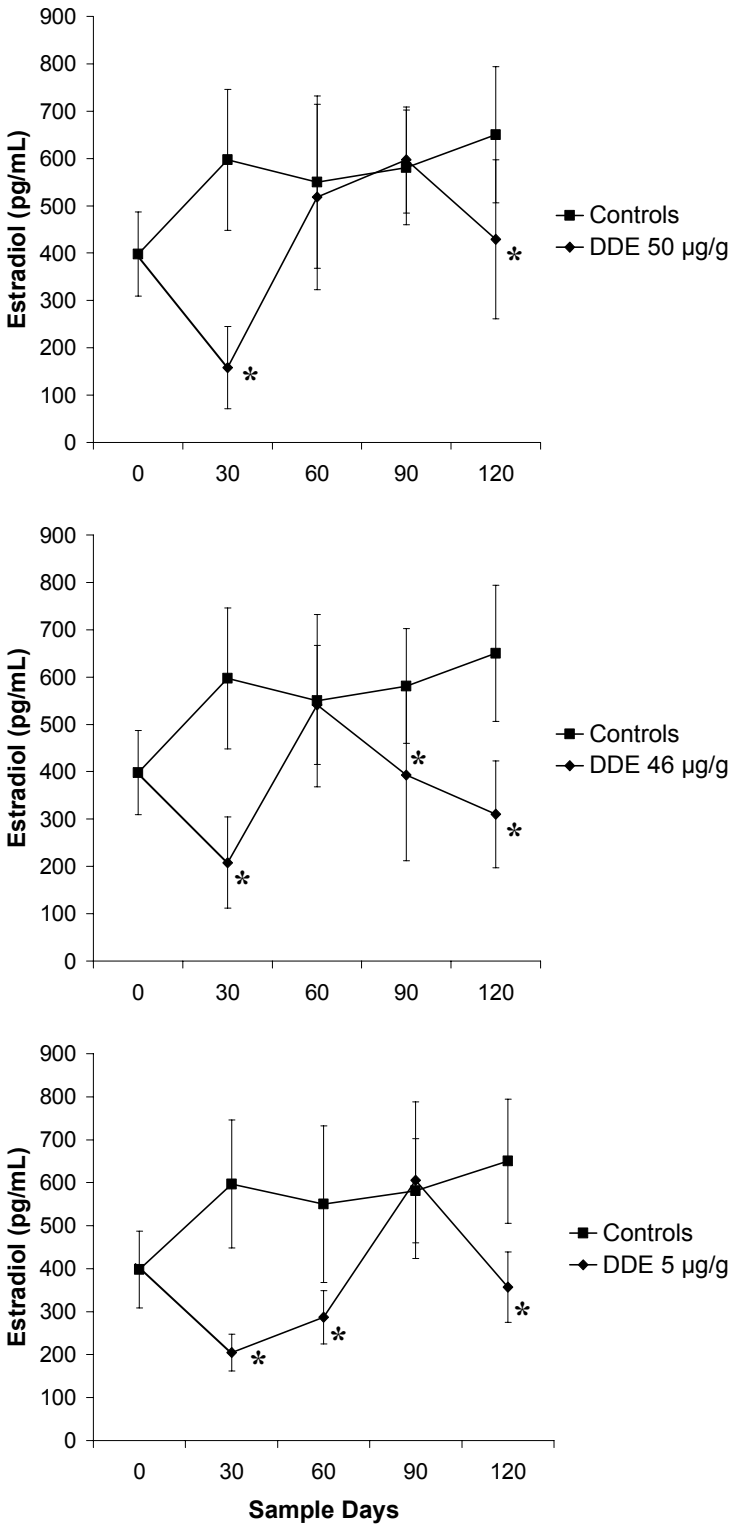


Figure 3-1. Female estradiol concentrations, on days 0, 30, 60, 90, and 120 for the 50, 46, and 5 µg/g *p,p*'-DDE treatments (n = 6 largemouth bass per sample day, per treatment), compared to the Control. Asterisk represents significant difference ($p \leq 0.05$) from the Control.

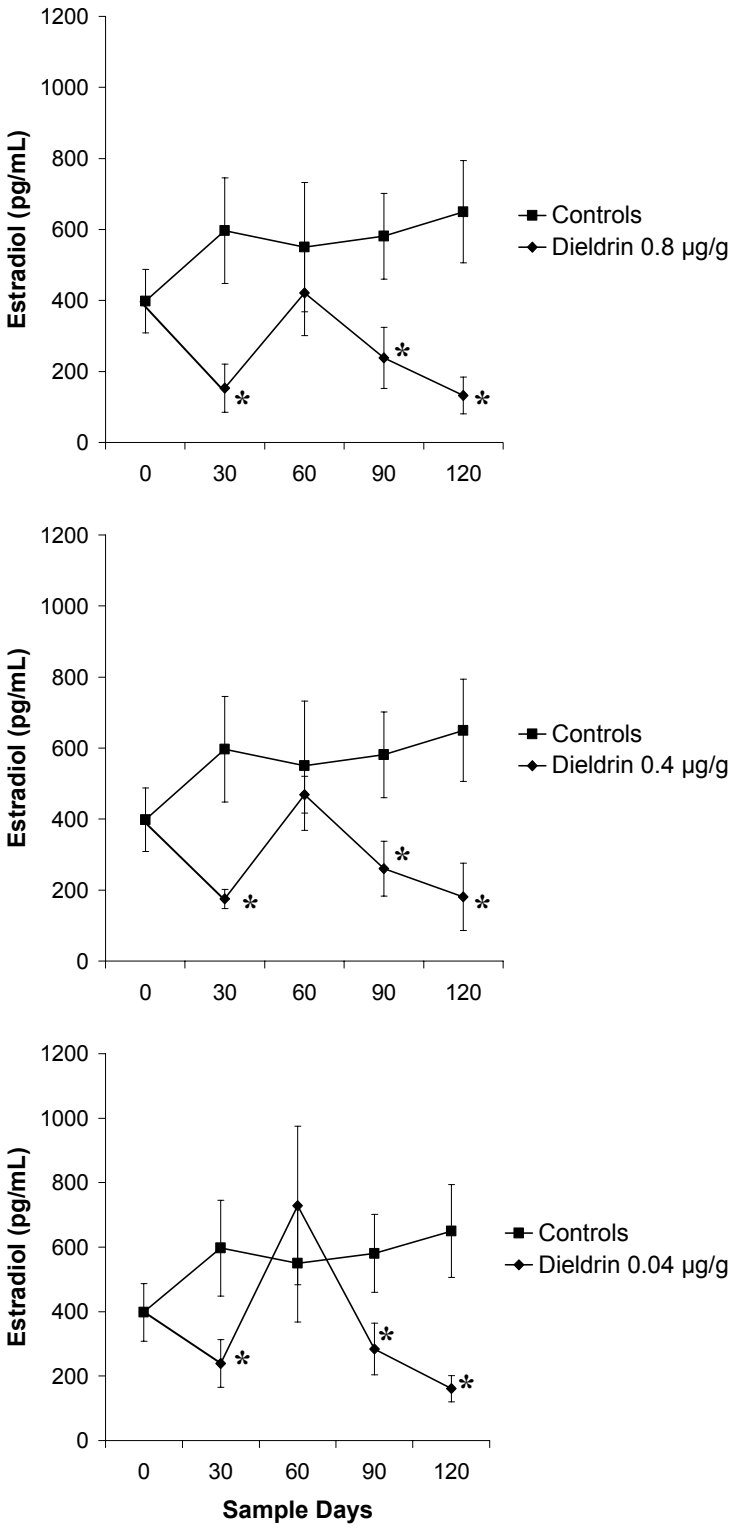


Figure 3-2. Female estradiol concentrations, on days 0, 30, 60, 90, and 120 for the 0.8, 0.4, and 0.04 µg/g Dieldrin treatments (n = 6 largemouth bass per sample day, per treatment), compared to the Control. Asterisk represents significant difference ($p \leq 0.05$) from the Control.

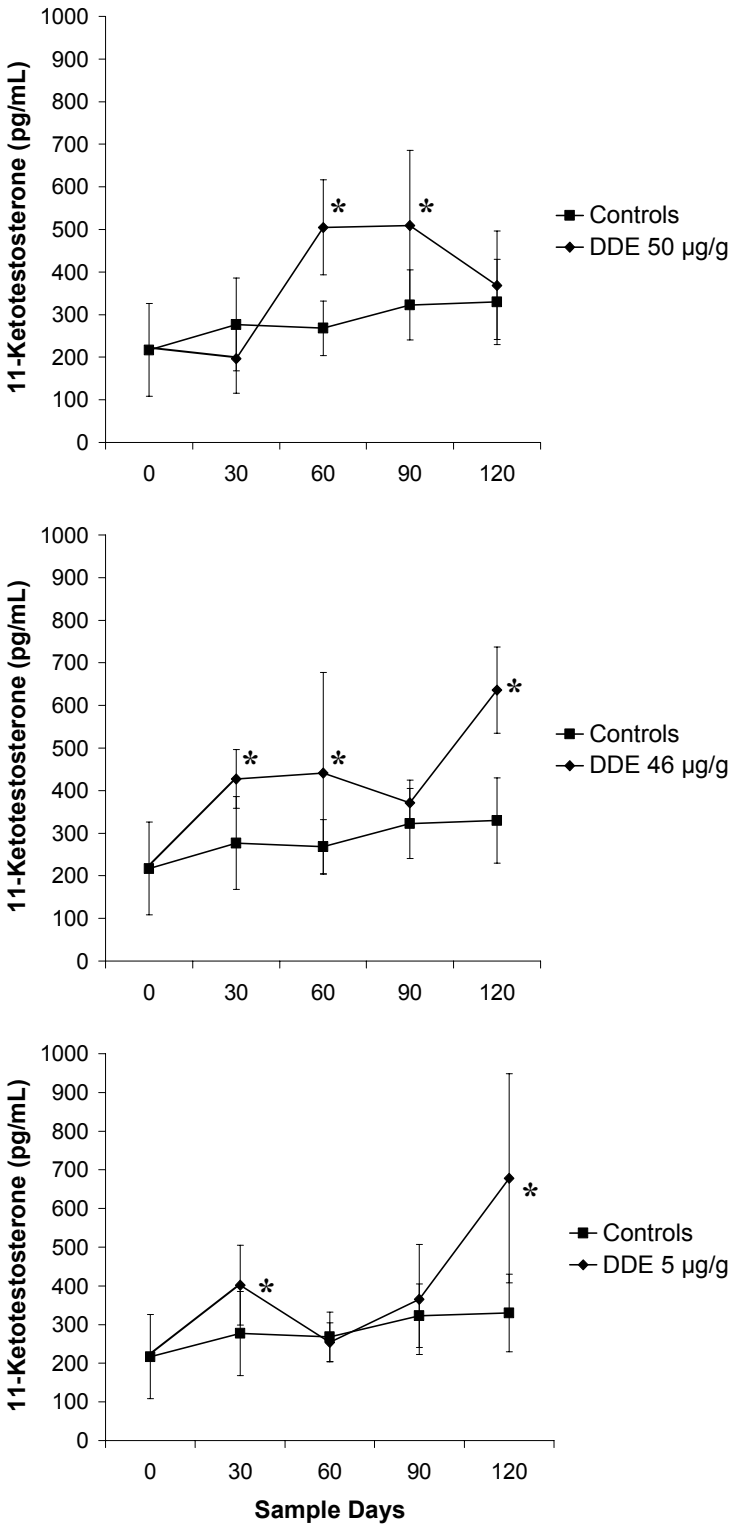


Figure 3-3. Female 11-ketotestosterone concentrations, on days 0, 30, 60, 90, and 120 for the 50, 46, and 5 µg/g *p,p*'-DDE treatments (n = 6 largemouth bass per sample day, per treatment), compared to the Control. Asterisk represents significant difference ($p \leq 0.05$) from the Control.

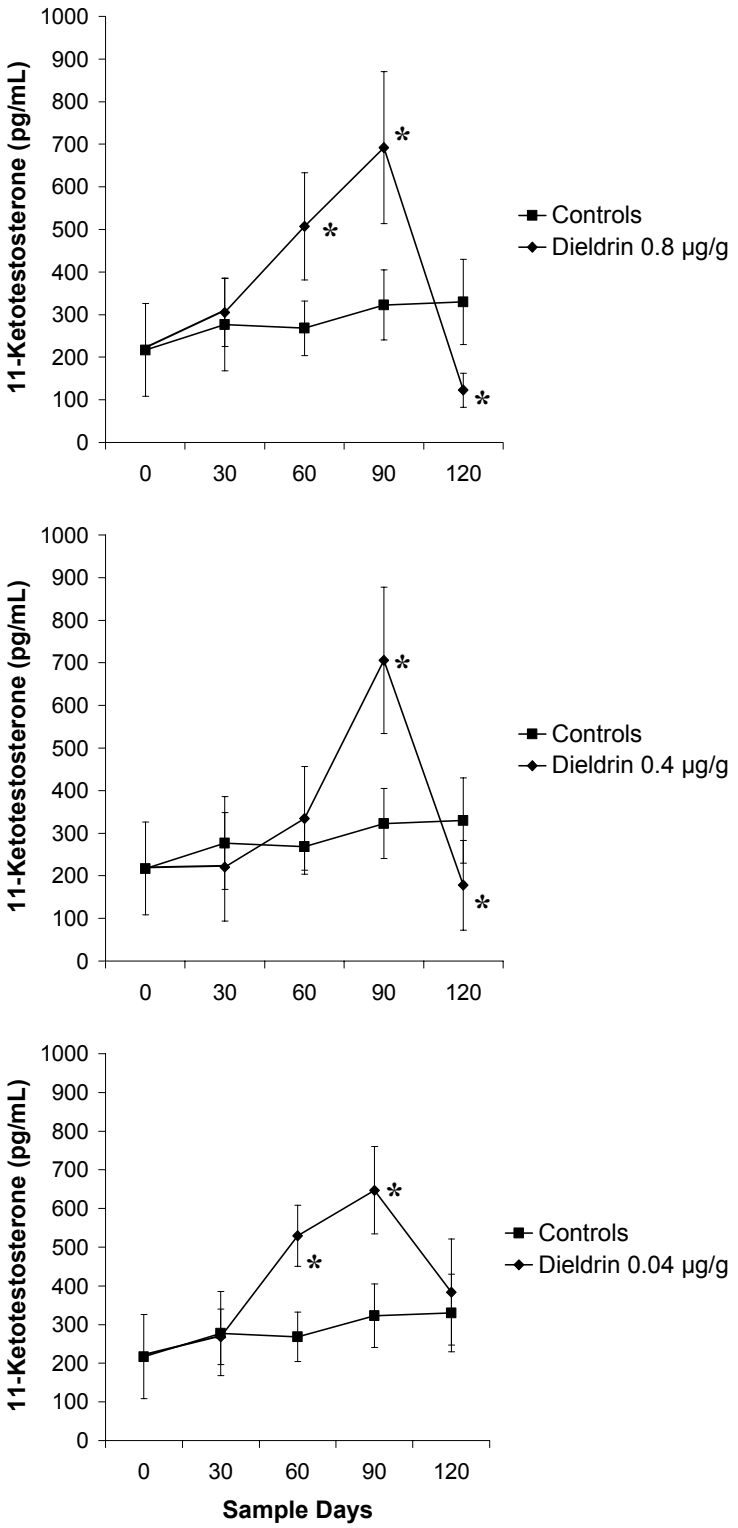


Figure 3-4. Female 11-ketotestosterone concentrations, on days 0, 30, 60, 90, and 120 for the 0.8, 0.4, and 0.04 µg/g Dieldrin treatments (n = 6 largemouth bass per sample day, per treatment), compared to the Control. Asterisk represents significant difference ($p \leq 0.05$) from the Control.

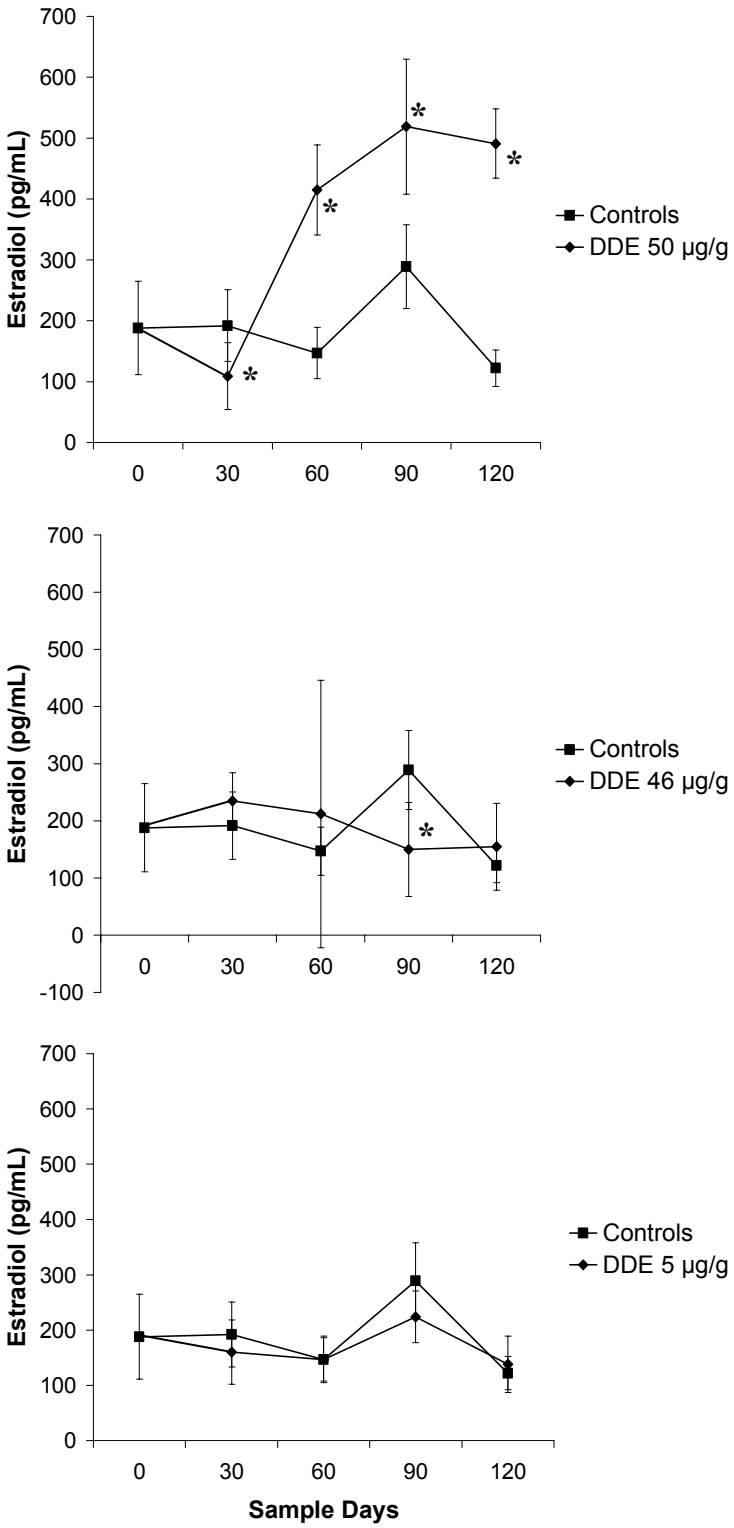


Figure 3-5. Male estradiol concentrations, on days 0, 30, 60, 90, and 120 for the 50, 46, and 5 µg/g *p,p*'-DDE treatments (n = 6 largemouth bass per sample day, per treatment), compared to the Control. Asterisk represents significant difference ($p \leq 0.05$) from the Control.

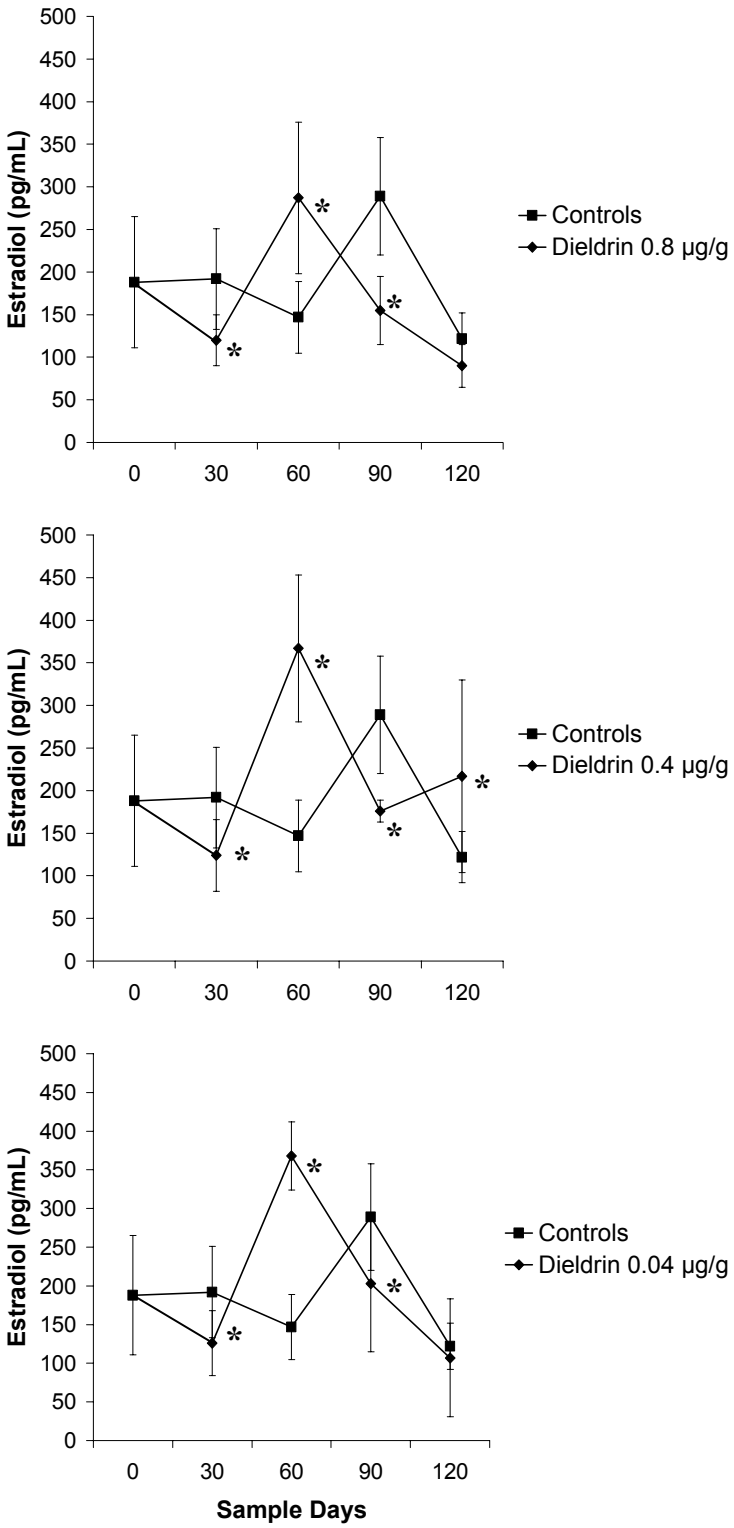


Figure 3-6. Male estradiol concentrations, on days 0, 30, 60, 90, and 120 for the 0.8, 0.4, and 0.04 µg/g Dieldrin treatments (n = 6 largemouth bass per sample day, per treatment), compared to the Control. Asterisk represents significant difference ($p \leq 0.05$) from the Control.

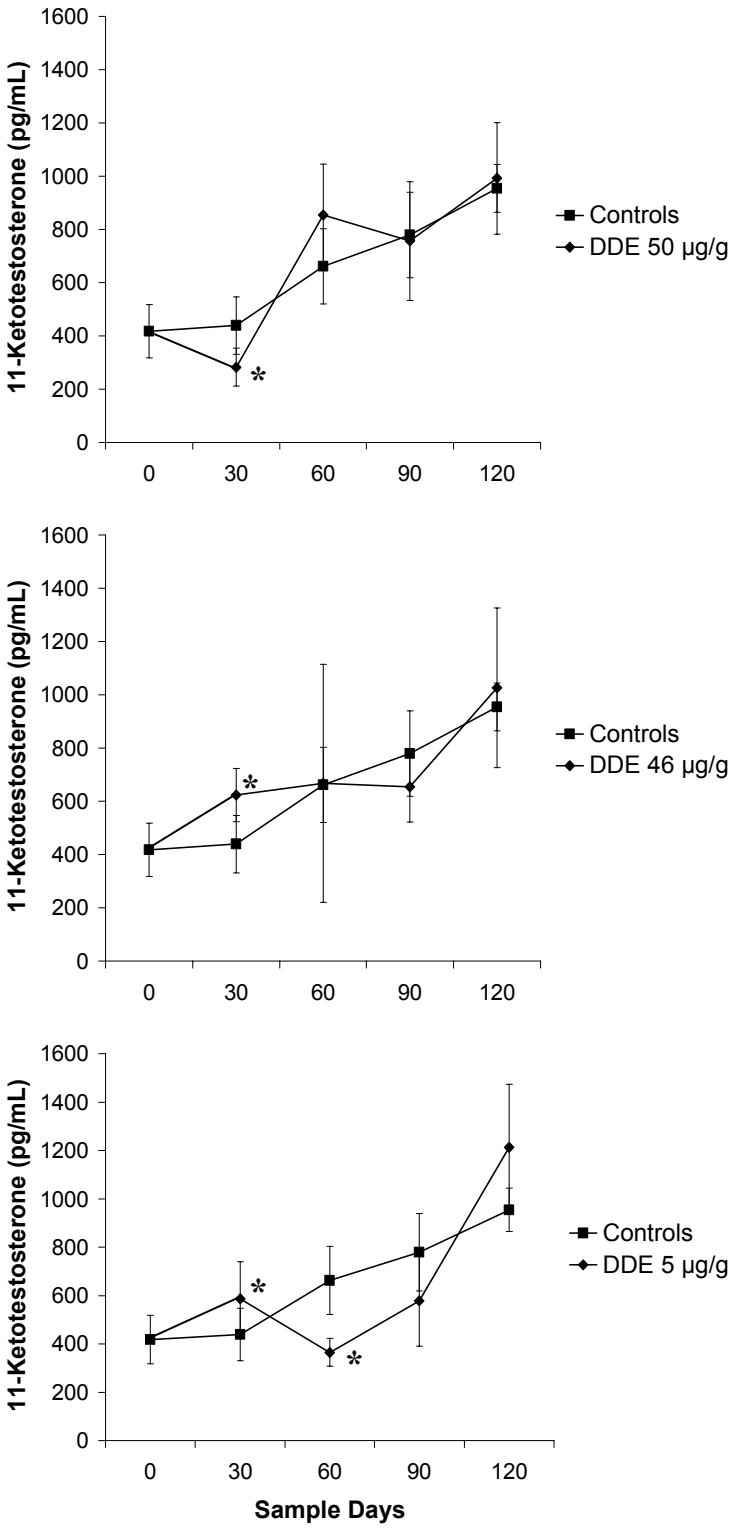


Figure 3-7. Male 11-ketotestosterone concentrations, on days 0, 30, 60, 90, and 120 for the 50, 46, and 5 µg/g *p,p*'-DDE treatments (n = 6 largemouth bass per sample day, per treatment), compared to the Control. Asterisk represents significant difference ($p \leq 0.05$) from the Control.

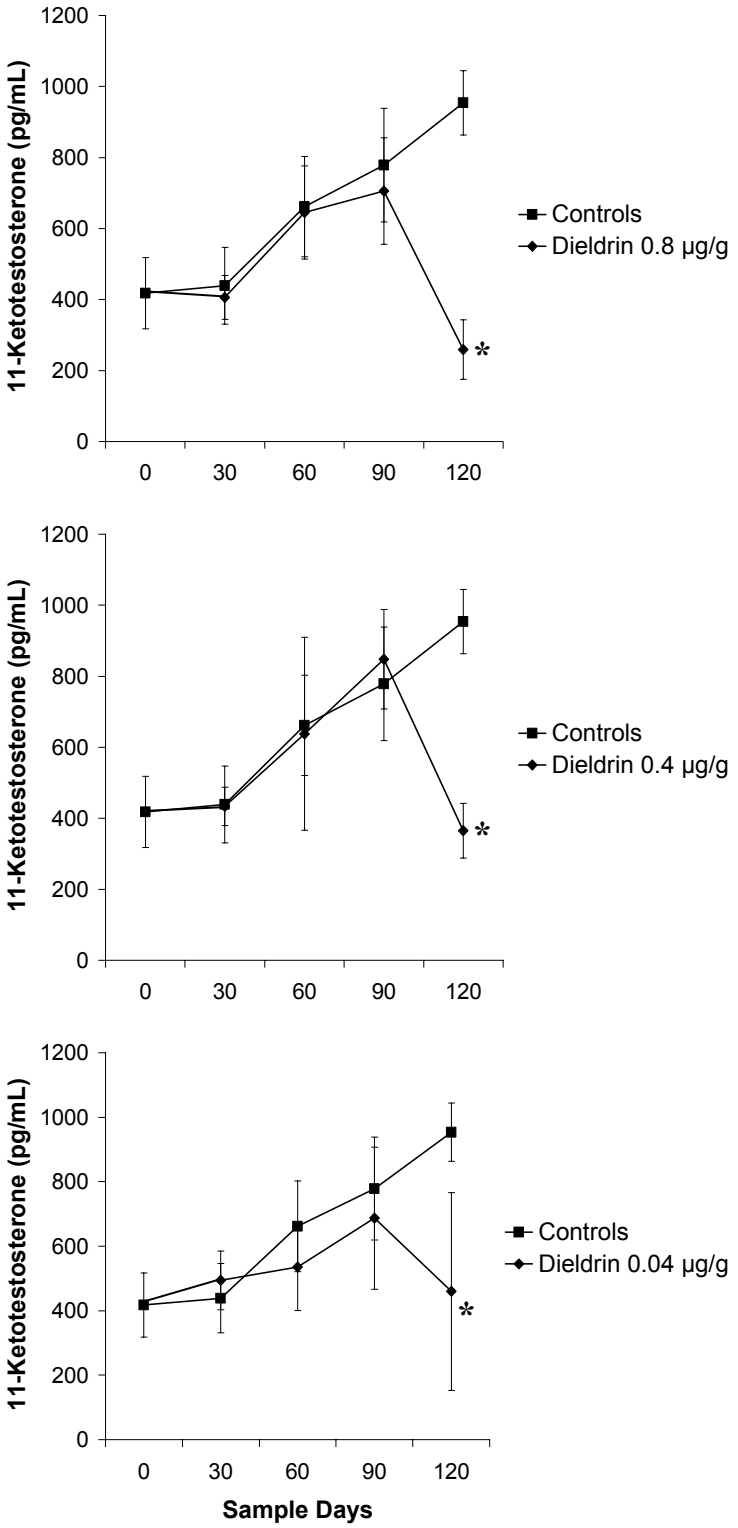


Figure 3-8. Male 11-ketotestosterone concentrations, on days 0, 30, 60, 90, and 120 for the 0.8, 0.4, and 0.04 µg/g Dieldrin treatments (n = 6 largemouth bass per sample day, per treatment), compared to the Control. Asterisk represents significant difference ($p \leq 0.05$) from the Control.

Table 3-5. Day-120 GC-MS mean ± SD results of both female and male largemouth bass *p,p*'-DDE and dieldrin concentrations (ng/g) in the carcass per treatment (n = 3 carcasses per treatment, for each sex).

| | Treatments (µg/g) | | | |
|---|---|---|---|---|
| *p,p*'-DDE | Control | 5 | 46 | 50 |
| Female Carcass | 19 ± 2 | 589 ± 614 | 8545 ± 1108 | 9015 ± 1042 |
| Male Carcass | 170 ± 222 | 1417 ± 372 | 7279 ± 1980 | 6763 ± 1430 |

| | Treatments (µg/g) | | | |
|---|---|---|---|---|
| Dieldrin | Control | 0.04 | 0.4 | 0.8 |
| Female Carcass | 2.3 ± 0.6 | 0.6 ± 0 | 216.7 ± 34.3 | 276.3 ± 57.1 |
| Male Carcass | 1.4 ± 1.4 | 1.4 ± 1.4 | 190.3 ± 44.8 | 254.3 ± 50.3 |

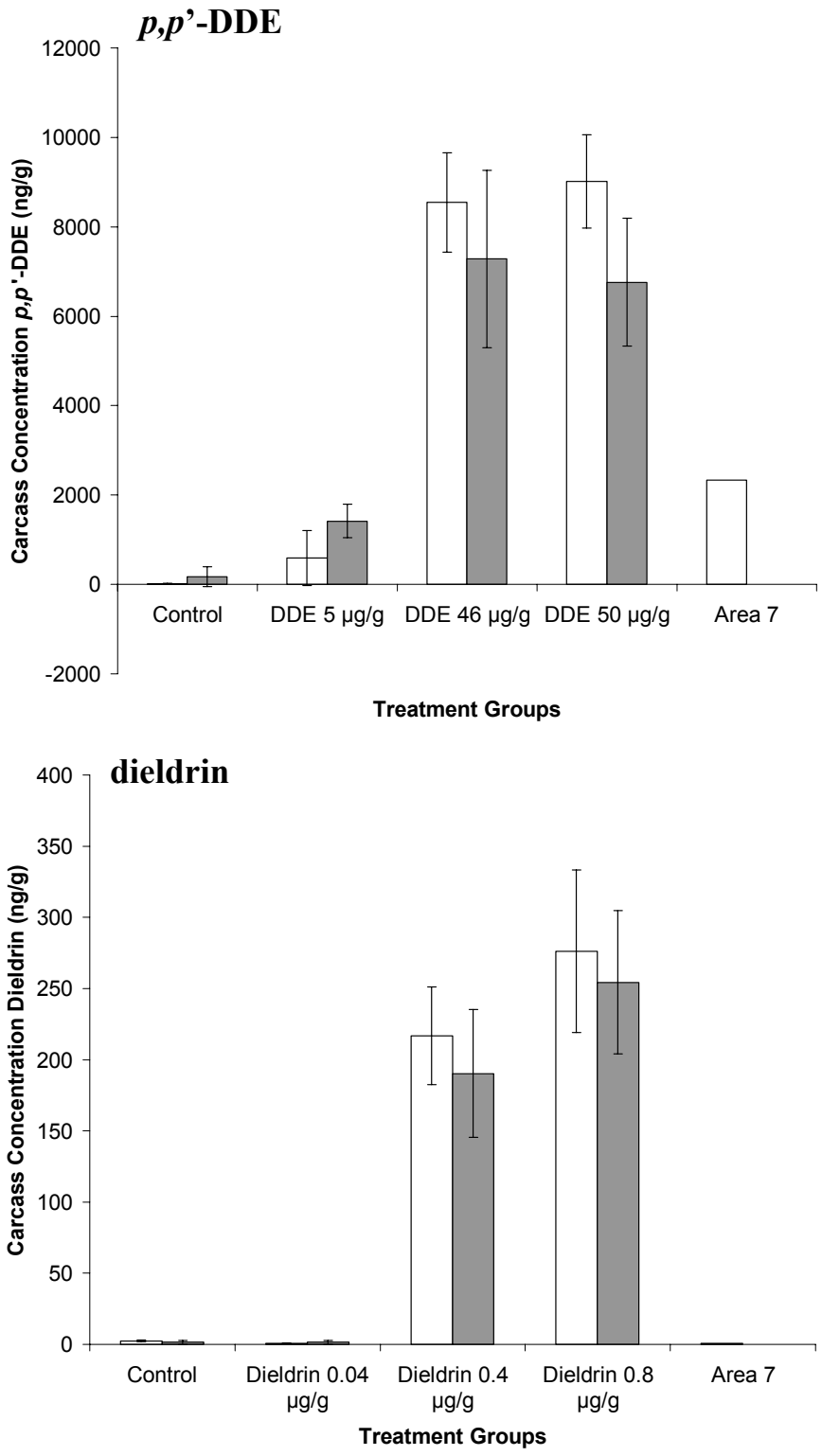


Figure 3-9. Female (white bars) and male (shaded bars) largemouth bass mean ± SD carcass concentrations of *p,p*'-DDE and dieldrin treatments (n = 3 carcasses per treatment, for each sex). Included is the mean carcass concentration of each organochlorine for the five female largemouth bass sampled from Area 7 (white bar) on February 23, 2004.

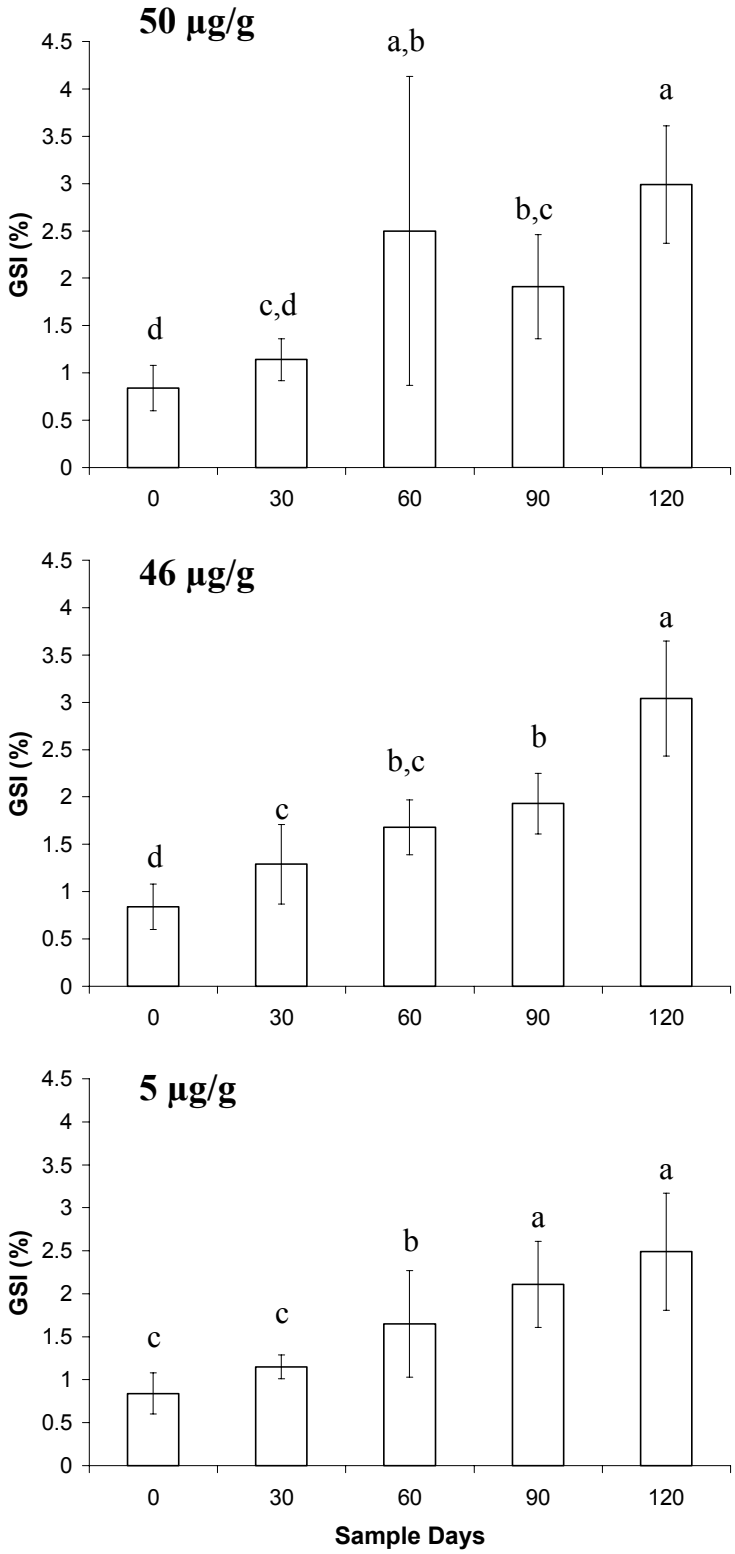


Figure 3-10. Change in female GSI (%) over the entire 120-day sampling period for the 50, 46, and 5 µg/g *p,p*'-DDE treatments (n = 6 largemouth bass per sample day). Sample days with the same lower case letter were not significantly different ($p > 0.05$).

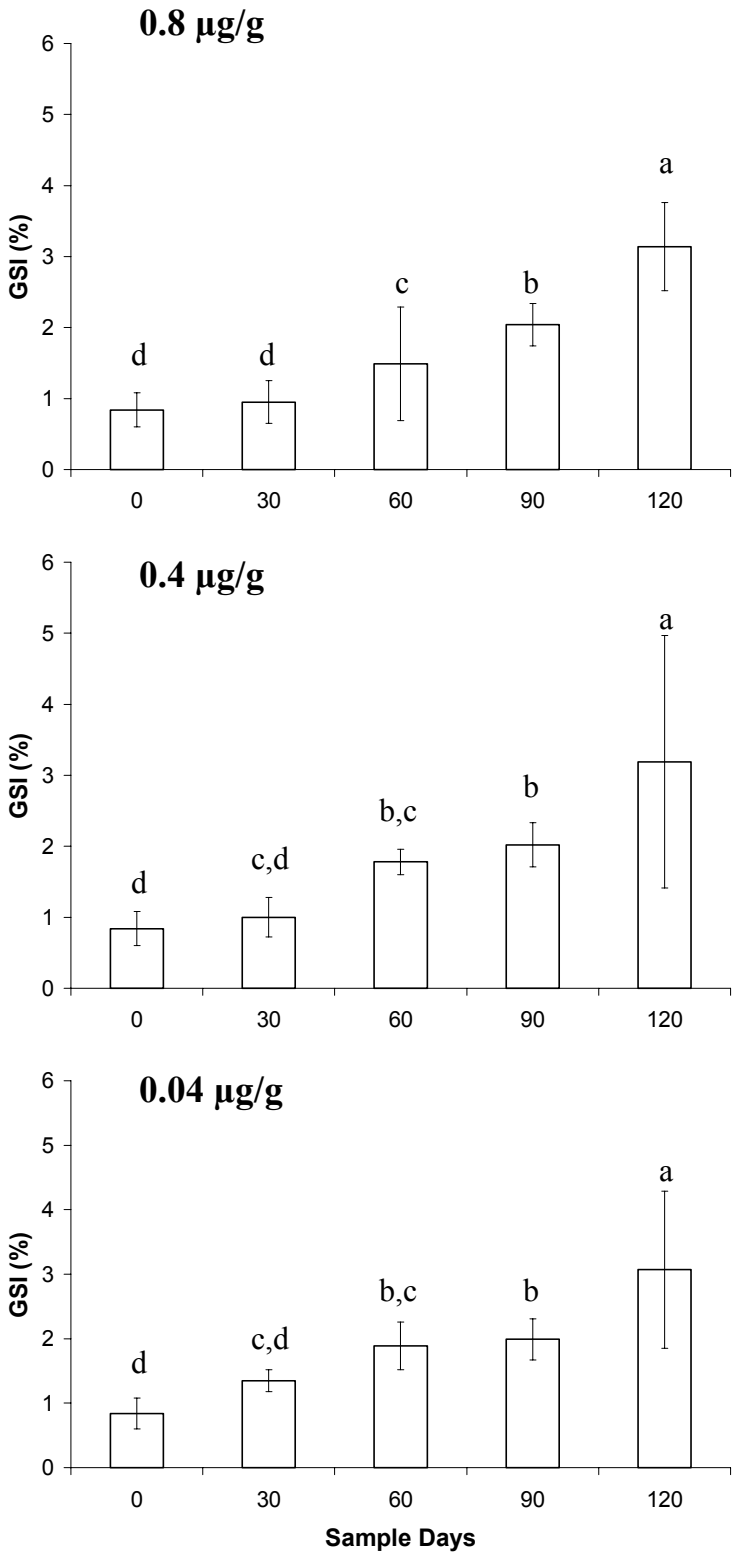


Figure 3-11. Change in female GSI (%) over the entire 120-day sampling period for the 0.8, 0.4, and 0.04 µg/g Dieldrin treatments (n = 6 largemouth bass per sample day). Sample days with the same lower case letter were not significantly different ($p > 0.05$).

Table 3-6. Day-120 mean ± SD results of percent hatch for the *p,p*'-DDE and dieldrin treatments (n = 6 clutches per treatment). Treatments with the same upper case letter were not significantly different ($p > 0.05$).

| *p,p*'-DDE Treatments (µg/g) | | | |
|---|---|---|---|
| Control | 5 | 46 | 50 |
| $51 \pm 15^{b}$ | $55 \pm 21^{a,b}$ | $52 \pm 9^{b}$ | $71 \pm 6^{a}$ |
| (36 – 74) | (23 – 77) | (39 – 61) | (63 – 80) |
| **Dieldrin Treatments (µg/g)** | | | |
| Control | 0.04 | 0.4 | 0.8 |
| $51 \pm 15^{b}$ | $85 \pm 6^{a}$ | $58 \pm 10^{a,b}$ | $61 \pm 29^{a,b}$ |
| (36 – 74) | (81 – 89) | (47 – 75) | (10 – 84) |

# CHAPTER 4
# GENERAL CONCLUSIONS

Research from this study provides evidence that dietary exposure to the OCPs *p,p*'-DDE and dieldrin can create internal carcass and gonad concentrations of these two pesticides at levels similar to largemouth bass taken from reclaimed Florida agriculture areas that are believed to have impaired endocrine and reproductive function (Benton and Douglas, 1996; Marburger *et al*., 1999). In addition, my study demonstrated that length and timing of pesticide dietary exposure is important in replicating sex steroid hormone concentrations reported for largemouth bass sampled from the EMCA. In my 30-day dietary exposure study, there was no evidence of a dose-response decrease in GSI or circulating sex steroid hormones. The results may have been influenced by OCP exposure taking place during a portion of the annual reproductive cycle of the largemouth bass after their reproductive organs were fully developed; thus, causing my first study to miss critical events in the reproductive cycle that could have facilitated gametogenic and steroidogenic changes. Perhaps, endocrine system changes that initiate gonad maturation, including surges in $E_2$ and 11-KT sex steroid production, had already taken place and were already on a seasonal decline by the time pesticide exposure in the 30-day study began. OCP exposure length for my second study (120 days) was, therefore, extended to encompass a larger portion of the annual reproductive cycle.

Extension of exposure length demonstrated reductions in female largemouth bass $E_2$ concentrations, a lack of expected seasonal increasing trend in $E_2$ concentrations, and abnormal increases in 11-KT. These changes were similar to the reproductive

abnormalities reported for largemouth bass sampled from the EMCA (Marburger *et al*., 1999). Reductions in female plasma $E_2$ concentrations demonstrated by my treatments (*p,p*'-DDE and dieldrin) averaged 2 to 3 times less than the Control, indicating that both OCPs induced significant biological reductions in $E_2$ concentrations. Attained OCP carcass concentrations and achieved depressions of female $E_2$ concentrations, however, did not cause a reduction of percent hatch between the *p,p*'-DDE and dieldrin treated fish and those feed a control diet. Consequently, my study did not provide any strong evidence that two of the predominate OCPs (*p,p*'-DDE and dieldrin) found in soils and largemouth bass tissues, sampled from the EMCA, cause dose-response decreases in the percent hatch of eggs produced by spawning treated fish. This may indicate that a lack of reproductive success by adult Florida largemouth bass, stocked into the EMCA, is not the primary reason for the failure of the development of the EMCA into a quality largemouth bass fishery.

Future research on the reproductive effects of *p,p*'-DDE and dieldrin on largemouth bass could focus on the application of a dosing experiment over an entire calendar year. Even when exposure length in my second study was extended to a 120-day period, effects were only demonstrated at the hormonal or biochemical level. Largemouth bass in EMCA, reported to have impaired endocrine and reproductive function, are environmentally exposed these pesticides on a yearly basis, spanning all portions of the steroidogenic and gametogenic phases of the reproductive cycle. Extending OCP exposure length to an entire year will allow researchers to cover all portions of the reproductive cycle of the largemouth bass.

In addition, my studies only sought to characterize single chemical dose-response effects for two of the predominate OCPs, *p,p*'-DDE and dieldrin, found in the soils and various tissues of largemouth bass from the EMCA. Fish in this system are environmentally exposed to multiple pesticides (e.g., toxaphene and chlordane) that may not only contribute to reductions in hormone concentrations, but also to decreased reproductive success. Future research on the reproductive effects of OCPs on largemouth bass could also focus on pesticide mixture exposure studies and the effects that multiple pesticide exposure may attribute to hormone depression and reproductive success, not just on the single chemical exposure to *p,p*'-DDE and dieldrin. The application of studies using multiple single chemical doses, coupled with mixture exposures, will enable researchers to pinpoint what OCPs are responsible for causing endocrine disruption.

The apparent limited reproductive success by stocked adult Florida largemouth bass or recruitment of fish to the fingerling stage in the EMCA might not be attributed to OCP endocrine disruption or toxicity at all. An ecosystem assessment was conducted at the EMCA in 2001 to address other factors that might influence poor recruitment of largemouth bass in this system including, spawning habitat and phytoplankton, zooplankton, and macroinvertebrate communities. Preliminary results of the assessment report that a low abundance of invertebrates might be contributing to poor sport fish production in the EMCA because planktonic and benthic invertebrates are important components of the diets of larval and juvenile sport fish, including largemouth bass (William E. Johnson, Florida Fish and Wildlife Conservation Commission, personal communication). There is also evidence from this ecosystem assessment that poor

habitat (i.e., muck sediments and a scarcity of aquatic plants) might also be contributing to largemouth bass reproduction and recruitment problems in the EMCA.

Lastly, it is important that biologists continue to monitor OCP concentrations and population dynamics of largemouth bass in the EMCA. This will enable researchers to gain insight into whether or not improvements in largemouth bass reproduction and/or recruitment are related to changes in OCP levels or other environmental factors. Based on the results of my study, if money is a limiting factor, I would focus all research efforts on OCP mixture exposure experiments.

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## BIOGRAPHICAL SKETCH

Kevin G. Johnson was born September 26, 1979, and grew up in Mt. Dora, FL. He attended Mt. Dora High School and graduated in May 1998. Kevin then attended the University of Central Florida and received his Bachelor of Science degree in August 2002, with a major in biology and a minor in environmental studies. During his undergraduate study, Kevin was a biological technician for Dr. Linda Walters at UCF, working on oyster reef ecology in the Indian River Lagoon on the east coast of Florida. In the fall of 2002, Kevin began working in the ecotoxicology laboratory of Dr. Timothy S. Gross at the United States Geological Survey in Gainesville, FL. Then, in the spring of 2003, he enrolled as a graduate student at the University of Florida with Drs. Timothy S. Gross and Daniel E. Canfield, Jr. as his advisors, focusing his work on the reproductive effects of organochlorine pesticides on largemouth bass. Kevin's passion for the study of fisheries and aquatic sciences has translated into his passion as a fresh and saltwater fisherman, a pastime he plans to pursue for many years to come.