Hepatic Mixed-Function Oxidase Enzymes in White Suckers (*Catostomas commersoni*) over a Seasonal Cycle and Following Injection with PCB Congener 77.

BY

RANDALL JAMES BOYCHUK

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HEPATIC MIXED-FUNCTION OXIDASE ENZYMES IN WHITE SUCKERS (*Catostomas commersoni*) OVER A SEASONAL CYCLE AND FOLLOWING INJECTION WITH PCB CONGENER 77

BY

RANDALL JAMES BOYCHUK

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

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To Mom and Dad

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Abstract

The seasonal cycles of two cytochrome P-450-associated catalytic activities, ethoxyresorufin-O-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH), were studied in white suckers from a lake in Northwestern Ontario over two years. Also, the dosage dependency of induction of these same activities was determined at high and low points in the natural cycle. Both activities were lowest during the period just prior to and during spawning. Fish were caged in the lake during the spawning period in the spring and given doses of ^{14}C-3,3',4,4'- tetrachlorobiphenyl (PCB congener 77) in corn oil by intraperitoneal injection for an exposure period of five days. Similar experiments were conducted late in September. EROD and AHH induction were noted only at the highest dosage of 1000 μg kg^{-1} in both spring and fall treatments. Organs most highly contaminated with congener 77 (from ^{14}C content) were the fattier tissues: liver, gonads, and intestine, with lower levels in the leaner tissues: muscle, heart and gill. The dose-response induction in the fall (non-spawning) did not occur at a lower dosage than the spring (spawning) treatments. If induction is affected by hormonal activity, the fall induction response should have been at a lower PCB concentration. The reason for no induction response at lower PCB concentrations in the fall is unknown, but the lower fall water temperatures may have been responsible. An increase in the exposure period from five days to ten days at 100 μg kg^{-1} PCB in the spring showed significant enzyme induction and suggested that the enzyme response may occur at lower concentrations of an inducer with increased exposure time.

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I. Introduction

In the field of aquatic toxicology much study has been concentrated on the transport and fate of chemical contaminants in the aquatic environment. Contaminants are not always easily detectable and, therefore, some studies have attempted to determine not only contaminant presence and but also subtle biological effects within aquatic organisms (Goksøyr and Förlin, 1992). These studies can range from the biochemical or molecular level to that of the physiology of the individual organism. Change in these biological systems can indicate exposure to pharmacologically active pollutants and can help identify the compounds and possibly their sources (Stegeman et al., 1992). Since the first response to environmental change is often seen at the biochemical or molecular level, the study of biotransformation enzymes has become important in the early detection of changes induced by organic contaminants. The biotransformation process appears to be an attempt to eliminate organic contaminants by a two-stage process involving phase I and phase II metabolism (Goksøyr and Förlin, 1992; Jimenez and Stegeman, 1990). Usually, the phase I metabolism is an oxidative step where a polar group (generally containing one of the oxygen atoms from O_2) is added to an organic contaminant by the cytochrome P-450 (CYP) monooxygenase system (Goksøyr and Förlin, 1992; Jimenez and Stegeman, 1990). This increases the solubility of the compound so that it can combine with an endogenous group (eg. glycosides, sulfates, amino acid conjugates (James, 1987; Pritchard, 1993)) in a phase II metabolism to produce a water soluble end product that can be easily excreted through the bile or over the gills (Goksøyr and Förlin, 1992; Jimenez and Stegeman, 1990; James, 1987). The phase I metabolism by the cytochrome P-450 system, then, is usually one of the first detectable responses to an organic contaminant.

The cytochrome P-450 monooxygenases belong to a superfamily of heme proteins embedded in the endoplasmic reticulum with the active site in the cytoplasm (Goksøyr and Förlin, 1992; Stegeman et al., 1992; Brown and Black, 1989; Jimenez and Stegeman, 1990). The reactions that these proteins catalyze are often referred to as mixed function oxygenase (MFO) reactions (Stegeman et al., 1992). Organic compounds are oxidized by NADPH electron transfer to insert one atom of oxygen or a hydroxyl group (OH)(Goksøyr and Förlin, 1992; Jimenez and Stegeman, 1990; Lech et al., 1982) to the compound. Substrates that are oxidized can include foreign compounds such as pesticides and polycyclic aromatic hydrocarbons (PAHs) or endogenous compound such as steroid hormones, vitamins, and bile acids (Payne et al., 1987). The most responsive member of the cytochrome P-450 system to aromatic and chlorinated hydrocarbons is the subfamily CYP1A (Goksøyr and Förlin, 1992). The presence of such a substrate or a compound closely related to a substrate will induce this subfamily. An induction response occurs when a chemical stimulates the amount of P-450, generally by increasing the rate of transcription, synthesizing new messenger RNA, and therefore new enzyme protein (Goksøyr and Förlin, 1992; Stegeman et al., 1992; Kleinow et al., 1987). The factors involved in this gene activation in fish are becoming better known (Goksøyr and Förlin, 1992); several known P-450 inducers in fish are common aquatic pollutants (eg. PCBs, chlorinated dioxins and furans, PAHs)(Goksøyr and Förlin, 1992; Kleinow et al., 1987; Stegeman and Kloepper-Sams, 1987).

Many studies of the MFO system in mammals have identified genes and specific proteins involved primarily in the liver microsomal P-450 system (Stegeman et al., 1992). Most studies have determined the activities of the enzymes produced by exposures to inducers rather than by detecting changes in mRNA. Similar but less complicated P-450 systems are present in fish livers and other tissues (gills, Miller et

al. (1989), heart, Payne et al. (1987), kidneys, Pesonen et al. (1987)) and are also inducible. Activities often used for detecting induction responses in the PAH-inducible CYP1A subfamily, have been ethoxyresorufin-O-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH)(Goksøyr and Förlin, 1992; Collier and Varanasi, 1991). These are relatively specific in their response to inducing chemicals (Stegeman et al., 1992; Lech et al., 1982; Elcrombe and Lech, 1979). Thus, "MFO activity" is a generic term that can be expressed as EROD activity when ethoxyresorufin is used as a substrate or as AHH activity when benzopyrene (B(a)P) is used as a substrate, or as others not covered in this review.

Most of the organic contaminants that induce the MFO system in the aquatic environment are toxic (Stegeman et al., 1992). Early studies used pure compounds or mixtures at high dosages to determine induction responses (Addison et al., 1978; Payne and Penrose, 1975; Sivarajah et al., 1978; Elcrombe and Lech, 1978; Förlin, 1980; Stegeman et al., 1981; Vodicnik et al., 1981). Later, dose-response studies showed that induction occurred at much lower levels of some pure contaminants, ranging from 0.1 to 1.0 mg kg^{-1} (Pesonen et al., 1987; Monosson and Stegeman, 1991; Collier and Varanasi, 1991; Gooch et al., 1989; Zhang et al., 1990). For example, significant increases in enzyme activity occurred with treatments of 3,3',4,4'-tetrachlorobiphenyl (PCB congener 77 or CB77) as low as 0.01 mg kg^{-1} (Melancon and Lech, 1982). CB77 is one of 209 possible PCB structural congeners and one of at least sixteen congeners that induce MFO enzymes (Niimi and Oliver, 1989a). The PCB is coplanar (has a structure with two *para* and at least one *meta* substituents (Gooch et al., 1989)) and allows the molecule to attain, at times, a flat stereochemistry similar to dioxins or furans which are highly toxic. When flat the CB77 would resemble one of the most toxic dioxins 2,3,7,8-TCDD (Janz and Metcalf, 1991; Niimi and Oliver, 1989b). With a low

solubility of 0.6 μg L^{-1} (Dickut et al., 1986), Koc of 34,500, and Henry's Law Constant of 9.4 x 10^{-3} (Huckins et al., 1988), CB77 is highly lipophilic and can bioaccululate quite readily in tissues (Huckins et al., 1988). Several studies have monitored the effects of CB77 on the MFO system and its fate in the tissues of fish (Gooch et al., 1989; Huckins et al., 1988; Melancon et al., 1989).

In most studies the fish were treated with a single intraperitoneal (I.P.) injection and the results suggest that contaminants at environmental concentrations could effect the MFO activities. In fact, elevated levels of P-4501A in many fish in the environment have been observed, confirming that environmental concentrations of some chemicals are indeed causing induction (Stegeman et al., 1992; Collier et al., 1992). The MFO system is one of the earliest biological responses that can be recognised following exposure and it is sensitive enough to detect concentrations of contaminants found in the environment. Consequently it has been rationalized that the MFO system could be used as an environmental monitor or "biomonitor" for exposure of fish to certain organic contaminants.

Numerous investigations have used the MFO system in fish as a biomonitor to detect exposures to organic contaminants in the aquatic environment (Chambers and Yarbrough, 1976; Goksøyr and Förlin, 1992; Stegeman et al., 1992; Payne and Fancey, 1982; Jimenez and Stegeman, 1990; Payne et al., 1987; Kleinow et al., 1987; Lech et al., 1982; Jimenez et al., 1991). The MFO system has been hypothesized to serve as an early warning before more serious pathologies are produced (Jimenez and Stegeman, 1990; Payne et al., 1987). The use of the MFO system as an environmental monitor is still far from perfect. There are still many unknown inducers which need to be identified, but more importantly, the concentrations of individual inducers required to

initiate induction must be addressed for different fish species. Experimentally, this could be accomplished with more studies of dose-response relationships for inducers and suspected inducers (Stegeman et al., 1992). Given these relationships the magnitude of induction could be compared with the environmental induction and tissue concentrations of the inducers.

One of the problems in using the MFO activities as an indicator of pollution is a lack of baseline data for the enzyme activities in the many species available. Finding suitable reference sites with 'uncontaminated' fish has proven difficult since even remote systems usually have measurable amounts of pollution from domestic, industrial, and agricultural sources (Jimenez and Stegeman, 1990). Such sites are important in interpreting the response of the biomonitor (Jimenez et al., 1991). The use of a baseline for enzyme activities under natural pristine or near pristine conditions are required as references for the comparison of fish thought to be exposed to contamination. Such baseline data must be species and sex and season specific to account for variations in activities that occur due to species (Goksøyr and Förlin, 1992; Kleinow et al.; 1987), sex (Hansson et al., 1980; Goksøyr and Förlin, 1992; Jimenez et al., 1991; Stegeman and Chevion, 1980) and water temperature (Goksøyr and Förlin, 1992; Stegeman 1979; Andersson and Koivusaari, 1985) which is linked to differences in season or hormonal status (Goksøyr and Förlin, 1992; Pajor et al., 1990; Gray et al., 1991; Jimenez and Stegeman, 1990; Jimenez et al., 1991). This "baseline" information could then be ulitized to help determine whether the fish from other systems had been exposed to inducing contaminants.

In principle, any species could be used, but some offer greater potential than others as environmental sentinels (Marshall et al., 1987; Munkittrick and Dixon, 1989);

desirable traits include wide distribution, known habitat requirements and niche characteristics, and suitability for laboratory experimentation. The white sucker (*Catostomus commersoni*) meets these criteria and has been used in environmental monitoring (Munkittrick and Dixon, 1989). This species, however, does have a high tendency to accumulate fat-soluble organic compounds like PCBs (Huckins and Schwartz, 1988; Smith et al., 1991; Maccubbin et al., 1985), though not as readily as fish of higher trophic levels. The white sucker is a benthic forager that can be the dominant biomass of a fish community (Trippel and Harvey, 1986) and is one of the most widely distributed fish species in North America (Scott and Crossman, 1973). The diet of the white sucker is a combination of aquatic invertebrates and detritus (Ahlgren, 1990). Hydrophobic organic contaminants will sorb to natural particles (Voice, 1983; Karickhoff et al., 1979) in the detritus and are transferred from the sediments into invertebrate animals (Larsson, 1986), especially those that move within the sediment (Connell et al., 1988; Oliver, 1987; Landrum, 1989). The white suckers then consume detritus and benthic invertebrates and are exposed through their diet (Maccubbin et al., 1985). Indeed, other benthic fish have also been used as bioindicators to detect contaminant exposure via MFO activities (Stein et al., 1992). Previous studies have shown the white sucker MFO activities to be inducible (Smith et al.,1991; Munkittrick et al., 1991; McMaster et al., 1991), but more information about the natural variations in MFO activities is needed.

In this study experiments have been performed to gain more information about the MFO system in the white sucker and possibly to improve its use as a biomonitor. The focus of the experiments was, first, to establish baselines for the seasonal changes in the MFO activities for male and female white suckers caught in an uncontaminated lake. The hypotheses being tested were set to answer questions about the natural seasonal

variation in P-450 catalytic activity and inducability of the system at different stages in the natural cycle. Stated as null hypotheses, these are:

> There are no differences in EROD and AHH activities in suckers taken at different times of the year.

and,

> EROD and AHH activities of white suckers do not differ between the range of dosages of CB77 injected.

and,

> There are no differences in inducability of sucker EROD and AHH activities between spring and fall samples.

Dose-response experiments were conducted to determine the sensitivity of white suckers to MFO induction by I.P. injections of CB77. As well, other dose-response experiments were conducted to test intermediate dosages, longer exposure periods, and seasonal effects on the induction response. These experiments were done using the EROD and AHH activities of hepatic microsomes since they are established and reliable methods of MFO measurement (Goksøyr and Förlin, 1992) and they have been shown to be very sensitive to induction by CB77 (Hahn et al., 1993).

Previous studies that have charted the seasonal variation of other species have shown declines in the MFO activities before and during the spawning periods (Jimenez and Stegeman, 1990; Luxon et al., 1987; Walton et al., 1983), especially in females (Lindström-Seppä, 1985; Koivusaari et al., 1981). This pattern during spawning may also exist in white suckers. Since the reproductive steroid hormones are in higher concentrations at spawning (Stegeman and Chevion, 1980; Mathieu et al., 1991), the

decline of the MFO activities at spawning may be attributed to mechanisms inhibiting the activities to avoid the excessive metabolism of the hormones (Luxon et al., 1987) that would affect reproductive function (Lech et al., 1982). If the MFO activities are inhibited at spawning, then, a higher concentration of an inducer may be required before the enzymes are induced.

II. Methodology

A. Seasonal variation study with white suckers

White suckers (*Catostomus commersoni)* were obtained from Roddy Lake (L468) at the Experimental Lakes Area (ELA) in northwestern Ontario (Latitude 93°44', Longitude 49°40'). Roddy Lake is a precambrian shield lake with a maximum depth of 105 feet (32 metres) and has populations of white suckers.

Fish were collected at intervals over the period from April 1990 until August 1991 (Table 1). Sampling intervals were more intensive just prior to, during, and just following spawning season in an attempt to record enzyme activity changes coincident with spawning. Samples obtained a few weeks prior to spawning were designated 'prespawn' while those obtained after spawning were designated 'postspawn'. Fish that exhibited definite spawning reactions (immediate gonad discharge when handled) were labelled 'spawning'. All other labelled samples were referred to by the season when they were obtained (Table 1).

The intent was to obtain 5-7 fish of each sex during each period. White sucker sexes can be distinguished by external appearance during the spawning seasons (Mohr, 1982) but no external differences are evident at other times.

Fish were captured with $2^1/_4$-to $3^1/_2$-inch (60mm to 90mm) mesh gill nets set at various sites for intervals of twenty minutes to three hours in open water or for intervals of three to six hours under the ice in winter. During the spawning season (late May to

Table 1. Fish sampling dates and descriptive phases by which they are described thoughout the text.

Description of fish	Dates	Year
Late winter	April 3,4	1990
Prespawn	May 9,10,11,17	1990
Spawn	June 7	1990
Postspawn	June 19	1990
Summer	July 24	1990
Winter	December 12,13	1990
Late winter	April 5	1991
Prespawn	May 9	1991
Spawn	May 31	1991
Postspawn	June 19	1991
Summer	August 20	1991

early June), fish were captured over a spawning site on May 9-11th, 1990, June 7-8th, 1990, and May 31st, 1991 with a 4x4 foot $^1/_2$-inch (13mm) mesh trap net (Beamish, 1972). During the open water season fish for enzymatic analysis were placed in water-filled containers and transported to shore. In the winter, fish were placed in ice-filled coolers to prevent freezing and were taken indoors to a field laboratory. Fish were killed without anaesthesia by a blow to the head, and the spinal cord was severed. Fork length, whole weight, eviscerated weight, and sex were recorded. Liver and gonad weights were also recorded except in instances when wind prevented reliable balance recordings outdoors. Livers were removed quickly, minced, separated usually into two or three Whirl-pak® bags, and frozen between slabs of dry ice within five minutes.

B. Experimental liver microsomal enzyme induction of white suckers

Fish were treated by intraperitoneal injection with universally carbon-14 ring-labelled 3,3',4,4'-tetrachlorobiphenyl (CB77) with a specific activity of 37.1 mCi/mmol. (Sigma Chemical Company). Higher dosages were diluted with non-radioactive CB77 (Ingram and Bell Scientific) to keep the use (and hence the cost) of labelled material to a minimum and the purity of the congener checked by gas chromatography (GC). The labelled and unlabelled components were separately dissolved in 5 and 25ml liquid scintillation grade toluene (Baker Chemical Company) respectively. Required amounts were mixed from each of these vials to yield the target quantities (Table 2). The mixtures containing required amounts of labelled and unlabelled CB77 were evaporated to near dryness and redissolved in corn oil (Sigma) with sonication. These corn oil solutions were injected intraperitoneally into the fish. The experimental exposures were conducted on suckers held in cages suspended in Roddy Lake. Fish cages were

Table 2. Mix of labelled and unlabelled congener 77 (μg congener 77 per kg fish) used for injections.

Spring 1990	labelled	0.1	1	10	10	10
	unlabelled	0	0	0	90	990
	target dose (total)	0.1	1	10	100	1000
Spring 1991	labelled	5	5	7.5	10	10
	unlabelled	5	25	92.5	290	990
	target dose (total)	10	30	100	300	1000
Fall 1991	labelled	1	10	10	10	10
	unlabelled	0	0	90	290	990
	target dose (total)	1	10	100	300	1000

constructed of untreated spruce (2x4-inch) dimension lumber held in place with 3-inch (75mm) Robertson® screws; cages measured 4x4x8 feet (1.2 x 1.2 x 2.4 m). They were surrounded by 1-inch (25mm) metal mesh and lined with a 4x4x10 feet (1.2 x 1.2 x 3 m) $^1/_4$-inch (65mm) mesh nylon net (Leckies Net & Twine Ltd). The cages floated in Lake 468 (Roddy L.) with styrofoam tied to the top of two sides.

B.1. Experimental induction of white suckers in Spring, 1990

Sixty-one fish (61) were collected from a trap net set over a spawning site on May 17 and 24 and June 1, 1990. These fish were divided randomly among four floating cages set offshore in about 3m of water. Cages 1 and 4 held seven males and eight females each; cage 2 held eight males and seven females; cage 3 held eight fish of each sex. Treatments of these fish with CB77 began on June 3 for cages 1 and 2 and concluded on June 4 for cages 3 and 4. For treatment, fish were removed from cages, transported in 20-L containers of water to shore where they were anaesthetised with 150 mg L^{-1} methanesulfonic acid salt (MS222). When sufficiently anaesthetised (ie. righting reflex lost), fork length, weight, and sex (Mohr, 1982) were recorded and the appropriate dosage of CB77 (Table 2) was injected interparietally into the right side of the ventral surface of the fish behind the pectoral fin. The first few rays of a fin were removed from each fish with a different fin clipped for each dosage to submit subsequent identification of the PCB concentration each fish received. After injection, fish were placed in fresh water for recovery from anaesthesia and then transported back to the cage. The mixture of fish with different sexes and dosages in each cage is shown in Table 3.

Table 3. Summary of the number of white suckers in holding cages by sex and dosage (μg kg^{-1} PCB congener 77) for Spring 1990.

		Cage 1	Cage 2	Cage 3	Cage 4	
Dosage (μg kg^{-1})	Sex	Exposed June 3-8	Exposed June 3-8	Exposed June 4-9	Exposed June 4-9	Total
Control	males	1	1	1	2	5
	females	1	2	1	1	5
0.1	males	2	1	1	1	5
	females	1	1	2	1	5
1	males	1	2	1	1	5
	females	1	1	1	2	5
10	males	1	1	2	1	5
	females	2	1	1	1	5
100	males	1	2	2	1	6
	females	1	1	1	2	5
1000	males	1	1	2	1	5
	females	2	1	1	1	5
Total		15	15	16	15	61

The fish from Cages 1 and 2 were sacrificed on June 8, five days following injection. The fish from Cages 1 and 2 were sacrificed on June 8. On June 9 the remaining fish from Cages 3 and 4 were removed.

B.2. Experimental induction of white suckers in Spring, 1991

Fish were collected from a trap net set over a spawning site on May 20. These fish were again randomly allocated to four floating cages as in 1990. Cage 1 held eleven males and eleven females; cages 2, 3, and 4 held ten males and ten females each. Treatment of these fish began on May 28 with Cage 4. Treatments of 10, 30, 100, 300, and 1000 μg CB77 kg^{-1} body weight over five days began on May 30, (Table 4). Fish in Cages 2 and 3 were treated on May 31. Treatments with the appropriate dosages were carried out as in the spring of 1990. As well, to compare exposure duration, 10 fish (five males and five females) from Cage 4 were injected with CB77 at 100 μg kg^{-1} body weight and 10 fish with corn oil. These fish were sacrificed after 10 days.

Five days following injection the surviving fish from Cages 1,2 and 3 were sacrificed and tissues were removed. The fish from Cage 1 were sacrificed on June 4. On June 5 the fish from Cages 2 and 3 were removed and processed. On June 7, following 10 days of treatment, the fish in Cage 4 were sacrificed and processed.

Table 4. Summary of the number of white suckers in holding cages by sex and dosage (μg kg^{-1} PCB congener 77) for Spring 1991

		Cage 1	Cage 2	Cage 3	Cage 4	
Dosage (μg kg^{-1})	Sex	Exposed May 30-June 4	Exposed May 31-June 5	Exposed May 31-June 5	Exposed May 28-June 7	Total
Control	males	1	2	2	5	10
	females	2	2	1	5	10
10	males	2	1	2	-	5
	females	2	2	1	-	5
30	males	2	2	1	-	5
	females	2	1	2	-	5
100	males	2	1	2	5	10
	females	1	2	2	5	10
300	males	1	2	2	-	5
	females	2	1	2	-	5
1000	males	2	2	1	-	5
	females	1	2	2	-	5
Total		20	20	20	20	80

B.3. Experimental induction of white suckers, Fall 1991

Fish were collected using gill nets set at various sites between September 16th and September 19th, and assigned to cages as in the earlier experiments. There were problems in the fall with fish survival before the experiment began. Consequently fewer fish were available for the experiment than originally planned. Cages 1 and 2 each held 13 fish and Cage 3 held 11 fish. Sex determination of these fish prior to dissection was not possible. Treatment of these fish began on September 28th. Fish from Cages 1,2, and 3 were injected with concentrations of 10, 30, 100, 300, and 1000 μg CB77 kg^{-1} body weight. Given the limited number of fish available, fewer fish were injected with each dosage, especially 10 and 30 μg CB77 kg^{-1} body weight which were not expected to show any induction effects. Each dosage and the control was to be represented in each cage but the 10 μg kg^{-1} dosage was not present in Cage 1 and there were no controls in Cage 3 (Table 5) since there were not enough surviving fish to treat.

Fish treatments were conducted as with the earlier treatments. Five days following injection (October 3rd) the fish from Cages 1,2 and 3 were sacrificed and tissues were removed.

Some fish perished during the exposure periods. The actual numbers of fish injected on each occasion and the number surviving to the sampling date are given in Table 6.

Table 5. Summary of the number of white suckers in holding pens by dosage (μg kg^{-1} PCB congener 77) for Fall 1991

Dosage (μg kg^{-1})	Cage 1	Cage 2	Cage 3	Total
Control	3	3	-	6
10	-	1	2	3
30	2	1	1	4
100	3	3	2	8
300	2	3	3	8
1000	3	2	3	8
Total	13	13	11	37

Table 6. Number of white suckers injected and survived from PCB exposure experiments.

		Treatment Exposure (μg kg^{-1})					
		Control	10	30	100	300	1000
Spring 1990	injected	10	10	-	11	-	10
	survived	10	10	-	11	-	10
Spring 1991	injected	10	10	10	10	10	10
	survived	6	9	8	7	9	7
10 Day 1991	injected	10	-	-	10	-	-
	survived	9	-	-	9	-	-
Fall 1991	injected	6	3	4	8	9	8
	survived	3	3	3	4	6	6

C. Collection of samples from white suckers from cage experiments

Individual fish were removed from the cages and transported to shore in 45-L containers of water. The fish were then anaesthetised with MS222 (150 mg methanesulfonic acid salt L^{-1}), and the spinal cord was severed with a scalpel. Fork length was measured to the nearest mm and fish were weighed to the nearest gram. The fin clip made at the time of treatment was read and sex was determined. After removal of the liver, it was separated into 2 or 3 labelled Whirl-Pak® bags and frozen between slabs of dry ice. Occasionally wind conditions prevented the use of the small balance to weigh the liver and gonads. Samples of gonad, gill, intestine, heart, and muscle were each placed in individually labelled bags and frozen for future analysis of carbon-14. The right pectoral fin ray was removed for use in age determination and the eviscerated body weight recorded. Precautions were taken to prevent contamination of one sample by another; dissecting tools were washed, gloves were changed, and plastic wrap covering scales and dissecting surfaces were replaced after every fish.

Following dissections, frozen tissues were transported on dry ice to the Freshwater Institute. Liver samples were stored in a Revco ultrafreezer at -80°C and other tissues were stored in a walk-in-freezer at -35°C in the Freshwater Institute. Fish remains and contaminated equipment were incinerated.

D.1. Preparation of microsomes

Subsamples of 2-3 g of frozen liver samples were weighed in pre-weighed petri dishes. Liver weights and buffer volumes were calculated for each sample with 4 mL

of KCl-HEPES buffer per g of liver and rounded to the nearest mL. The KCl-HEPES buffer was 0.02M HEPES (N-[2-Hydroxyethyl]-piperazine-N'-[2-ethanesulfonic acid)(Sigma Chemical Company) and 0.15M potassium chloride (KCl)(BDH Chemical Company). The solution was prepared by dissolving 22.36 g KCl and 9.50 g HEPES in less than 2000 mL redistilled water and then adjusting the volume to 2000 mL; then the pH was brought to 7.5 with potassium hydroxide (KOH). In a cold room at 4°C, partially thawed liver tissues were minced with scissors and placed in glass homogenizer tubes. The calculated volume of buffer was added and tissues were homogenized with 5-7 passes with a motorized glass tissue homogenizer with a Teflon® pestle. Homogenates were transferred to cold polycarbonate tubes and centrifuged at 12,000 x g for 20 minutes in a refrigerated centrifuge at 2°C (Sorvell RC2-B, SM-24 head; 10,000 rpm). Supernatants were transferred to ultracentrifuge tubes and centrifuged at 105,000 x g for 75 minutes at 2°C in a Sorvall OTD-2 ultracentrifuge (Sorvall ODT-2, T1270 head; 39,000 rpm). Supernatants were removed and the pellet was washed with the appropriate buffer by pouring a small amount of buffer over the pellet and draining twice. KCl-HEPES was used for immediate analysis; alternately pellets were suspended in TRIS buffer (pH 7.4) if microsomes were to be stored in liquid nitrogen and analyzed later. The TRIS resuspension buffer was made to 0.05M TRIS (Tris[hydroxymethyl]methylamine)(BDH) with 1mM dithiothreitol (Sigma), 1mM ethylenediaminetetraacetic acid (EDTA,Sigma) and 20% glycerol (Sigma). The solution was prepared by dissolving 12.11 g TRIS, 0.3048 g dithiothreitol, 0.5844 g EDTA, and 500 g glycerol in less than 2000 mL redistilled water, then bringing the volume to 2000 mL, and finally adjusting the pH to 7.4 with KOH. Pellets were scraped out of the tubes for KCl-HEPES buffer or the tubes were vortexed with TRIS buffer to remove the pellets. Pellets were resuspended in the appropriate buffer with a hand held glass homogenizer and Teflon® pestle. Buffer was added to achieve a protein concentration

near 10 mg/mL. TRIS-suspended microsomes were transferred to labelled cyrovials and immersed in liquid nitrogen for storage. Some of the resuspensions for were stored in an ice bath and used in the analysis for protein concentration on the day of preparation. HEPES-suspended microsomes were used on the day of preparation for EROD and AHH analysis. Liver microsomes from fish sampled between April 1990 and April 1991 were not frozen in liquid nitrogen before analysis for enzyme activity; they were analyzed when they were prepared. These samples include the seasonal data for 1990 and the spring injection experiment for 1990. Liver microsomes fom fish sampled after April 1991 were stored in liquid nitrogen before enzyme analysis these included the seasonal data for 1991 and the spring and fall injection experiments for 1991. Experiments conducted at Freshwater Institute showed no significant difference between either the HEPES fresh or TRIS stored preparations for EROD and AHH activities (Appendix A, D. Metner, unpublished)

D.2. Analysis for Protein (modified Lowry Assay) (Markwell et al., 1981)

The protein assays required 100 μL of microsomal suspension from each sample. Two separate dilutions were made from each sample with 50 μL microsomal suspension added with an Eppendorf® pipette to 5 mL redistilled water for each replicate, and dilutions were vortex mixed. For a blank, 50 μL of the buffer used for resuspension, either HEPES or TRIS, was added to 5 mL of redistilled water. The absorbances from the blanks were subtracted from the protein absorbances to get the absorbances attributed to protein.

The assay reagent was made by mixing together two solutions, the Lowry reagent and a copper sulphate solution in the proportion of 100:1. The Lowery reagent was prepared with 2% sodium carbonate (Na_2CO_3, BDH), 0.4% sodium hydroxide (NaOH, BDH), 0.16% sodium tartrate (BDH), and 1% sodium lauryl sulphate (SDS, BDH). This was done by dissolving 40g Na_2CO_3, 8g NaOH, 3.2g sodium tartrate, and 20g SDS in 2000 mL of redistilled water. Four per cent copper sulfate was prepared by dissolving 4g ($CuSO_4 \cdot 5H_2O$) in redistilled water and adjusting the volume to 100 mL. Fresh reagent (Lowry + $CuSO_4$ solution) was made each day by mixing these solutions (100:1 Lowry:$CuSO_4$). Each dilution of microsomal suspension was run in triplicate so that six assay test tubes were run for each sample. To each assay tube 1 mL of the Lowry/$CuSO_4$ reagent was added with an Eppendorf® repeater pipette. Then 60 μL of the appropriate microsomal dilution were added to each assay tube and vortexed. After this mixture had stood for 10 minutes at room temperature, 100 μL of $33^1/_3$% (v/v) phenol (BDH) (water:phenol 2:1) were added while mixing on the vortex mixer to ensure immediate and thorough mixing. The tubes were then allowed to stand for at least 30 minutes and absorbance at 600 nm was measured against redistilled water on a Beckman DU-7 spectrophotometer.

The absorbance was set to 0.000 for redistilled water. Starting with the blank and check samples (samples with known protein content), followed by the microsomal suspensions, samples were pipetted into disposable plastic cuvettes, the tops sealed, and the absorbances read. Standards consisting of known concentrations of bovine serum albumin served as checks for the analytical accuracy and consistency. The absorbance for a sample was entered into the spectrophotometer computer memory and then the cuvette was drained with a vacuum line and the next sample added. Absorbances were converted to protein concentrations by reference to a calibration curve prepared by the

assay of a series of known protein concentrations and calculated protein concentrations were printed and entered manually into an S20/20 spreadsheet run on the Freshwater Institute VAX-6000 model computer. The buffer blanks' average absorbance was subtracted from the sample absorbances and the corrected value calculated as a concentration of mg protein in 1 mL of suspension based on a calibration curve. The standard calibration curve was prepared using known concentrations of bovine serum albumin in ascending order run through the analysis in the same manner as the microsomal samples. The regression equation from the standards relating absorbance and protein concentration was used to calculate the protein concentrations in the microsomal suspensions. These calculations were done automatically by the spectrophotometer computer. These protein contents were used in calculating enzyme activity per mg protein from the EROD and AHH analysis.

D.3. Analysis for Ethoxyresorufin O-Deethylase (EROD) (Pohl and Fouts, 1980)

The EROD analysis required 300 μL of fresh or thawed microsomal suspension. Frozen microsomal suspensions were placed in an ice bath and allowed to thaw for 30 minutes. Six Corex glass centrifuge tubes, three replicate samples and three blanks were used for each assay. Mixed into each of these tubes were the reagents needed for the analysis: 1100 μL HEPES buffer (0.1M), 10 μL magnesium sulphate ($MgSO_4$) solution, 50 μL bovine serum albumin (BSA) solution (4g dL^{-1}), 10 μL β-nicotinamide adenine dinucleotide phosphate (NADP) solution, 10 μL $_{DL}$-isocitric acid solution, and 20 μL isocitrate dehydrogenase solution (8.0 mg mL^{-1}) added with an Eppendorf® repeater pipette. The 0.1M HEPES buffer was prepared by dissolving 23.83 g HEPES (Sigma) to a total volume of 1000 mL with redistilled water and the pH adjusted to 7.8 with KOH. The $MgSO_4$ solution was prepared by dissolving 15.4 g $MgSO_4$ (Fisher Scientific) to a total volume of 100 mL with redistilled water. BSA solution was prepared by dissolving 4.0 g BSA (Sigma) to a total volume of 100 mL with redistilled water. The isocitric dehydrogenase solution was prepared by dissolving 8.0 mg isocitric dehydrogenase (Sigma) in a solution of water:glycerol (Sigma) (1:1). The NADP and $_{DL}$-isocitric acid solutions were made freshly for each new analysis or run by dissolving 98.3 mg NADP (Sigma) and 193.58 mg $_{DL}$-isocitric acid (Sigma) mL^{-1} redistilled water. 50 μL of the microsomal suspension were added to each of the six tubes, vortexed, and incubated for at least 5 minutes in a water bath at 25°C. 2.5 mL of methanol (HPLC grade, Caledon Laboratories) were added to the blanks. With the overhead laboratory lights off, 10 μL 7-ethoxyresorufin solution (prepared by mixing 0.1 mg 7-ethoxyresorufin (Sigma) mL^{-1} dimethylsulphoxide (DMSO, Caledon)) from a repeater pipette were added to a test sample every 10 seconds by a stopwatch, vortexed, and returned to the water bath. After exactly 2 minutes 2.5 mL of methanol were added to

each sample to stop the reaction. 10 μL 7-ethoxyresorufin were then added to the blanks and vortexed. The tubes were centrifuged for five minutes in a Sorvell RC2-B refrigerated centrifuge at 24,000 x g (Sorvell RC2-B, SS34 Head, 15,000 rpm). Tubes were held in racks covered with aluminum foil to prevent light exposure. After being centrifuged a precipitate would settle at the bottom. Tubes were handled carefully so as to not disturb the precipitate.

Fluorescence of the treated samples was measured against the blank samples with an Aminco-Bowman spectrofluorometer. The excitation wavelength was 530 nm and the emission wavelength was 580 nm. As well, triplicate low and high check samples with .001 (low) and .05 (high) mg resorufin (7-hydroxy-3H-phenoxazine-3-one, Sigma) per mL DMSO standards were made by diluting 10 μL resorufin solution in 2.5 mL methanol and stored in the dark for a minimum of one hour before measurement on the day of analysis.

Before measurement began, the glass cuvette was cleaned with methanol. With the chart recorder running, the low check (0.001 mg mL^{-1}) was measured by transferring a low check sample into the cuvette, opening the instrument shutter, allowing the chart recording to stabilize, then closing the shutter and returning the check sample to the original test tube. After three low check samples at the amplifier setting of 0.003 were read, the three high check samples (0.05 mg/mL) were run at the amplifier setting of 0.1. These checks were run before and after the protein samples to assure consistent performance by the instrument. After the checks were run, the glass cuvette was rinsed with methanol twice and sample measurements began. The treated samples were measured first to determine which amplifier setting was required for on-scale measurement. The blanks were then measured at the same amplifier setting. Special care

was taken not to transfer or agitate any precipitate. The chart readings were measured in mm from the base. Blank readings were subtracted to get the fluorescence not due to hydrolysis of substrate. The enzyme activity was calculated by the formula below:

activity = (f x h) / ((p x 0.050) x t)

where

activity = enzyme activity in nanomoles mg^{-1} protein min^{-1}.

f = instrument response factor in nanomoles mm^{-1} of chart peak height for the reaction volume (3.75 mL) and the instrument amplifier setting used.

h = measured peak height of sample minus peak height of blank in mm.

p = protein concentration of the tissue preparation in mg mL^{-1}.

0.050 = volume of microsomal suspension (mL).

t = incubation time in minutes.

The units of activity are nanomoles mg^{-1} protein $minute^{-1}$.

D.4. Analysis for Aryl Hydrocarbon Hydroxylase (AHH) (van Cantfort et al., 1977)

The Aryl hydrocarbon hydroxylase analysis required 900 μL of fresh or recently thawed microsomal suspension. Six glass test tubes with screw top caps consisting of three replicant samples of microsomal suspension and three blanks were used for each sample. Mixed into each of these tubes were 800 μL Tris-HCl buffer, 10 μL magnesium chloride ($MgCl_2$) solution, 10 μL NADP solution, 10 μL $_{DL}$-isocitric acid solution, and 20 μL isocitrate dehydrogenase solution added with an Eppendorf® repeater pipette. 0.1M TRIS buffer was prepared by dissolving 12.1 g TRIS (BDH) to a total volume of 1000 mL with redistilled water and the pH adjusted to 7.5 with hydrochloric acid (HCl). The $MgCl_2$ solution was prepared by dissolving 4.77g $MgCl_2$ (BDH) to a total volume of 100 mL with redistilled water. The isocitric dehydrogenase solution was the same as that used for the EROD analysis. The NADP and $_{DL}$-isocitric acid solutions were made freshly for each run by dissolving 78.7 mg NADP and 154.86 mg $_{DL}$-isocitric acid in 1 mL redistilled water. These contents were mixed on a vortex mixer and allowed to incubate at room temperature for at least ten minutes, then 150 μL of the microsomal protein were added to each tube. Before the substrate was added to the mixture, 2 mL of a DMSO-KOH solution were added to each blank tube and vortexed to prevent any enzymatic activity there. The 0.15M KOH in 85% DMSO solution was prepared by mixing 16.833 g KOH with 300 mL redistilled water and adjusting the volume to 2000 mL with DMSO (Caledon). 20 μL of tritiated benzo[a]pyrene (Specific activity 20.3081 μCi μmol^{-1}, Dupont) solution was then added to each tube and vortexed. The tritiated benzo[a]pyrene solution was prepared with 5070.906 μCi tritiated benzo[a]pyrene in 55.4mL of acetone (Fisher Scientific) to produce a solution with a concentration of 1.831μCi benzo[a]pyrene μL^{-1}. These tubes were then covered and incubated in a 25°C water bath for exactly 30 minutes. The reaction was then stopped by the addition of 2

mL DMSO-KOH to each reaction tube with vortex mixing.

Three millilitres of hexane (HPLC grade, Caledon) were added to each tube to extract any unchanged substrate and leave product in the aqueous layer; then each was vortexed, and capped with a Teflon lined cap. They were then shaken on a Burrell auto shaker for five minutes. Once taken off the shaker, the caps were removed and the tubes centrifuged in a clinical centrifuge for three minutes. The hexane layer was aspirated off and 3 additional mL of hexane was added to each tube. The procedure was repeated and the hexane layer aspirated off. The tubes were then centrifuged again for 5 minutes. The remaining liquid was transferred by pipette from the inoculation tubes to clean disposable 12x75 mm glass tubes. From each tube 200 μL were transferred to each of two mini scintillation vials; 5 mL Atomlight® scintillation cocktail (Dupont) were added to each. Vials were then capped, shaken, labelled and radioactivity in each was counted on a Beckman LS7500 liquid scintillation counter. The activity for each replicate, with the protein concentration, was used to calculate the AHH activity in nanomoles product formed mg^{-1}protein $minute^{-1}$ with the following formula:

$$\text{activity} = [(((v/a)*c)*s)/(p \times 0.150)]/t$$

where

activity = enzyme activity in nanomoles mg^{-1} protein min^{-1}.

v = total volume of extract in μL.

a = volume of aliquot counted in μL.

c = mean dpm for all replicate aliquots - mean blank dpm for sample.

s = specific activity in nanomoles dpm^{-1}.

p = protein concentration of the tissue preparation in mg mL^{-1}.

t = incubation time in minutes.

0.150 = volume of microsomal suspension (mL)

The units of activity are nanomoles mg^{-1} protein $minute^{-1}$.

E. HPLC analysis for CB77

Liver tissues from injected fish were weighed in individual disposable aluminum cups. Tissues were then freeze dried for four days. Each of the freeze dried samples was broken up and placed in a test tube where in which they were crushed to powder. Four mLs of liquid scintillation counter grade toluene (Baker) were added to the tissue and the mixture was ground with a Polytron mixer (which was cleaned between samples). The tubes were then centrifuged in a clinical centrifuge for ten minutes. The supernatant was removed by pipette and put aside; the pellet was washed twice more with 1 mL toluene each time; the combined supernatants were used for further analysis. 100 μL of the supernatant was added to 5 mL of Atomlight® scintillation cocktail and the radioactivity counted, with two replicates for each sample.

The extracted toluene solutions were evaporated to approximately 0.5 mL and then analyzed by reverse-phase high pressure liquid chromatography (HPLC). 50 μL of the concentrated sample were injected into the Waters HPLC C-18 reverse-phase column (part# T11782K10) which was eluted with methanol. A LKB 2111 Multirac fraction collector was used to collect effluent in mini scintillation vails every thirty seconds following a sixty second delay postinjection. 50 μL of the original concentrated sample was assayed directly by LSD in order to compare recovery. Recoveries ranged from 80 to 86%. Five mLs of Atomlight® scintillation cocktail was added to each minivial, the contents mixed, and counted for radioactivity (^{14}C) on a liquid scintillation counter. A pure standard of 5 μL of the ^{14}C-CB77 was run though the HPLC column to define the retention time of the parent compound.

F. Oxidation to recover $^{14}CO_2$

Tissues from injected fish were placed in combustible paper cups. Tissue weight was recorded for each cup; triplicate samples from each tissue were oxidized when possible. Tissues sampled were liver, muscle, intestine, gill, gonad, and heart. Tissues were left to desiccate for at least two days. When dry, cups were placed individually in a Packard 306D (Packard Instuments) oxidizer and burned for 15 to 30 seconds. CO_2 from combustion was trapped in 7 mL Carbosorb (Packard) and mixed with 13 mL scintillation fluor (2:1 PCS (Amersham) to xylene (Fisher Scientific)) in the instument. These liquids are dispensed by the oxidizer into 25-mL scintillation vials. Each vial was then labelled and replaced with a new vial. Blanks (paper cups alone) and known standards (5000 dpm ^{14}C paper, Packard) were also run intermittently with each group of samples. When oxidation of all the samples was completed the vials were washed in methanol, wiped and allowed to air dry to remove any excess fluor on the vials. They were then placed in the Beckman 7500 scintillation counter to record radioactivity. Each vial was counted for up to ten minutes. PCB content per gram wet weight was calculated from the original specific activity of the starting material. This was based on the average counts for the tissue per gram wet weight and ^{14}C-PCB content of the PCB given to the injected fish.

G. Effects of sampling and assay temperatures on EROD activity

Liver samples from the winter of 1990-91 (water temperature = 0-4°C) and summer of 1991 (water temperature = 20°C) were removed from the freezer and

separated into four groups: winter male, winter female, summer male, and summer female. For each group, 6mL of microsomal suspension was prepared from a composite prepared by combining all the liver samples from that group. This homogenate was then separated into six cyrovials of 1 mL each and frozen in liquid nitrogen. Vials were thawed and analyzed as described ealier with EROD analysis performed on each group with five minutes incubation at 5°, 10°, 15°, 20°, and 25°C.

H. Statistical analysis

The mean enzymatic activities of different sampling periods and injection concentrations were compared using SAS software run on the Freshwater Institute VAX-6000 computer. The lsd multiple means test was used to compare the log transformed means (geometric means) of the sample periods for seasonal variation within the PROC GLM procedure. Examples of the analysis programs are given in Appendix B. The use of log transformed values "normalized" the data so that standard statistical treatment could be applied. The PROC CORR procedure was used to calculate the regression analysis to compare EROD vs AHH and the temperature gradient experiment. For the temperature of incubation experiment, analysis of covariance (ANCOVA) was used to compare the slopes of the four separate regression analyses. The means of the injection concentrations were compared using the lsd contrast statements within PROC GLM procedure (Appendix B). ANCOVA was also used to analyze the 1990 to 1991 seasonal patterns of MFO activity (Appendix B) and the patterns of PCB residues in the tissues (Appendix B). The critical level of significance for all statistical analysis was $\alpha=0.05$.

III. Results and Discussion 1. Seasonal Variation

A. Seasonal variation

1. Females 1990

The mean EROD and AHH activities for the various groups are charted in Figures 1 and 2; actual mean and standard deviation (S.D.) values are listed in Table 7. Letters above the bars indicate LSD comparisons and differences between means. Values for individual fish are listed in Appendix C. During 1990 the samples from spawning females had the lowest average AHH activity of 0.019 nmol mgP^{-1} min^{-1}. The mean of log transformed values of spawning samples was significantly ($P \leq 0.05$) different from other sample period means except for the winter and postspawning samples. The EROD average activity of spawning females was also the lowest at 0.005 nmol mgP^{-1} min^{-1} and the mean differed significantly from the other periods except for the prespawn sample. The prespawn samples were collected over the spawning site rather than in open water and may not be "prespawn" samples but "spawning" fish. Clearly both EROD and AHH activities for these females were depressed around the spawning period relative to the rest of the year.

2. Females 1991

During 1991 the spawning females once again had the lowest average AHH activities at 0.029 nmoles mgP^{-1} min^{-1} and the geometric mean was significantly different from all but the winter and prespawning females. The EROD average activities of 0.001 nmol mgP^{-1} min^{-1} (the limit of enzyme activity measurement) during spawning were the

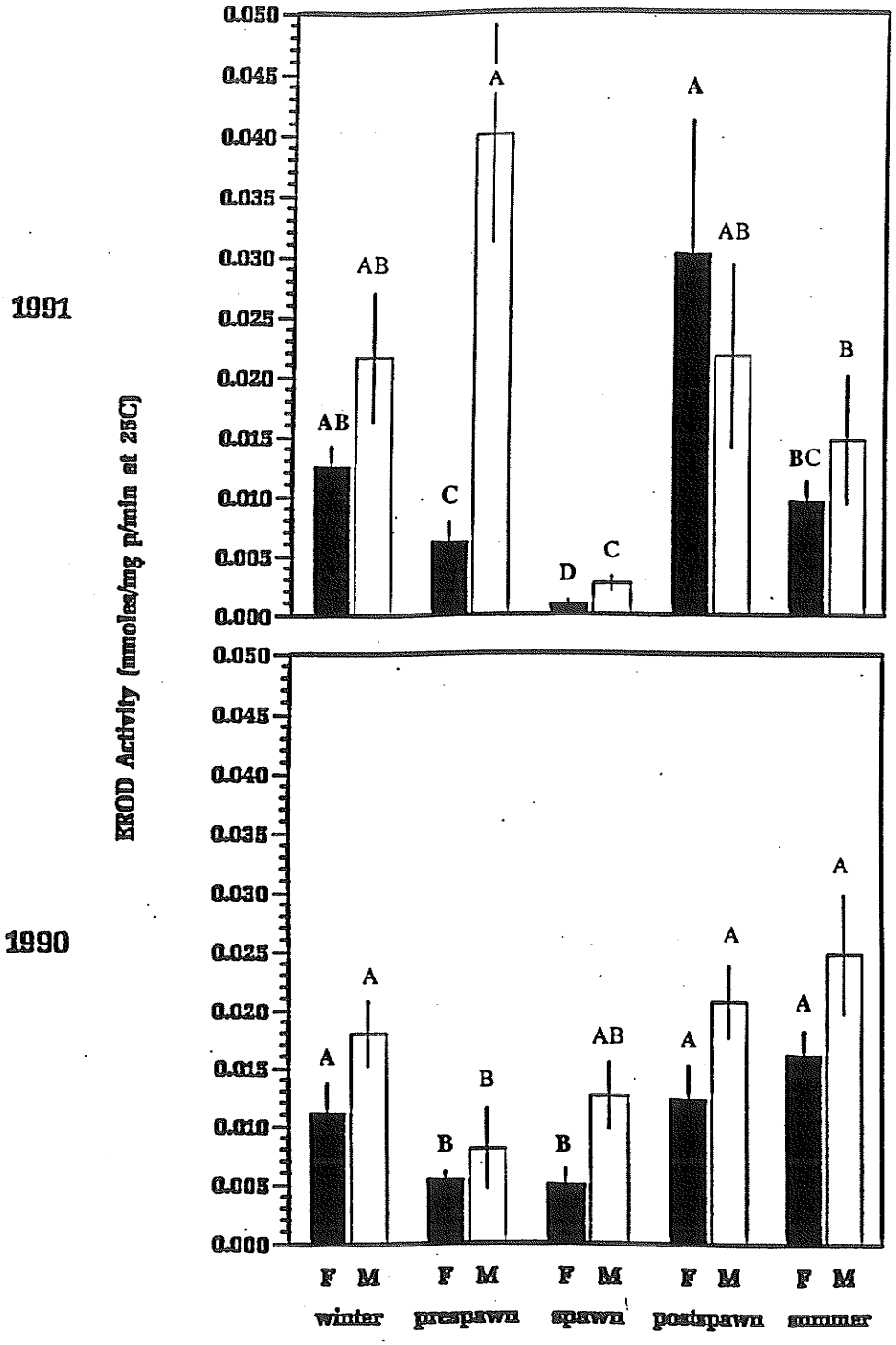

Figure 1. Seasonal variation of mean EROD activities (nmoles mg protein^{-1} minute^{-1} at 25°C) in male (M) and female (F) white suckers for 1990 and 1991. Sampling period dates are given in Table 1. Letters indicate Significant differences by LSD comparisons.

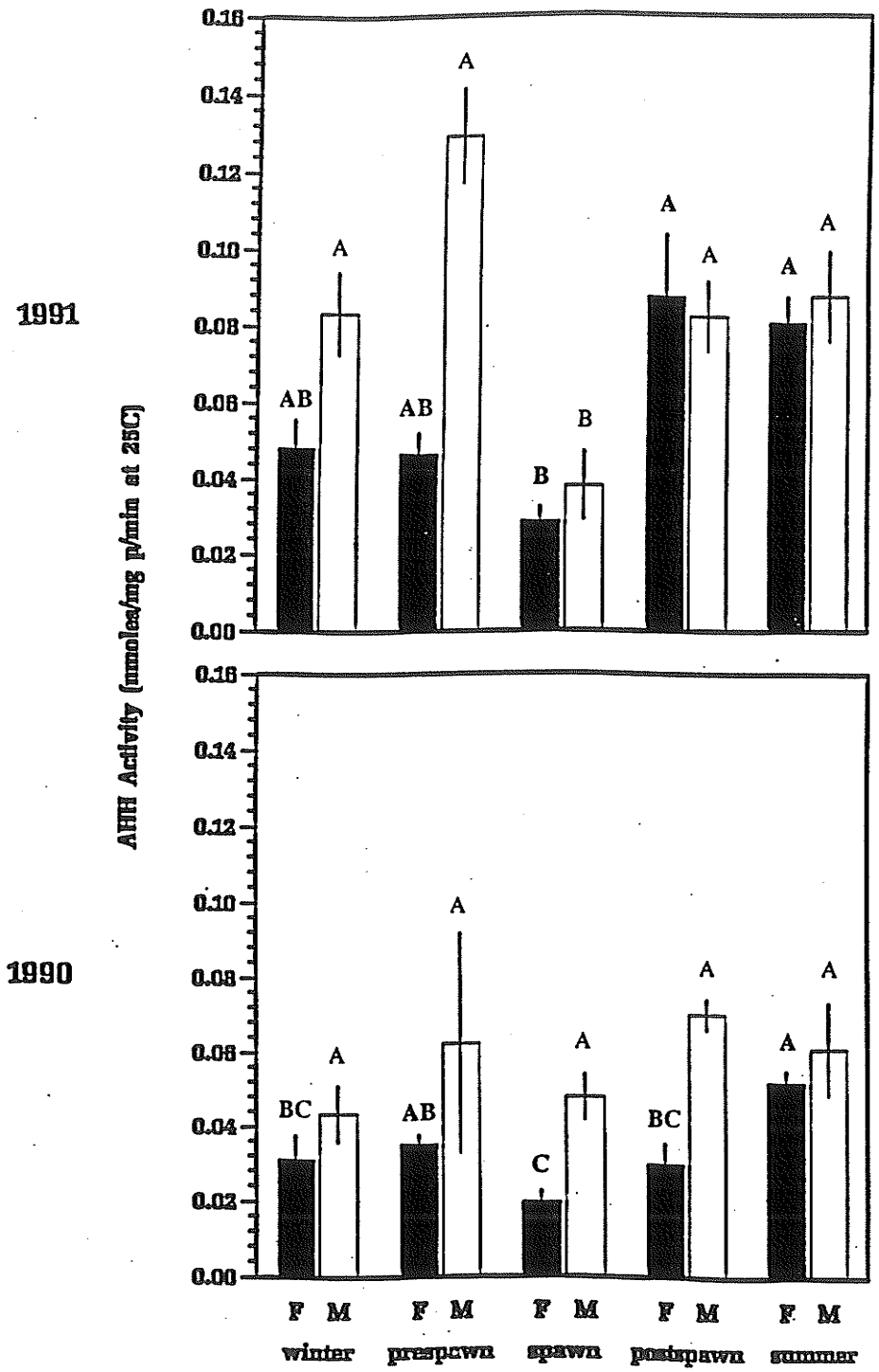

Figure 2. Seasonal variation of mean AHH activities (nmoles mg protein^{-1} minute^{-1} at 25°C) in male (M) and female (F) white suckers for 1990 and 1991. Sampling period dates are given in Table 1. Letters indicate Significant differences by LSD comparisons.

Table 7. Natural variation of mean enzyme activities (nmol/mg p/min at 25°C) and standard deviations for white suckers for 1990 and 1991. Dates for sample periods appear in Table 1.

		AHH				EROD			
Year	Period	Female	SD	Male	SD	Female	SD	Male	SD
1990	winter	0.031	±0.016	0.044	±0.015	0.011	±0.006	0.018	±0.006
	prespawn	0.035	±0.008	0.062	±0.059	0.006	±0.002	0.008	±0.007
	spawn	0.019	±0.006	0.048	±0.014	0.005	±0.003	0.013	±0.006
	postspawn	0.029	±0.014	0.070	±0.008	0.012	±0.007	0.021	±0.006
	summer	0.052	±0.007	0.061	±0.028	0.016	±0.005	0.025	±0.011
1991	winter*	0.050	±0.022	0.083	±0.019	0.012	±0.005	0.022	±0.009
	prespawn	0.046	±0.015	0.129	±0.032	0.006	±0.004	0.040	±0.023
	spawn	0.029	±0.009	0.038	±0.020	0.001	±0.000	0.003	±0.001
	postspawn	0.088	±0.040	0.082	±0.016	0.030	±0.027	0.022	±0.013
	summer	0.081	±0.017	0.088	±0.021	0.010	±0.004	0.015	±0.009

* winter sample includes fish taken on December 12 & 13, 1990 and April 5, 1991 due to low sample number for each sampling date. Samples from both dates were captured under ice.

lowest for 1991 and significantly differed from all other sampling periods. The lowest activities again occurred around the spawning season.

3. Males 1990

The variation between periods for the males was not as pronounced as with females. During 1990 the male spawning and prespawning samples had the lowest average activities for EROD. Statistically, the log transformed AHH activities did not differ significantly throughout the year. With geometric means of EROD activities however, the prespawn mean activity of 0.008 nmol mgP^{-1} min^{-1} was significantly different from the other periods except for spawning males. As mentioned earlier, the prespawn samples may not be "prespawn" samples but "spawning" fish since they were collected over the spawning site rather than in open water. This could then be the same sample period with lower activity fish accumulating over the spawning site.

4. Males 1991

The mean AHH activity in males of 0.038 nmol mgP^{-1} min^{-1} for the spawning period of 1991 was significantly different from all the other periods, unlike the males in 1990. The prespawn mean was high at 0.129 nmol mgP^{-1} min^{-1} and significantly different from all other periods. The mean EROD activity for spawning males, 0.003 nmol mgP^{-1} min^{-1}, was also significantly different from all other sample periods. The spawning mean was significantly different from all other sample periods for both the AHH and EROD enzymes. The mean activity for the spawning period was the lowest

for the year.

Previous studies have shown seasonal variations in the activities of the AHH and EROD enzymes in several fish species with declines in activity before and during the spawning periods (Jimenez and Stegeman, 1990; Luxon et al., 1987; Walton et al., 1983), especially in females (Lindström-Seppä, 1985; Koivusaari et al., 1981). The null hypothesis for the present two-year series of tests was that there would be no change in AHH or EROD enzyme activities in white suckers with the change in seasons. The null hypothesis for EROD activity is rejected; there were clear differences in both males and females in both years. There were also significant differences among the means within each year for AHH activities (Table 8), except for the males in 1990. The periods at or near the spawning period were consistently lower in both activities in both years, except for males in 1990. The reason for the failure of males in 1990 to differ in mean AHH activities are speculative. Overall, there is evidence to reject the null hypothesis. In all four cases with EROD, differences were found; in 3 of the 4 cases with AHH, differences were found (Table 8). As with the literature, lower activities were found during and near the spawning period for this species. Reproductive steroid hormone levels have seasonal cycles linked with gonadal cycles and hormone increases at spawning (Stegeman and Chevion, 1980) as much as 10-fold (Mathieu et al., 1991). Elevated sex hormones in fish at spawning are necessary for healthy spermatogenesis and vitellogenesis (Lech et al., 1982). Mixed function oxygenases hydroxylate steroids (Truscott et al, 1983) so that the MFO activity may be regulated to avoid the excessive metabolism of the hormones (Luxon et al., 1987) and affect reproductive function (Lech et al., 1982). The decline in EROD and AHH activities before and during the spawning period may be attributed to changes in the concentration of reproductive hormones. The lower MFO activities may be a response to prevent undesired degradation of the sex hormones at that time.

Table 8. Statistical analysis to determine if there were statistical difference among seasonal sampling periods for each sex and year. LSD pair comparisons shown in Figures 1 and 2.

			AHH		EROD	
		n	F value	Pr>F	F value	Pr>F
Female	1990	25	6.12	0.0011	7.32	0.0004
	1991	27	8.05	0.0010	5.00	0.0095
Male	1990	17	0.69	0.6106	3.07	0.0470
	1991	21	12.37	0.0003	5.17	0.0129

B. Males vs females.

Sex differences have been noted in hepatic MFO activities and cytochrome P-450 systems in several fish species (Kleinow et al., 1987; Koivusarri et al., 1984; Stegeman and Chevion, 1980; Lindström-Seppä, 1985; Schell et al., 1987) with higher MFO activities in the males. Comparable to previous research, the average enzyme activities in the males in the present study were almost always higher than the average enzyme activities in females for the same sample. Of the 20 paired comparisons of males and females in Figures 1 and 2 and Table 7, males exceeded females in 18 comparisons. The null hypothesis that there would be no difference between the male and female mean activities for both enzymes is thus rejected. The mean activities in males were consistently higher than those in females; often the difference was two-fold. The only exception to higher mean activity in males was during the 1991 postspawn sample. Statistically, the differences in the cycles are highly significant (Table 9) between the sexes. Work done on gonadally mature trout species showed significant differences in the MFO activity between males and females (Stegeman and Chevion, 1980) leading to research that demonstrated cytochrome P-450 and EROD depression with estradiol 17β (Vodicnik and Lech, 1983) and increase with testosterone (Stegeman et al., 1982). This finding suggests that androgens and estrogens influence the regulation of MFO activity. The EROD and AHH activities would then be expected to be higher in males and lower in females, with the greatest difference between the two sexes at the point when hormone levels are highest: just before and during the spawning period. This is evident especially in 1991 when the difference in MFO activities between the sexes was the greatest.

Table 9. Statistical comparison of males vs females seasonal cycles.

	AHH		EROD	
	F	Pr>F	F	Pr>F
Males vs Females	2.71	0.0068	2.67	0.0074

C. 1990 vs 1991

The patterns or cycle of mean enzyme activities during 1990 and 1991 were also compared for each sex by ANCOVA by adding **Year*Period** to the model statement in SAS and which therefore allowed the cycles to be statistically analyzed while still using the enzyme activity means for each period. The null hypothesis for this comparison was that for each sex the AHH and EROD cycles for the two years would not be significantly different. The results of these comparisons are outlined in Table 10.

For the males the log transformed AHH and EROD cycles for 1990 and 1991 were significantly different. The 1990 and 1991 patterns for log transformed EROD activities in the females were also significantly different. The 1990 and 1991 cycles for the female AHH, however, did not show a significant difference with a F value of 2.38 and a Pr > F of 0.0629. There is evidence to reject the null hypothesis for the AHH and EROD cycles for males and for the EROD cycles of the females. The 1990 and 1991 cycles are different for the AHH and EROD activities for the males and for the EROD activities in the females. Although the cycles appear to have lower mean activities for both enzymes for both years, this is not a strong enough pattern to make the two years statistically similar for these enzymes. The years have too much variability within them even after log transformation. This may be due to the low sample size or an inherent variability that occurs when the fish are not spawning. Since fewer than ten fish of each sex were sampled for each sample period, the variability of the means is much larger, especially when some of the male sample periods are represented by only four fish. The females, with a larger sample size, show similar AHH cycles for both years, and therefore the null hypothesis is not rejected for this comparison. A larger sample size for each sex at each sampling period would reduce the variability and may, as with

Table 10. Statistical comparison of 1990 vs 1991.

		AHH		EROD	
		F	Pr>F	F	Pr>F
1990 vs 1991	Female	2.38	0.0629	4.49	0.0034
	Male	3.22	0.0245	11.04	0.0001

these females, provide a clearer picture of the seasonal cycles. However, the lack of a significant difference between the two years may be due to a variability in the enzyme activities that is inherent to the cycle. Several studies that have examined MFO seasonal cycles in various fish species for more than one season, such as winter flounder (Edwards et al., 1988), freshwater venace (Lindström-Seppä, 1985), and lake trout (Luxon et al., 1987). All of these examinations have determined that there was a seasonal cycle with the most abrupt change at or before spawning, but the MFO activity results showed considerable variation and were not otherwise constant for different times of the year (Lindström-Seppä, 1985). The results from those studies indicated that the fluctuation in the MFO activities was due more to the hormonal changes before and at spawning than to any seasonal affect. The cycles that appear in the MFO activities for those fish and for the fish in the present study may be a reflection of the hormonal changes that occur with spawning and with a high variability in the MFO activities for the rest of the year.

D. EROD vs AHH

The relationship between the EROD and AHH activities for each fish when plotted (Fig. 3) is linear with the equation EROD = 0.301185 * AHH - 0.002664. There was a predictable regression relationship between the two natural enzyme activities with a correlation coefficient (r) of 0.75206 with a Prob>r of 0.0001. This correlation between EROD/AHH is similar to the range of correlations shown by Goksøyr and Förlin (1992) and Collier et al. (1991) with r values of 0.75-0.87 for nine benthic fish species captured in U.S. waters.

E. Temperature gradient

The temperature of incubation has been discussed in previous studies (Jimenez and Stegeman, 1990; Lindström-Seppä, 1985; Kleinow et al., 1987; Jimenez et al., 1988; Karr et al., 1985). MFO enzyme activities in white suckers have been shown to increase with increasing temperature to a maximum efficiency temperature between 20-25°C and then to decline at higher temperatures (Munkittrick et al., 1993). The present experiment was to test for any assay temperature differences. The null hypotheses for these two experiments were: 1) there would be no difference between the EROD activities of the homogenates from winter and summer samples at different assay temperatures, and 2) there would be no difference in the way EROD activities of the homogenates from males and females respond to temperature. The present study found that EROD activity increased linearly with temperature in all cases, up to about 20°C (Fig. 4), all with strong correlations and R values (Table 11). The increment between 20° and 25° was smaller in males than would be expected from the slope at lower temperatures, suggesting that the optimum assay temperature was near that seen

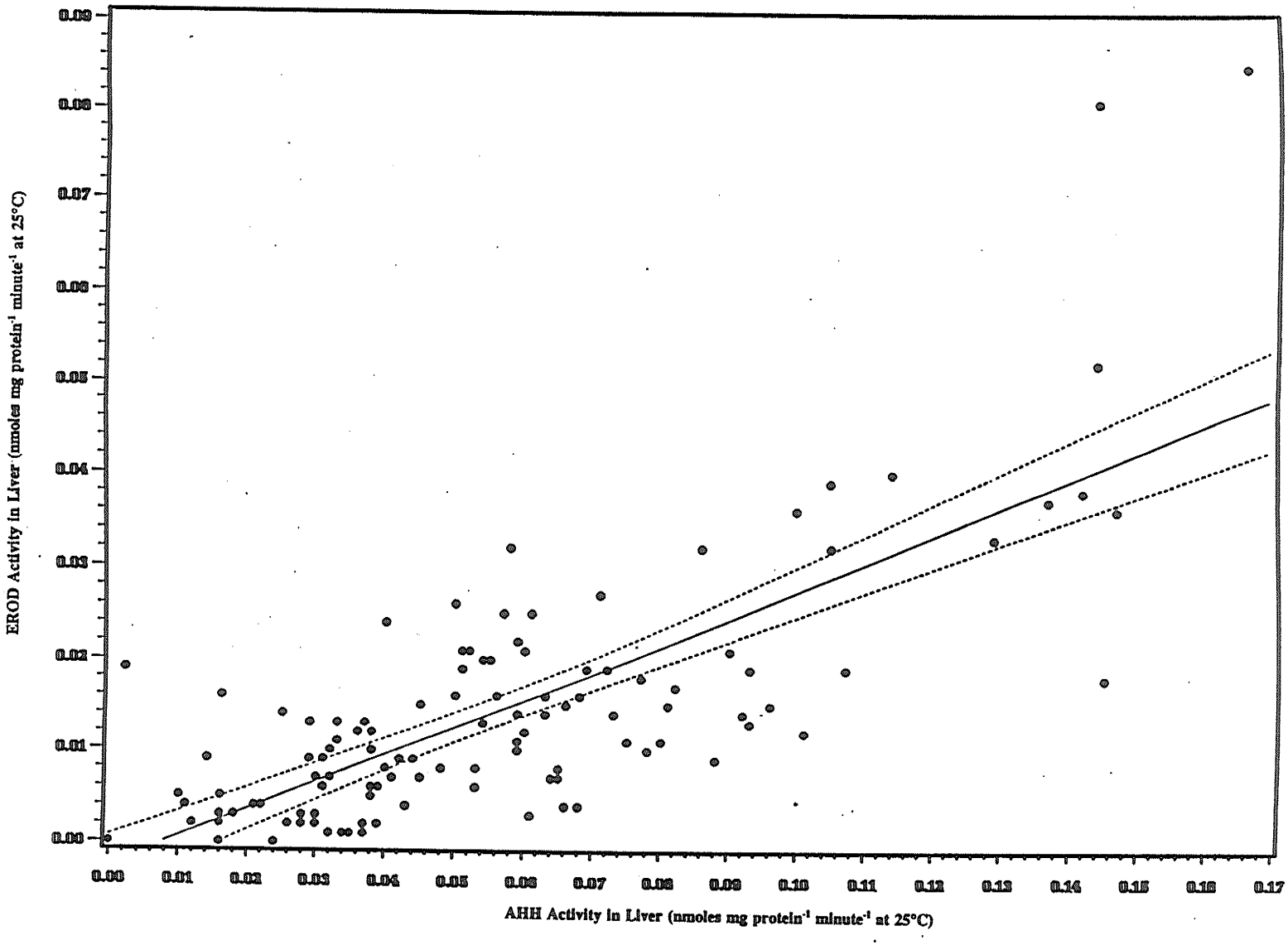

Figure 3. Relationship between EROD and AHH activities in the livers of white suckers with 95% confidence limits.

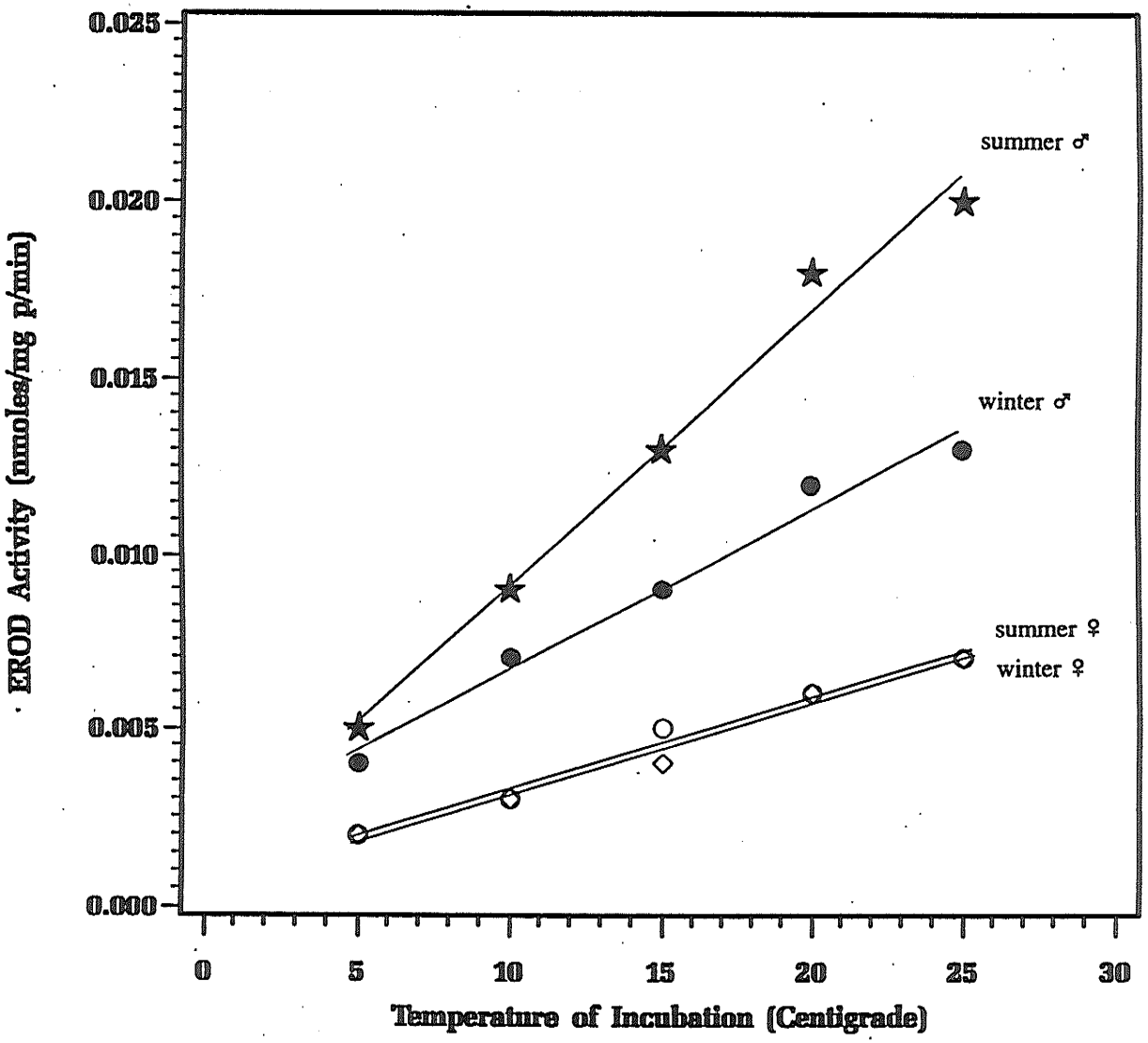

Figure 4. EROD activities (nmoles mg^{-1} protein $minute^{-1}$) of microsomal homogenates from males and females from winter (water temperature = 4°C) and summer (water temperature = 20°C) sampling with different assay temperatures.

Table 11. Correlations and equations from the temperature gradient experiment.

Correlation	R	Equation
Male Winter	0.98976	EROD=0.0021+0.00046(Temp)
Male Summer	0.99371	EROD=0.0013+0.00078(Temp)
Female Winter	0.99124	EROD=0.0005+0.00026(Temp)
Female Summer	0.99124	EROD=0.0007+0.00026(Temp)

in Munkittrick et al. (1993). The striking feature of these results is the much greater responsiveness to temperature of preparations from the males than the females. The EROD activity increased with increasing temperature for all homogenates as expected with the summer males showing the highest rate of increase. There was a significant difference between the slopes for the sexes (Table 12). The differences between the seasons were not as easy to discriminate. The slopes for the summer and winter homogenates for the females were not significantly different, but the slopes for the winter and summer males were significantly different from both the female homogenates and from each other. The first null hypothesis can be rejected for males since there appears to be a difference in enzyme activity between the winter and summer homogenates. The first null hypothesis cannot be rejected for females since there was no difference in the slopes of the winter and summer response to incubation temperature. There is also evidence to reject the second null hypothesis that there would be no difference between the male and female homogenates. There appears to be sex differences in the EROD activity response in this population of white suckers to incubation temperature.

From these data on four pooled homogenates it is clear that the temperature of incubation during the assay effects enzyme activities. This raises the question of whether to conduct the assays at a single standard temperature or at the temperature prevailing when the fish were captured. Generally, laboratories have opted to assay at a standard single temperature for reasons of analytical consistency and to facilitate comparisons among results from different laboratories. When this is done some of the changes in seasonal MFO activities could be attributed to the environmental temperature changes over the year. Previous studies have shown that temperature is important in the basal activities seen in fish when assayed at environmental temperature. Some species such

Table 12. Statistical results of ANCOVA comparing the regression slopes from the temperature gradient experiment.

	F value	Pr>F
Winter Male vs Summer Male	42.67	0.0001
Winter Female vs Summer Female	0.00	1.0000
Winter Female vs Winter Male	16.67	0.0015
Summer Female vs Summer Male	112.67	0.0001

as bluegill (Jimenez et al., 1988) have greater MFO activities at higher acclimation temperatures while other species exhibit comparable activities (Ankley et al., 1985; Koivusaari, 1983; Stegeman, 1979) at higher and lower acclimation temperatures. This is known as temperature compensation (Mathieu et al., 1991). When assayed at a constant temperature fish acclimated at a colder temperature appear to have greater enzymatic activities than those acclimated at higher temperatures (Kleinow et al., 1987).

In principle, factors could be established from temperature vs activity equations to determine seasonal activities related to capture temperature. For this project all analyses were performed at 25°C for consistency, with full recognition that this procedure does not portray the actual activity present in the fish at the time of capture. A factor was established for the population in this study using the equations calculated by regression analysis for the male and female white suckers (Table 11). The females maintained the same slope in the correction factor while a new slope had to be interpolated between the summer and winter slopes for males depending on the temperature of capture. The original male winter and summer slopes were used for males when dealing with winter or summer samples. The resulting EROD activities from seasonal variation data adjusted for water temperature at capture were charted by periods of sampling (Fig. 5) in the same manner as before (Fig. 1), with analysis at 25°C. Some activities after adjusted for water temperature at capture were negative and considered to be zero. The seasonal activity for EROD adjusted for temperature does not appear to exhibit temperature compensation with similar activities in winter and summer. However, the pattern seen in the seasonal activities (measured at 25°C) has an annual low at spawning time when water temperatures are rising, rather than in the winter with minimal water temperatures. Hence the seasonal cycle is strongly influenced by the spawning effects and not by environmental water temperature. The analysis with 25°C

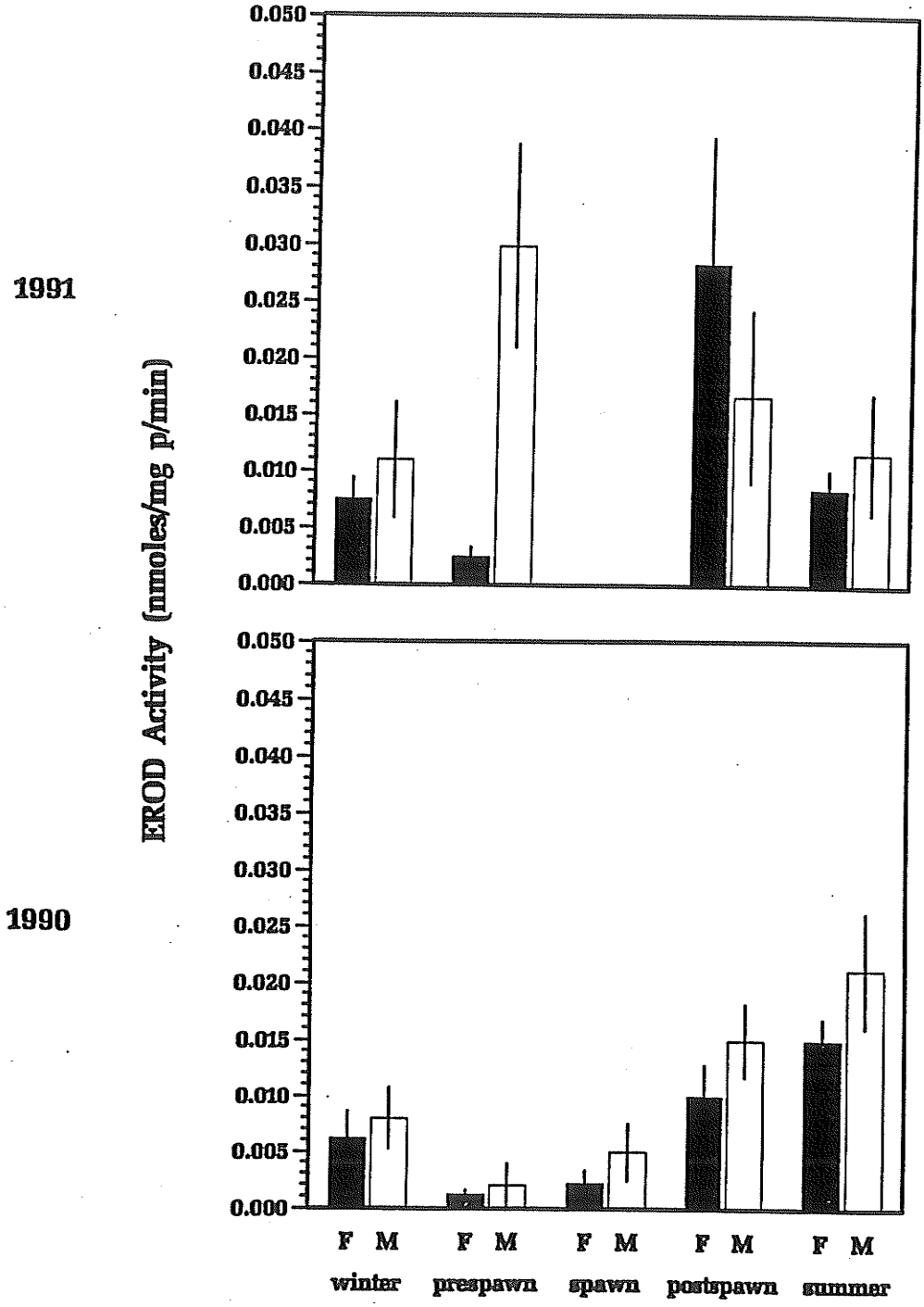

Figure 5. Seasonal variation of mean EROD activities (nmoles mg protein^{-1} minute^{-1}) in male (M) and female (F) white suckers for 1990 and 1991 adjusted to seasonal water temperatures. Sampling period dates are given in Table 1.

incubation was sufficient to determine any seasonal changes in the EROD activities.

IV. Results and Discussion 2. Injection and Fate of PCB Congener 77

A. Experimental with exposure to CB77 for 5 days

The null hypothesis for these experiments was that injection of a range of dosages of CB77 would have no effect on enzyme activities. This planar congener has been shown to induce MFO activities in rainbow trout (Melancon et al., 1989), scup (Gooch et al., 1989), and winter flounder (Monosson and Stegeman, 1991). A five day exposure was used because induction of the MFO enzymes had been reported to take place as early as three days following intraperitoneal injection (I.P.)(Janz and Metcalf, 1991; James and Bend, 1980; Zhang et al., 1990) or feeding (Addison et al., 1981) with a known inducer. Several other studies have used a five day exposure period for a reference (Gooch et al., 1989; Melancon and Lech, 1983). The few dose-response studies performed have shown that with doses of chlorobiphenyls, aromatic hydrocarbons, and β-naphthoflavone in the range of 100 to 1000 $\mu g\ kg^{-1}$ strong induction response will occur (Personenet et al., 1987; Zhang et al., 1990; Gooch et al., 1989). There has been little work done on the threshold levels of inducers in fish. The threshold induction concentration by I.P. injection of 3,3',4,4'-PCB in rainbow trout began below 1000 $\mu g\ kg^{-1}$ CB77 for both rainbow trout and carp (Melancon and Lech; 1983) and below 100 $\mu g\ kg^{-1}$ CB77 for scup (Gooch et al., 1989; Hahn et al., 1989) after five days exposure. Hence, for this experiment a five day exposure with PCB dosages ranging up to 1000 $\mu g\ kg^{-1}$ was used.

1) Spring 1990 (5-day exposure)

This experiment was planned as a range-finding investigation with 10-fold differences between dosages. Fish were injected on June 3 and 4 when the water temperature was 15°C and sampled on June 8 and 9 when the water temperature was 18°C, with no mortalities during the exposure period. The results are plotted in Figures 6 and 7 and summarized in Table 13. Values for individual fish are listed in Appendix C. There was no significant difference between the sexes in response to the injection of CB77. The 1000 μg kg^{-1} injection was the only dosage to produce a significant response in enzyme activities relative to the corn oil control (PROC GLM in SAS with F values of 46.73 and 92.21 and the Pr > F of 0.0001 and 0.0001 for the mean EROD and AHH activities respectively). The AHH and EROD means were 8-fold and 35-fold higher respectively. These results suggest that in white suckers a threshold dosage for the induction of these enzymes after five days by I.P. exposure must lie between 100 and 1000 μg CB77 kg^{-1} body weight for this season. Consequently, for the exposures in 1991, an intermediate dosage was inserted between 100 and 1000 μg kg^{-1}. In rainbow trout the dosages of 200 μg kg^{-1} for Melancon and Lech (1983), 300 μg kg^{-1} for Melancon et al. (1989) and 500 μg kg^{-1} for Janz and Metcalfe (1991) were associated with induction, hence, the intermediate dosage chosen was 300 μg kg^{-1}.

2) Spring 1991

There were several mortalities, including controls, in the cages over the exposureperiod for each injection concentrations in the spring of 1991. The reasons for these are unknown, but may be attributable to handling and cage stresses from the high water temperatures and lower oxygen concentrations that occurred during this treatment.

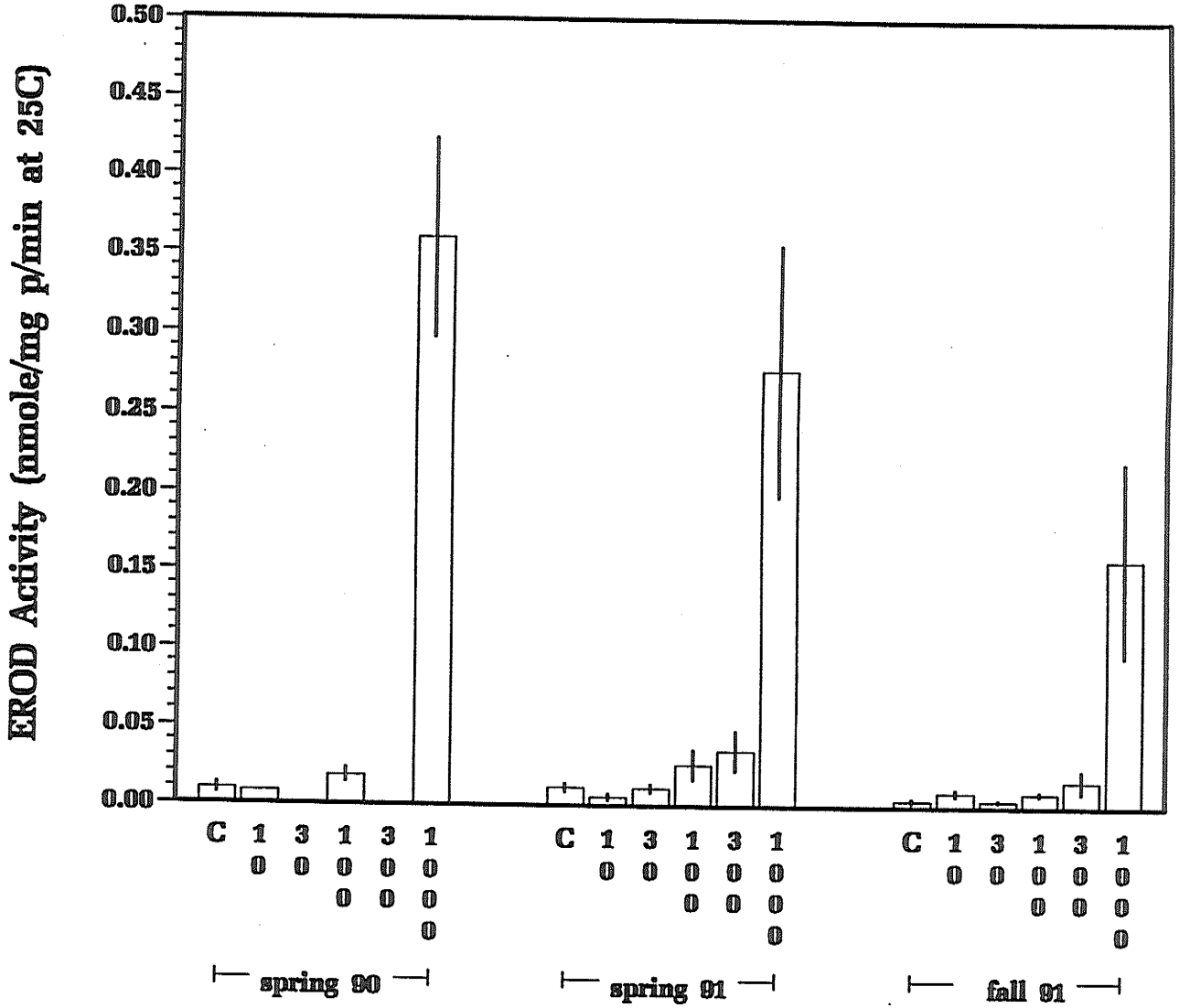

Figure 6. Mean EROD activities (nmoles mg^{-1} protein $minute^{-1}$ at 25°C) for white suckers treated with PCB Congener 77 ($\mu g\ kg^{-1}$ body weight) at dosages from C = control to 1000 $\mu g\ kg^{-1}$ for five days.

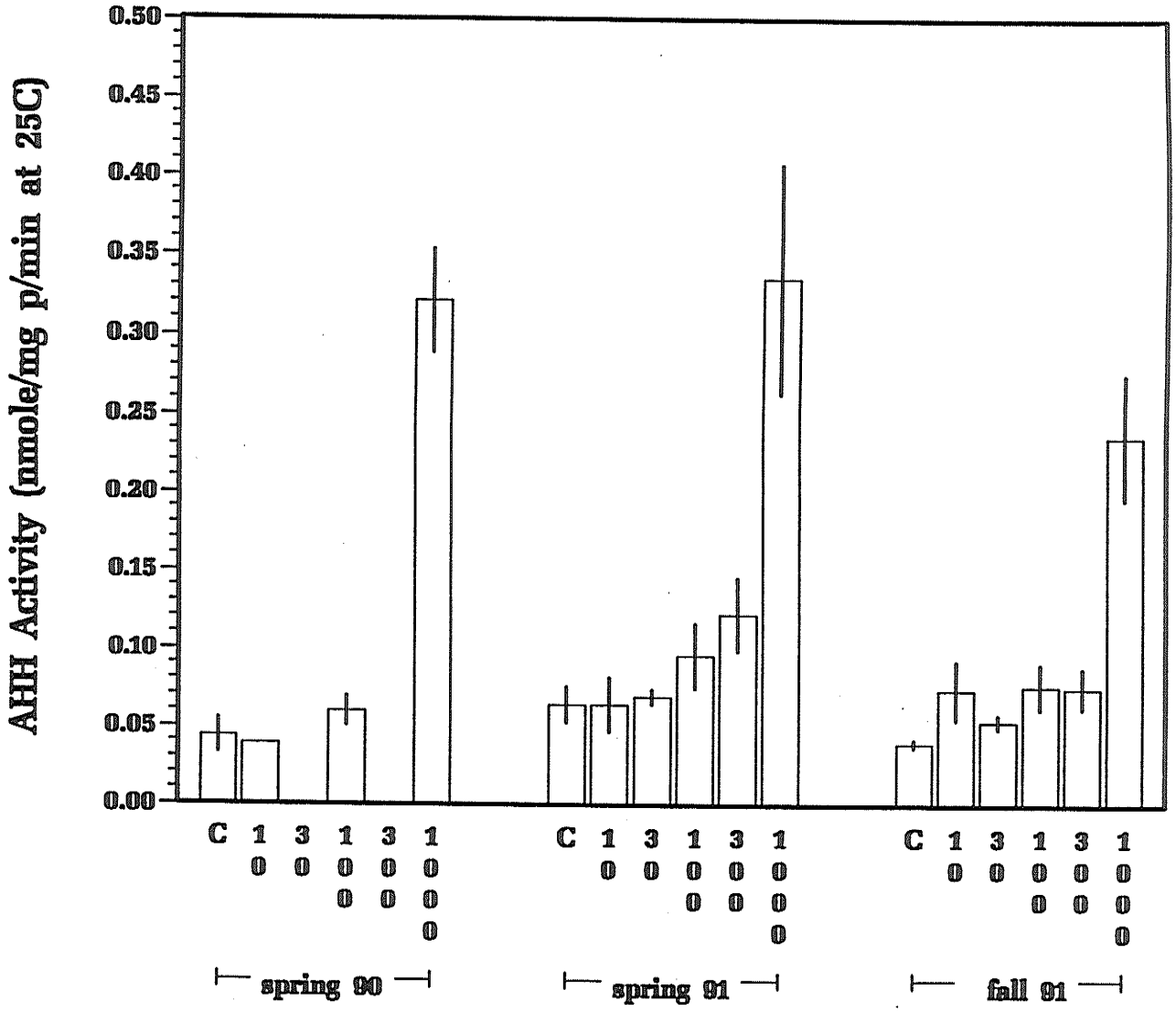

Figure 7. Mean AHH activities (nmoles mg^{-1} protein $minute^{-1}$ at 25°C) for white suckers treated with PCB Congener 77 ($\mu g\ kg^{-1}$ body weight) at dosages from C = control to 1000 $\mu g\ kg^{-1}$ for five days.

Table 13. Mean enzyme activities and standard deviations for pooled sexes of treated fish.

	EROD (nmole mg protein^{-1} min^{-1})							
Treatment Exposure (µg kg^{-1})	Spring 1990		Spring 1991		Fall 1991		10-Day Spring 1991	
	Activity	SD	Activity	SD	Activity	SD	Activity	SD
Control	0.010	±0.011	0.010	±0.008	0.004	±0.003	0.007	±0.005
10	0.008	-	0.005	±0.005	0.009	±0.004	-	-
30	-	-	0.011	±0.004	0.004	±0.002	-	-
100	0.019	±0.016	0.032	±0.033	0.009	±0.004	0.019	±0.010
300	-	-	0.031	±0.044	0.016	±0.018	-	-
1000	0.375	±0.198	0.276	±0.209	0.158	±0.139	-	-

	AHH (nmole mg protein^{-1} min^{-1})							
Treatment Exposure (µg kg^{-1})	Spring 1990		Spring 1991		Fall 1991		10-Day Spring 1991	
	Activity	SD	Activity	SD	Activity	SD	Activity	SD
Control	0.044	±0.036	0.059	±0.030	0.039	±0.005	0.047	±0.021
10	0.039	-	0.064	±0.035	0.073	±0.033	-	-
30	-	-	0.069	±0.007	0.053	±0.009	-	-
100	0.063	±0.033	0.114	±0.065	0.076	±0.030	0.082	±0.024
300	-	-	0.109	±0.077	0.075	±0.033	-	-
1000	0.324	±0.099	0.335	±0.192	0.235	±0.090	-	-

The temperature of the lake water rose from 15°C to 19°C over this week. Two to four fish from every injection concentration died during the exposure period before sampling took place (Table 5). There were no mortalities for the Spring 1990 treatment when the water temperature was lower at the time of handling but rose slowly during the exposure period.

Again, there was no significant difference between the sexes in response to the injection of CB77. The 1000 $\mu g\ kg^{-1}$ injection was the only dosage to produce a significant response in enzyme activities relative to the corn oil control ($F=30.86$ and 29.30 for the mean EROD and AHH activities respectively with the $Pr>F$ of 0.0001 for both enzymes). The mean AHH and EROD activities were 6-fold and 20-fold higher respectively (Table 13)(Fig. 6 and 7). Mean values for 100 and 300 $\mu g\ kg^{-1}$ were clearly above those for lower dosages for both enzyme activities, but they failed to meet the criterion of statistical significance. The results from the spring of 1990 suggest that induction of these enzymes occurs after an injection between 100 and 1000 $\mu g\ kg^{-1}$ CB77 after five days exposure. The intermediate dosage of 300 $\mu g\ kg^{-1}$ added in 1991 effectively narrowed the range to between 300 and 1000 $\mu g\ kg^{-1}$.

As discussed earlier, reproductive steroid hormones have a seasonal cycle (Stegeman and Chevion, 1980; Mathieu et al., 1991) that seems to effect the seasonal cycle of the MFO activities of this population of white suckers. An induction experiment was performed with the same range of PCB concentrations in the fall of 1991 to determine if induction was being inhibited during the spawning period as seen in other fish induction studies (Jimenez and Stegeman, 1990; Folin and Lidman, 1981). If induction was inhibited by spawning then the threshold concentration for induction would be lower in the fall than the spring.

3) Fall 1991

Again there were several mortalities during the experiment. Most cages lost one to four fish (Table 5) attributed to stress from gill netting and not water temperature which was low, dropping from 12 to 9°C throughout the experiment. These effects and the stress resulting from an animal attack on the cages are assumed to have killed the twenty fish that died before treatments had begun. Following treatment, 12 of the 37 fish died before sampling.

There was no significant difference between the sexes in response to the injection of CB77. The 1000 μg kg^{-1} dosage was again the only concentration to produce a significant response in enzyme activities ($F=19.75$ and 48.75 with the $Pr>F$ of 0.0003 and 0.0001 for the mean EROD and AHH activities respectively). AHH and EROD means were 6-fold and 30-fold higher respectively (Fig. 6 and 7).

If there is a threshold concentration of PCB congener 77 for the induction of these enzymes after five days exposure in white suckers during this non-spawning period, then it presumably lies between 300 and 1000 μg kg^{-1}.

4) Comparison among treatment periods - Spring 1990 vs Spring 1991 vs Fall 1991

The responses of the fish to all injections at different periods were compared to test the null hypothesis, which was that the mean AHH and EROD activities for each treatment period and dosage (Spring 1990, Spring 1991, & Fall 1991) were not different. The bars representing mean activities for the 1000 μg kg^{-1} dosages appear to

have the greatest difference (Fig. 6 & 7), however when all the injections were analyzed by PROC GLM the three treatment periods were not significantly different ($F=1.16$; $P=0.3348$ for EROD; $F=0.53$; $P=0.8270$ for AHH). This does not provide evidence to reject the null hypothesis. The response to the PCB injections were similar and did not change with the different years or seasons.

Previous studies have shown decreased induction during the spawning period (Jimenez and Stegeman, 1990; Folin and Lidman, 1981; Lech et al., 1982), possibly linked to gonad development and sex hormone cycles (Stegeman and Chevion, 1980; Mathieu et al., 1991; Vodicnik and Lech, 1983). To avoid the excessive metabolism of the hormones during spawning the MFO activity may be regulated (Luxon et al., 1987) to prevent any effect on the reproductive functions (Lech et al. 1982). This suggests higher tolerances for inducing agents during the spawning season (Vodicnik and Lech, 1983). With the elevated levels of sex hormones and low basal activities of EROD and AHH in the spring, it was hypothesized that induction would require higher dosages at the spawning season. That is, induction might be suppressed to minimize hormone degradation at spawning. On this basis, one must expect induction at a lower dosage when the fish are not spawning. The experiments reported do not provide evidence to support this argument. Spawning and non-spawning periods were not significantly different; both required 1000 $\mu g\ kg^{-1}$ to effectively induce MFO activity, although more subtle differences might have been revealed by intermediate dosages. A possible explanation for the lower activities in the fall may be due to the colder waters involved. While the lake water warmed to 16-18°C during the spring treatment periods, the Fall 1991 treatment period became much colder, ending with 9°C when the fish were sampled. It has been found that a higher water temperature has higher induction with the same dose in bluegill (Jimenez et al., 1990), *Fundulus heteroclitus* (Stegeman, 1979),

largemouth bass, and catfish (Haasch et al., 1993) while there was a lag time to induction for other fish studies (James and Bend, 1980; Andersson and Koivusaari, 1985; Jimenez and Burtis, 1989) at colder temperatures. This suggests that maximum induction can be achieved in fish acclimated to low temperatures given sufficient time (Jimenez et al., 1990). Colder water temperatures can repress the induction response of MFO enzymes that may eventually occur over extended exposure. Fish have slower metabolisms at lower temperatures (Pritchard, 1993) with reduced absorption rates for inducing agents (James and Bend, 1980) and possibly low rates of protein synthesis (Andersson and Koivusaari, 1985), therefore, affecting rates of induction. The time needed to initiate induction may be longer at colder temperatures. For these reasons, the fall response to treatment may have been less reactive than was anticipated. Indeed, experiments by P. Delorme have shown that maximum induction is not reached for 11 weeks after injection (Delorme, 1994) of 2,3,4,7,8-pentachlorodibenzofuran (PCDF) in white suckers. Such evidence suggests that the lower response of the fall 1991 fish for 300 and 1000 μg kg^{-1} PCB treatments than the spring 1991 treatments may be due to colder water temperatures in the fall. It was hypothesized that the threshold concentration for induction would be lower during a non-spawning period. Although there was no significant difference between the induction threshold concentrations, the water temperature may have been cold enough to have had more effect on the uptake and induction rates than any sex hormone regulation effect on the MFO system. As seen in the temperature compensation experiment, water temperature does not seem to affect the normal seasonal cycle, but it may affect the rate of induction. Over a short period of five days the effect may be enough to delay induction at the lower dosages. At the same temperature, a non-spawning treatment could still have a lower threshold concentration of the PCB for induction of the MFO enzymes.

When the induction responses are compared to the natural seasonal activities, the induction of all three treatment periods at 1000 μg kg^{-1} was significantly higher than the highest seasonal period observed, the prespawn males of 1991. The induction response in these fish, then, could not be confused with the natural activities. When using this response as a biomonitor, the natural activities, even at their seasonal highest, are too low to be considered an induced response.

B. Induction results - ten day exposure

As discussed earlier, use of the 5-day exposure period was arbitrary and followed methods utilized by other studies (Gooch et al., 1989; Melancon and Lech, 1983). It was thought desirable to establish at least one other point on the time course of induction since some work estimated slower rates of absorption at cooler temperatures (James and Bend, 1980) and a longer exposure could provide induction at lower PCB dosages. The spring of 1991 ten-day exposure of 100 μg kg^{-1} PCB showed a significant difference in the mean EROD and AHH activities from the ten-day corn oil control exposure ($F=9.34$; $P=0.0085$ for EROD; $F=9.43$; $P=0.0083$ for AHH)(Fig. 8 and 9). Ten-day exposure to this dosage of CB77 resulted in significantly higher mean enzyme activities in the fish, although this dosage produced no induction over the five-day exposure. The higher activity for the ten day exposure at 100 μg kg^{-1} CB77 was less pronounced than the five day exposure at 1000 μg kg^{-1}. The higher activities suggest that any threshold concentration required to induce the EROD and AHH activities is lower for longer exposures. The longer exposure to the lower concentration seems to have resulted in weak but measurable induction. Chronic exposure of very low inducer concentrations have been shown to initiate induction after long periods of time (Delorme,

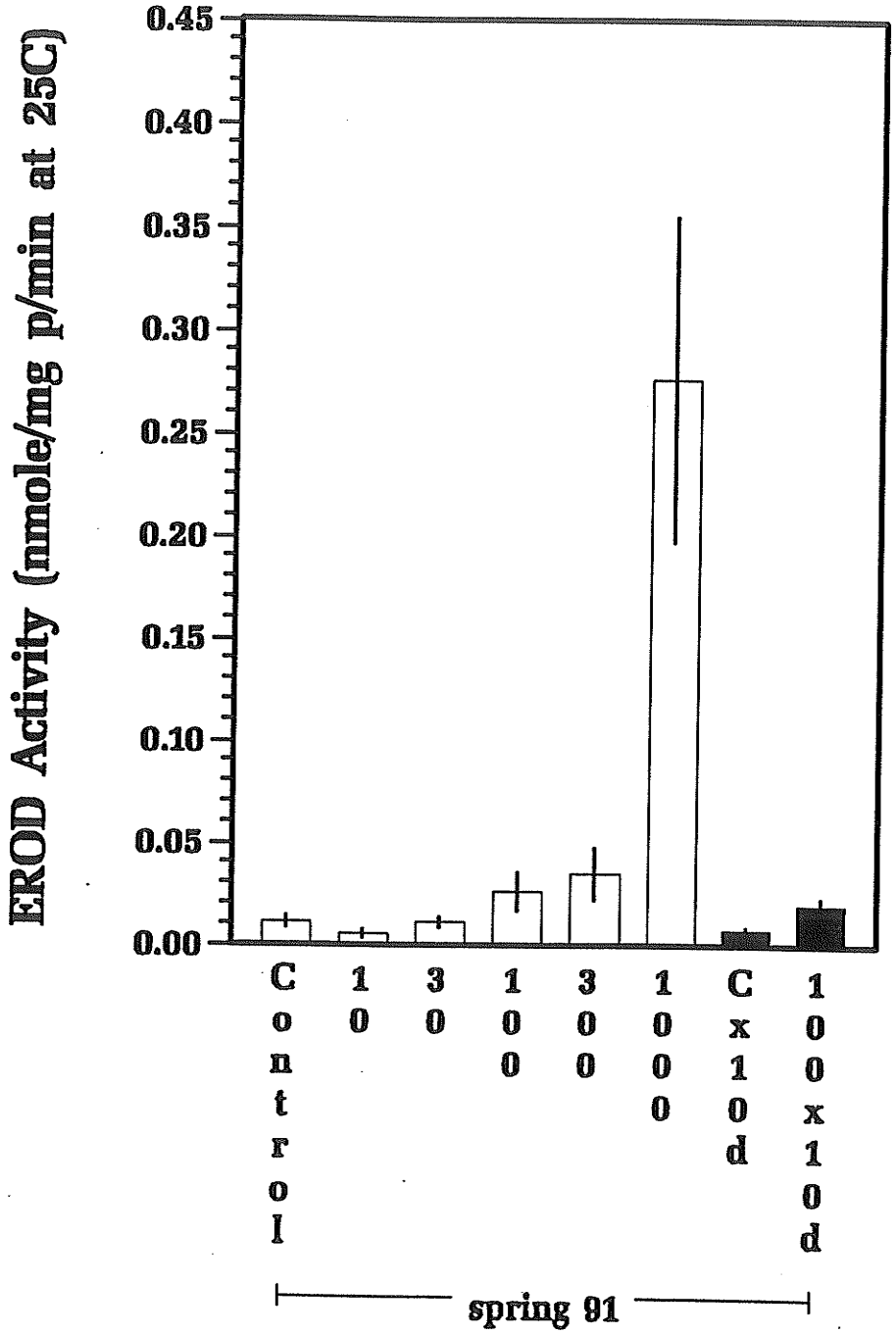

Figure 8. Mean EROD activities (nmoles mg^{-1} protein $minute^{-1}$ at 25°C) for white suckers treated with PCB Congener 77 (μg kg^{-1} body weight) in the Spring of 1991 with ten day (black)(x 10d) exposures. The five day exposure (white) is also seen in Fig. 5 and is used here to compare the response difference between five and ten day exposure.

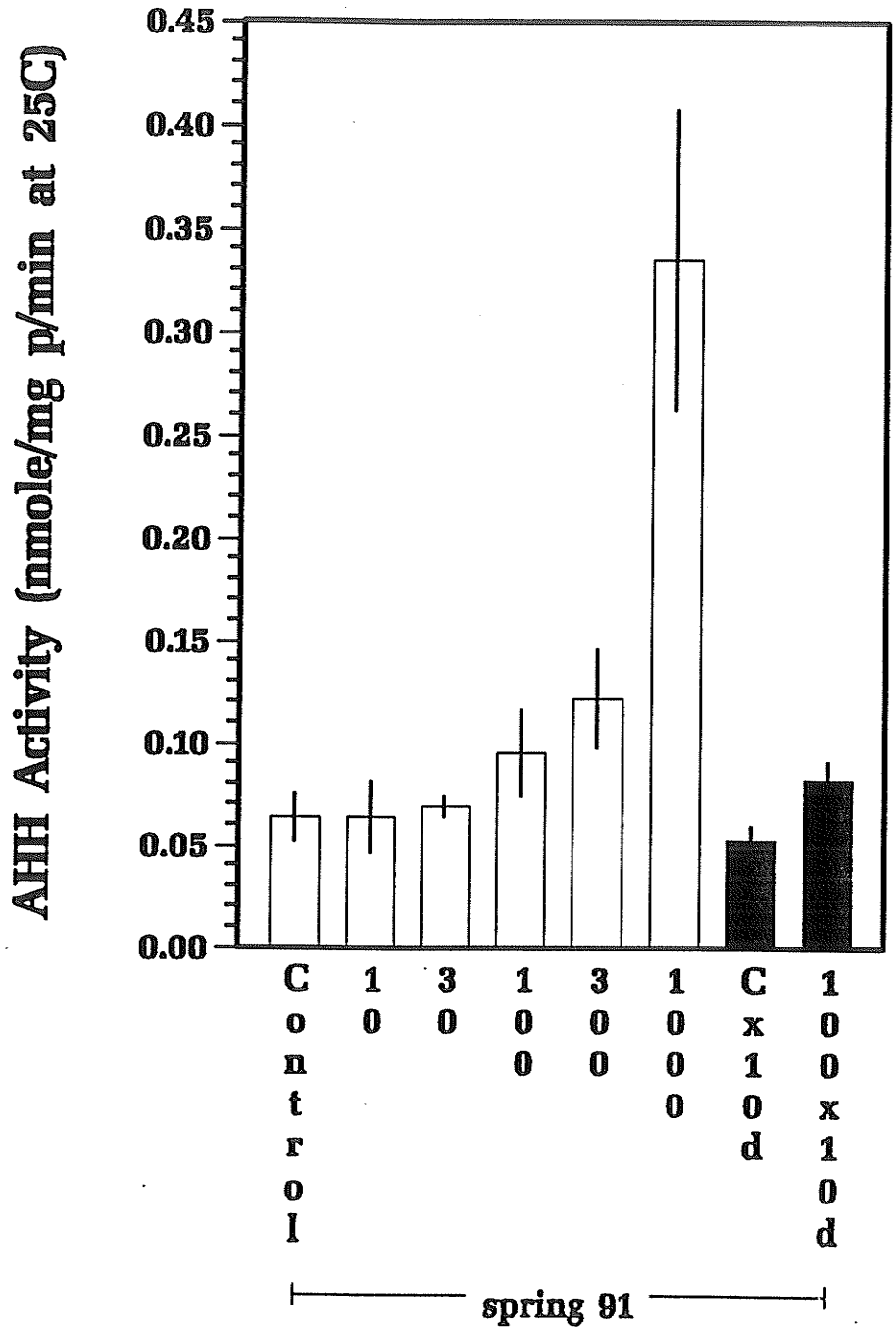

Figure 9. Mean AHH activities (nmoles mg^{-1} protein $minute^{-1}$ at 25°C) for white suckers treated with PCB Congener 77 (μg kg^{-1} body weight) in the Spring of 1991 with ten day (black)(x 10d) exposures. The five day exposure (white) is also seen in Fig. 6 and is used here to compare the response difference between five and ten day exposure.

1994). The mean activities for the five-day exposure of Spring 1991 gradually increased with increasing PCB concentration. Although the 100 and 300 μg kg^{-1} responses were not significantly different from the control in a statistical sense, they do suggest that a weak induction may have occurred. The low sample numbers and high variability within each exposure dosage have made detection of weak induction difficult. Without the evidence from the ten-day exposure, the possible weak induction from the lower dosages may have been ignored due to the high variability. Further work could be done with white suckers at these lower dosages with a larger sample size to distinguish any weak induction.

C. PCB fate in liver

The livers of the treated fish were first analyzed for PCB resides for the spring 1990 samples. The relationship between the PCB concentration and EROD and AHH activity (Fig. 10 and 11) for this treatment showed, excluding one outlier, that induction occurred in those livers where the PCB concentration exceeded 300 μg kg^{-1}. This finding coincides with results of other I.P. injection studies in rainbow trout where 200 μg kg^{-1} (Melancon and Lech, 1983) was the minimum CB77 concentration needed for induction after five days and 300 μg kg^{-1} (Melancon et al., 1989) of a PCB mixture residue in the liver caused induction after seven days.

The spring and fall 1991 treatment scatterplots (Fig. 10 and 11) were more inconsistent than for spring 1990 and did not produce as easily identifiable induction thresholds. This is not that unusual since with rainbow trout benzo(a)pyrene levels above 350 μg kg^{-1} showed clear cut induction whereas levels between 250-350 μg kg^{-1}

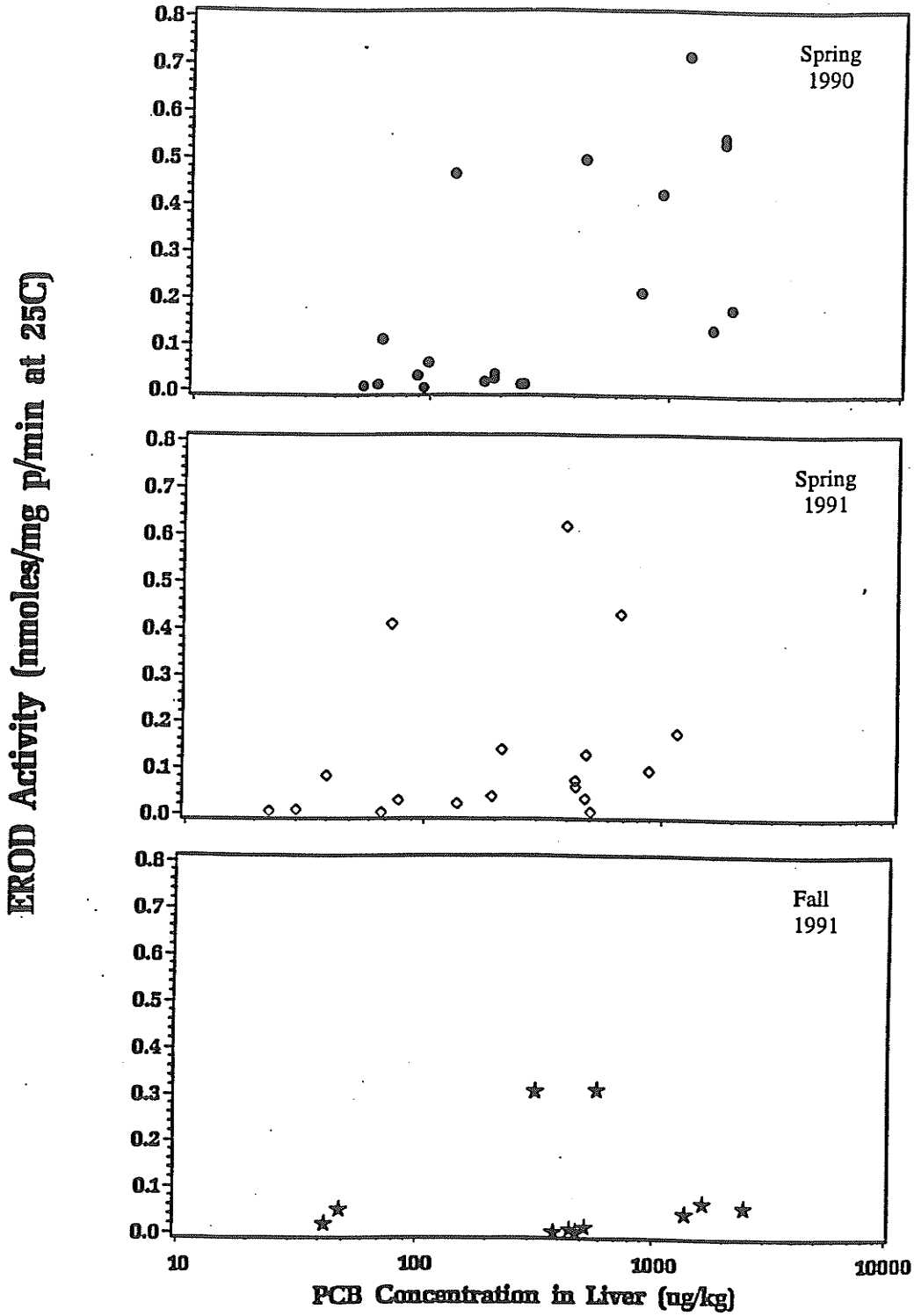

Figure 10. Relationship of PCB Congener 77 levels in liver to EROD enzyme activity in pooled male and female white suckers for Spring 1990 (●), Spring 1991 (◇), and Fall 1991 (★) after five days exposure.

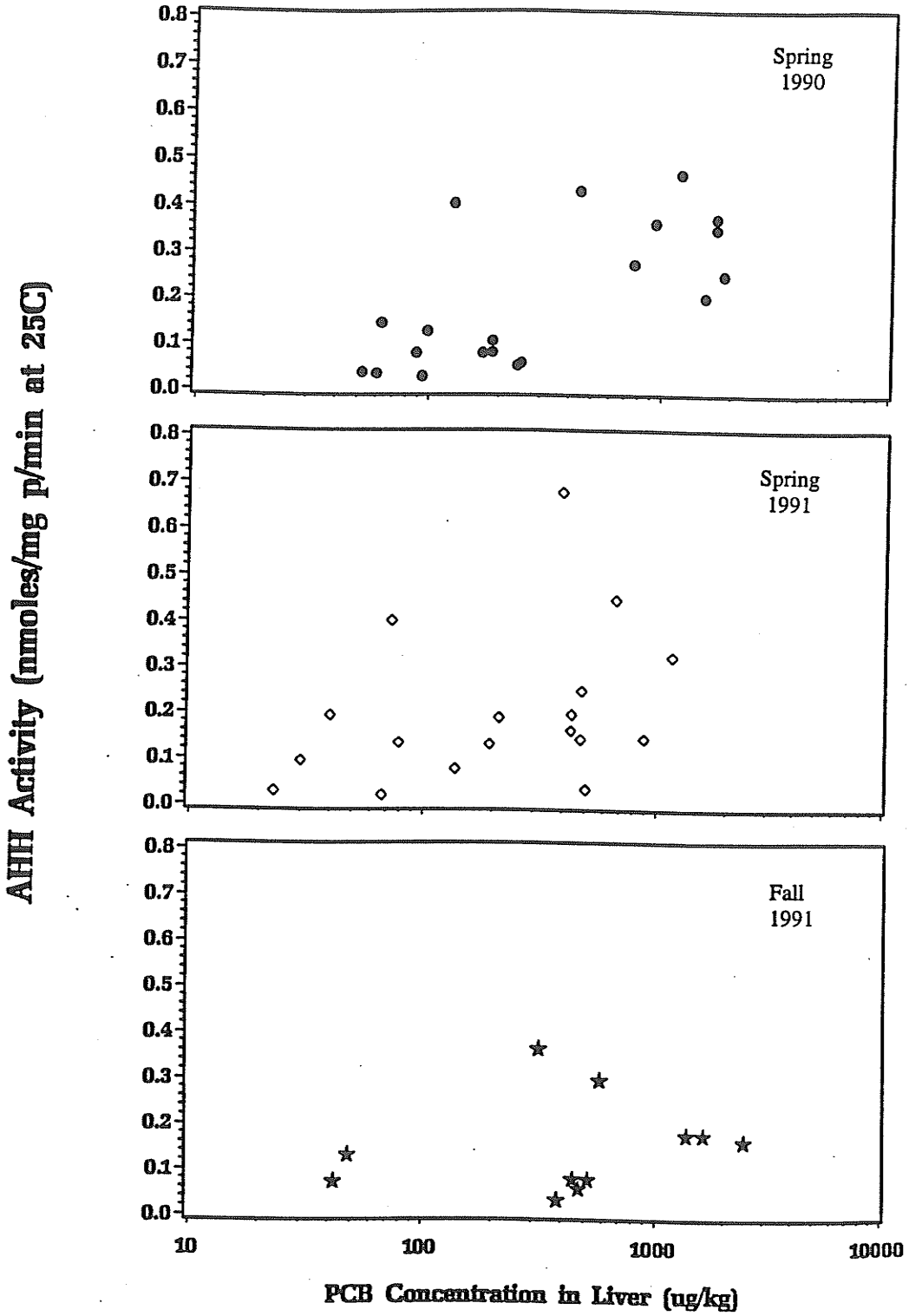

Figure 11. Relationship of PCB Congener 77 levels in liver to AHH enzyme activity in pooled male and female white suckers for Spring 1990 (●), Spring 1991 (◇), and Fall 1991 (★) after five days exposure.

corresponded to inconsistent increases between fish (Gerhart and Carlson, 1978). Theinconsistencies seen in these data show that some of the tissues may have needed more time before induction could take place. With the colder water temperature for the fall 1991 treatment the livers were able to accumulate the high PCB levels needed forinduction but would not have been able to induce within the treatment period of five days. The lower temperature would slow down the metabolism and/or the protein synthesis required for enzyme induction (Andersson and Koivusaari, 1985).

D. PCB fate in tissue

The average PCB concentrations in the tissues are shown in Figures 12, 13, & 14. Only the tissues from the three highest injection concentrations were analyzed to concentrate on the samples on either side of the threshold dosage for induction. As well, this reduced the number of samples analyzed in triplicate on the oxidizer. For all three dosages analyzed, 100, 300, and 1000 $\mu g\ kg^{-1}$, the muscle, gill, and heart tissues had mean PCB concentrations lower than the injected concentrations. The liver, intestine, and gonad tissues had mean PCB concentrations greater than the injected concentrations, indicating accumulation of the PCB in these tissues. This is not unexpected since the lipophilic PCB would accumulate in the lipids of these tissues and not in the leaner tissues such as the muscle, gill, and heart. As in other work, the levels of PCBs in the tissues were greater in the fish that received the larger dosages (Melancon et al., 1989; Monosson and Stegeman; 1991). This pattern of accumulation is remarkably similar in all three concentrations of PCB exposure. These three exposures were compared taking the separate tissues into account. The null hypothesis that the pattern of accumulation of the PCB in the tissues would be the same for each injection concentration was tested

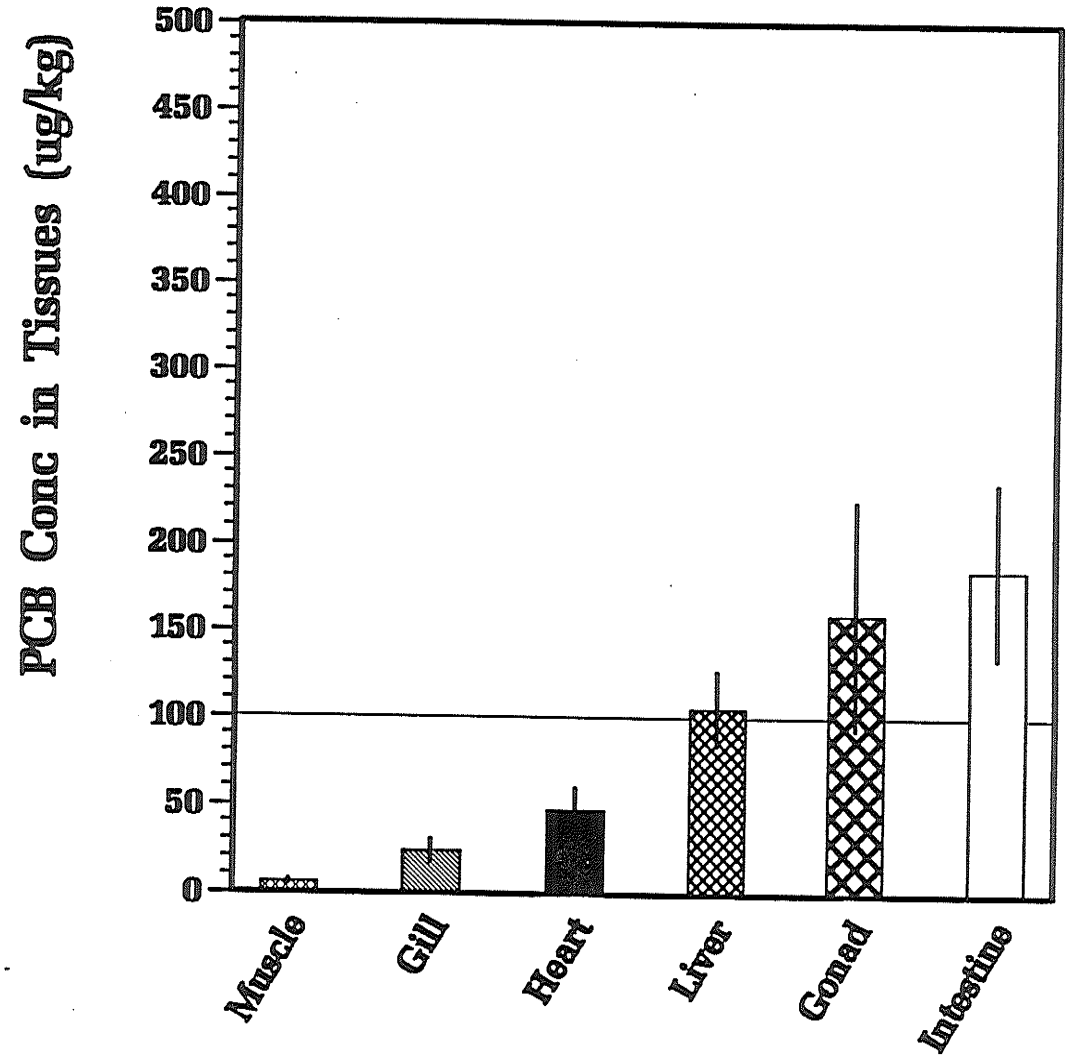

Figure 12. Mean PCB Congener 77 concentrations in tissues of pooled male and female white suckers after treatment with 100 μg kg^{-1} body weight (Reference line) after five days exposure.

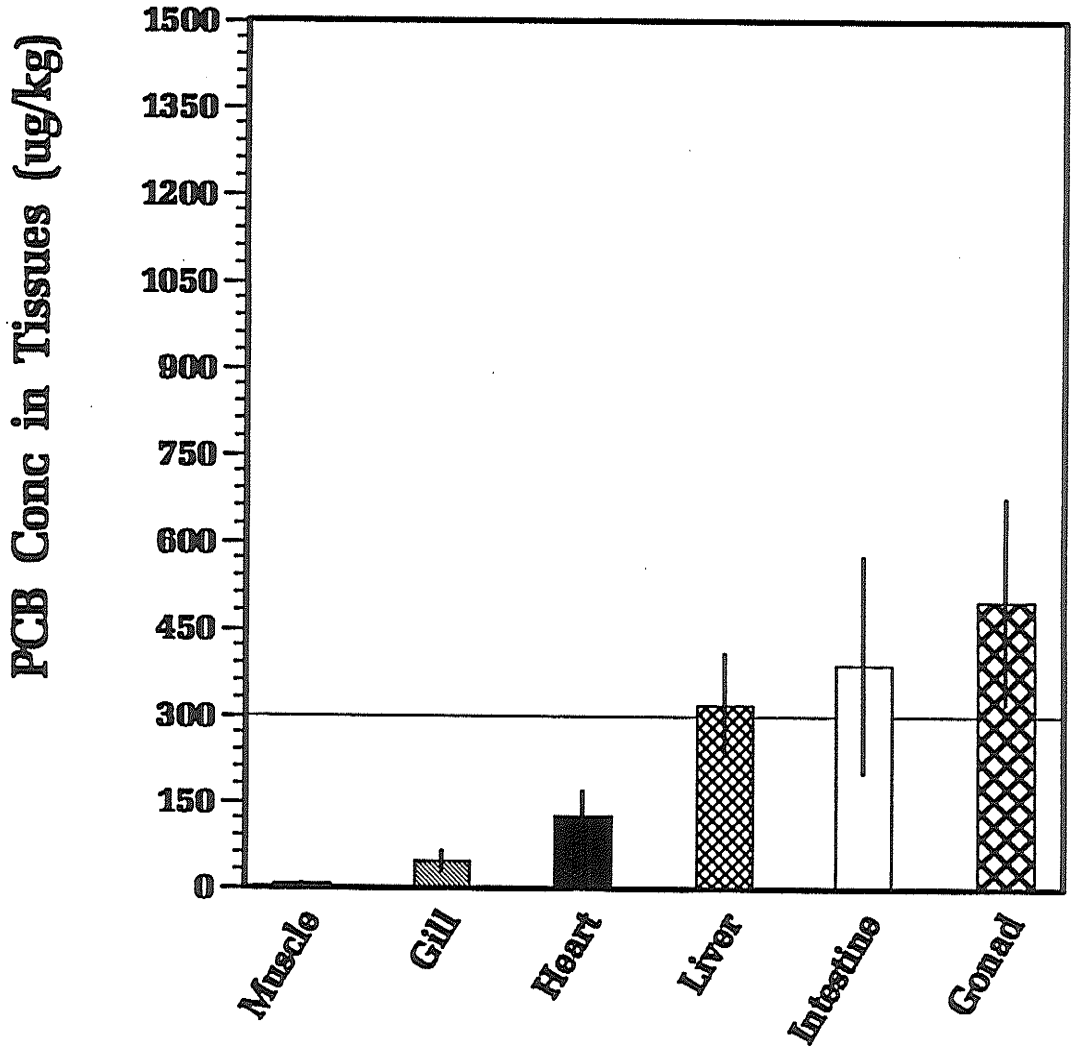

Figure 13. Mean PCB Congener 77 concentrations in tissues of poole2 male and female white suckers after treatment with 300 μg kg^{-1} body weight (Reference line) after five days exposure.

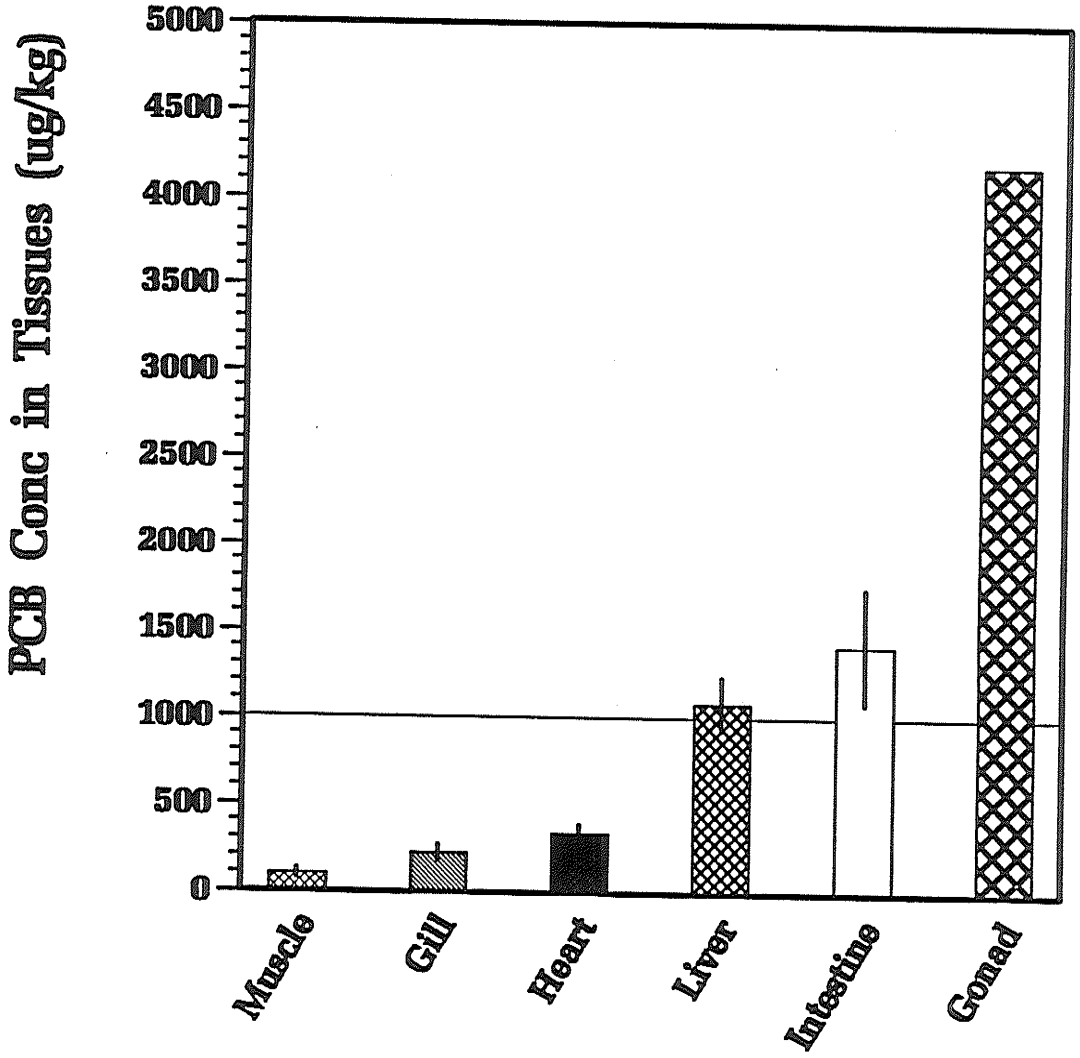

Figure 14. Mean PCB Congener 77 concentrations in tissues of pooled male and female white suckers after treatment with 1000 μg kg^{-1} body weight (Reference line) after five days exposure.

by normalizing the concentrations of PCB in the tissues to 100 μg kg^{-1} by using acorrection factor (for 1000 μg kg^{-1} tissue PCB concentration divided by 10 and for 300 μg kg^{-1} tissue concentration divided by 3). Use of ANCOVA and an additional **Concentration*Tissue** statement to the SAS model statement allowed the pattern to be statistically analyzed. There was no significant difference between the exposures for the pattern of the PCB concentration in the tissues. With an F value of 1.17 and Pr>F of 0.3140 there is no evidence to reject the null hypothesis. The pattern of accumulation in the tissues appears to be the same among these concentrations. The CB77 distribution among tissues does not differ as a function of exposure concentration and there is a distinct relationship between the injection and residue concentrations at these levels. This is similar to the CB77 residues in flounder where the dosages produced order-of-magnitude differences in the livers after five days of exposure (Monosson and Stegeman; 1991).

In the environment, other research has shown that there is a great range in the PCB concentrations in the muscle tissues of fish in the Great Lakes. In Lake Superior the white suckers had an average total PCB concentration of less than 80 ng g^{-1} (Smith et al., 1991) and Lake Huron white suckers ranged from 10 to 180 ng g^{-1} (Kononen, 1989). The total PCB concentration in the muscle of white suckers in Lake Ontario was much higher at 800 ng g^{-1} (Smith et al., 1991) and is similar to the lake trout mean of 751 ng g^{-1} (Janz et al., 1992) for the same lake. The CB77 in the fish, though, only accounts for a small percentage of the entire body burden (Janz et al., 1992; Niimi and Oliver, 1989a). The Janz et al. study (1992) found 3.32 ng g^{-1} CB77 in the muscle with a total PCB conc of 751 ng g^{-1} while Niimi and Oliver (1989a) only found 5 ng g^{-1} in muscle that contained 1066 ng g^{-1} total PCB. Though higher levels, white suckers in Lake Michigan, as well, had smaller concentrations of 50 ng g^{-1} CB77 relative to the

total PCB content of 41400 ng g^{-1} (Huckins and Schwartz, 1988). These totals should not be confused with inducable amounts since not all PCBs are inducing agents. Moderate correlations, however, have been found in comparisons between the total PCB residues and rates of EROD and AHH activity (Goksøyr and Förlin, 1992). These chemicals do not appear to be alone, though, but are part of a mixture (Janz et al., 1992; Niimi and Oliver, 1989a) including both inducing and non-inducing chemicals.

In this study only one PCB congener was present with resulting muscle residues averaging 93 ng g^{-1} in the fish with the 1000 μg kg^{-1} dosage. This is somewhat above the Superior and Huron but well below the Lake Ontario total PCB concentrations, but as an individual PCB it does not appear to be an environmentally legitimate concentration. The The toxic equivalent factor (TEF) for CB77 when compared to the most toxic dioxan, 2,3,7,8-TCDD, has been tested and calculated (Janz and Metcalf, 1991; Huckins et al., 1988; Niimi and Oliver, 1989b) This factor, when used on the 93 ng g^{-1} CB77 concentration in the muscle of the injected suckers in this experiment, would derive the amount of dioxan that would be needed to have the same toxic effects. A TEF of 0.0001 (Niimi and Oliver, 1989b) would have a product of 0.0093 ng g^{-1} equivalent TCDD. In Lake Ontario where 2,3,7,8-TCDD dioxan concentrations in whole fish range from 0.006 to 0.020 ng g^{-1} TCDD, an equivalent toxic concentration of 0.0093 could be considered to have environmentally legitimate toxic effects. The PCB dosages used in these experiments could be a good indication of the type of toxic contaminent effects, though all in one dose, that fish are subjected to in contaminated regions. The contaminant levels in the Great Lakes could be effecting these fish. If longer exposure periods allow for induction at lower concentrations of an inducer then such concentrations could be inducing higher MFO enzyme activities in Great Lakes' fish. Lake Superior and Lake Huron which are often used as "clean" references

(McMaster et al., 1991; Munkittrick et al., 1991) could have fish with elevated activities.

E. HPLC analysis

The HPLC analysis for the CB77 standard resulted with the highest peaks of recovery of ^{14}C radioactive material in the 12th and 13th vials (Fig. 15). As well, there were smaller peaks in the 2nd and 14th vials. These peaks when adjusted for background radiation and added together represented a 94% recovery of the injected standard. In comparison to this standard, the liver sample residues also had their highest peaks in the 12th and 13th vials (Fig. 15) with a few smaller peaks sometimes occurring in the 3rd or 4th vials. Although these samples had varying concentrations of ^{14}C between them, when compared to the radioactivity of the original sample (the white bars), the recovery rates were 80, 86, and 81% for the samples A, B, and C respectively. The lower recovery rates of the samples than the standard may be due to the much lower radioactivity in the samples and therefore are subject to less sensitivity by the scintillation counter. Recovery rates above 80% for such methodology are acceptable results. These results do, however, show a similar pattern of separation by the high pressure column and do not indicate degradation of the CB77 in the tissues. The radioactivity measured in the tissues by the oxidation analysis can be considered CB77 and not an accumulation of degradation products.

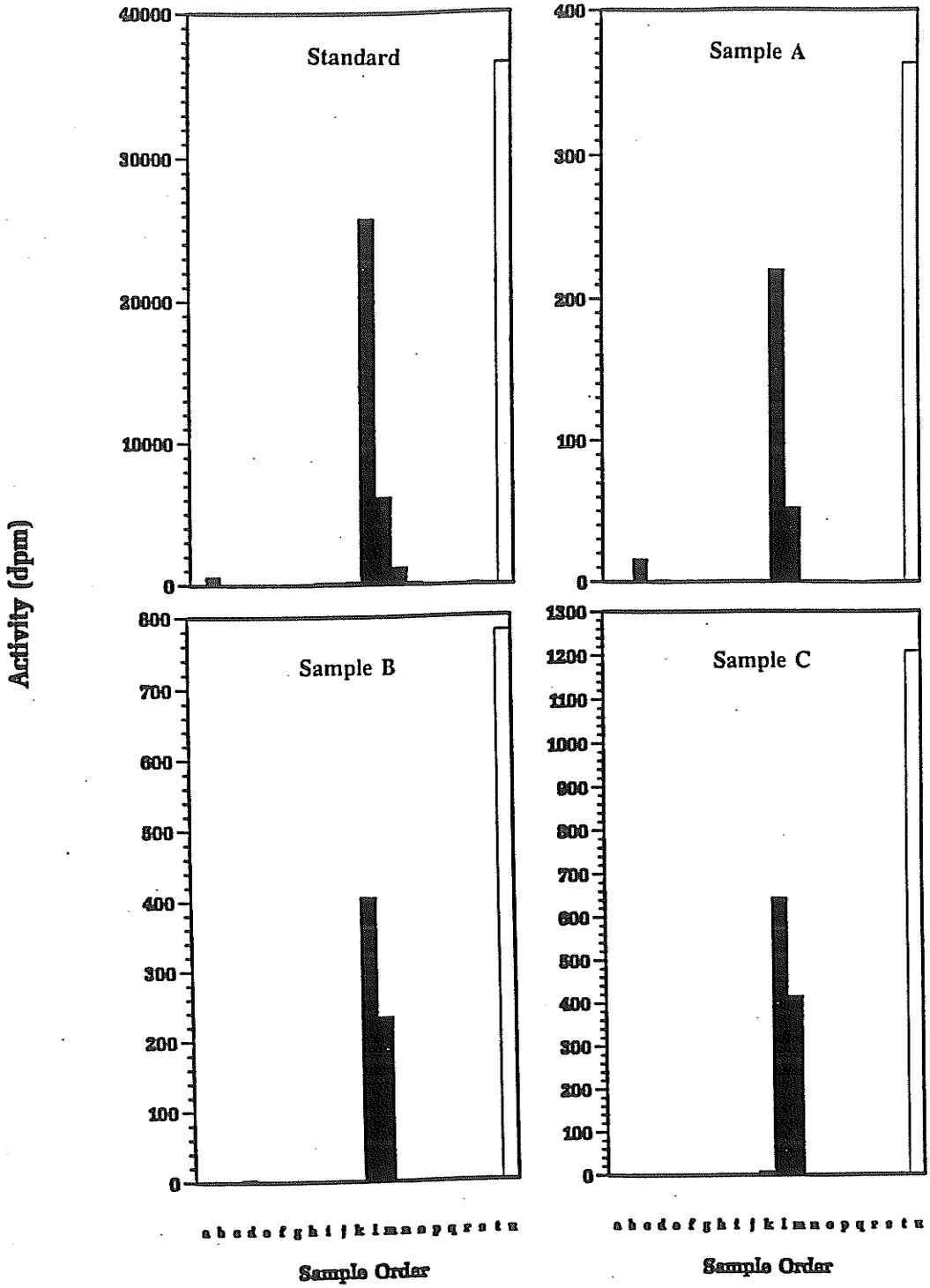

Figure 15. High pressure liquid chromatography (HPLC) elution profile. Radioactivity of ordered vials (a to t) after 5μL Standard or 50μL extracted sample (A, B, and C) of ^{14}C-PCB congener 77 passed though the column. The vial u represents the total radioactivity of sample injected into column.

V. Conclusions

In this study the hypothesis being tested were set to answer questions about the natural seasonal variation in P-450 catalytic activity and inducability of the system at at different dosages at different stages in the natural cycle. Stated as null hypotheses, these were:

> There are no differences in EROD and AHH activities in suckers taken at different times of the year.

and,

> EROD and AHH activities of white suckers do not differ between the range of dosages of CB77 injected.

and,

> There are no differences in inducability of sucker EROD and AHH activities between spring and fall samples.

These experiments were able to answer all three of these questions. A seasonal cycle in the activities of the MFO enzymes ethoxyresorufin-O-deethylase and aryl hydrocarbon hydroxylase has been described for white suckers from Roddy Lake (Lake 468). The trend over the two years of study showed a decline in the activities during spawning periods for both 1990 and 1991. When enzymatic activities were adjusted for water temperature at the time of capture the trend was unchanged. Significant statistical differences occurred between males and females for both enzyme activities with mean enzyme activities from males consistently higher than those from females for all field sample times and also for the temperature gradient experiment. The only exception to the general pattern of higher activities in males was one event during the 1991

postspawn sample. While the seasonal cycles were not precisely the same for the two years the general patterns were consistent.

When white suckers were treated with increasing doses of CB77 for five days, only the fish treated with the 1000 $\mu g\ kg^{-1}$ dosage exhibited statistically clear induction of EROD and AHH activities for both the spring treatments of 1990 and 1991 and for the fall treatment of 1991. The mechanism responsible for the low activities at spawning did not impair induction since there was no statistical difference between the response for spawning (spring) and non-spawning (fall) fish. If the water temperatures were equal, it may be possible to initiate induction in non-spawning fish during the fall with a lower dosage of CB77 than required by spawning fish in spring. A difference in induction response was not observed, though, in the colder water in the fall of 1991. The induction after five days exposure by I.P. injection appears to occur after a dosage between 300 and 1000 $\mu g\ kg^{-1}$ CB77, though these are injected dosages and not the true body burdens, and does not differ between spring and fall.

Although a five-day exposure during the spring of 1991 exhibited induction only at 1000 $\mu g\ kg^{-1}$, a ten-day exposure to 100 $\mu g\ kg^{-1}$ did show induction. The relationship between the exposure period and the threshold (if one really exists) for the induction is not known. The use of a five-day exposure period in these experiments was based on literature experiments (Hahn et al., 1989; Gooch et al., 1989; Melancon and Lech, 1983). The threshold concentration for induction was lower when the longer exposure was used. Longer exposure periods may give the liver time to accumulate the higher PCB levels required for induction. Weak induction at the lower dosages of 100 and 300 $\mu g\ kg^{-1}$ CB77 during the 1991 spring treatments may have occurred, though, a larger sample size would be needed to detect it with statistical confidence.

The fattier tissues absorbed more CB77 than the leaner tissues, as would be expected with the lipophilic properties of this compound. The concentrations in these tissues are higher than those found in Lakes Superior, Huron , Ontario, and Michigan but the toxic equivalence of these concentrations could be similar to that of the dioxan concentrations found in Lake Ontario fish. Such concentrations of dioxans could be inducing higher MFO activities in Great Lakes fish, especially if longer exposure periods cause induction at lower contaminant concentrations.

The white sucker has been used as a successful environmental monitor to distinguish some contaminated habitat in North America, notably near pulp mills. More research is still needed to determine the induction thresholds and dose-responses of other known and potential inducers. As well, more work is needed to set the seasonal cycles of the MFO activities seen here in the context of other regions. The sensitivity of the white sucker MFO enzymes to induction by an inducer at concentrations lower than some aquatic environments enhances its credibility as an indicator of pollution with organic contaminants having inducing structure.

VI. References

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APPENDICES

APPENDIX A.

Statistical analysis of HEPES vs TRIS buffers for the resuspension of microsomes.

DIFF1 = Fresh TRIS vs HEPES

DIFF2 = Frozen TRIS vs HEPES

DIFF3 = Frozen TRIS vs Fresh TRIS

Comparison of resuspension buffers for EROD assay

Variable	N	Mean	Maximum	Minimum	Variance	Std Dev	T	Prob>|T|
DIFF1	10	-0.0259	0.0400	-0.2390	0.0059	0.0769	-1.0648	0.3147
DIFF2	10	-0.0142	0.1320	-0.2510	0.0089	0.0942	-0.4765	0.6451
DIFF3	10	0.0117	0.0920	-0.0120	0.0009	0.0308	1.2031	0.2596

Comparison of resuspension buffers for BAP assay

Variable	N	Mean	Maximum	Minimum	Variance	Std Dev	T	Prob>|T|
DIFF1	10	0.0099	0.0530	-0.0390	0.0007	0.0268	1.1672	0.2731
DIFF2	10	-0.0262	0.0400	-0.1410	0.0040	0.0632	-1.3118	0.2221
DIFF3	10	-0.0361	0.0220	-0.1610	0.0045	0.0671	-1.7007	0.1232

Statistical analysis of HEPES vs TRIS buffers for the resuspension of microsomes.
Data logged to normalize.

Comparison of resuspension buffers for EROD assay

Variable	N	Mean	Maximum	Minimum	Variance	Std Dev	T	Prob>|T|
DIFF1	10	-0.0694	0.1823	-0.4568	0.0362	0.1904	-1.1525	0.2788
DIFF2	10	-0.2204	0.3365	-1.2040	0.1815	0.4261	-1.6358	0.1363
DIFF3	10	-0.1510	0.1542	-1.2993	0.1841	0.4291	-1.1131	0.2945

Comparison of resuspension buffers for BAP assay

Variable	N	Mean	Maximum	Minimum	Variance	Std Dev	T	Prob>|T|
DIFF1	10	0.1814	1.2528	-0.2231	0.1804	0.4248	1.3504	0.2099
DIFF2	10	0.2865	1.7047	-0.1969	0.3340	0.5779	1.5678	0.1514
DIFF3	10	0.1051	0.8650	-0.1957	0.1073	0.3276	1.0148	0.3367

APPENDIX B.

SAS program using General Linear Model (GLM) for EROD and AHH and their logs for seasonal data. Example for male white suckers.

```
 * input procedure

 options nodate;
 libname rb '[boychuk]';
 filename seamfo '[boychuk]season.dat';
 data mfosea;
      infile seamfo recfm=fb lrecl=48;
      input time $ 1-10 sex $ 14 sample 22-28
      erod 33-37 ahh 42-46 year 48;
      if year=2 or year=3 or sex='I' or sex='?' or sex='F'
      or time='fall'  then delete;
      lahh=log10(ahh); lerod=log10(erod);
      if time='prespawn1' then time=spawn1;
      keep time sex bap erod year lbap lerod;
 run;

 * title statements

 Title1 'MFO Analysis for Male White Suckers';
 Title2 'Over a Seasonal Cycle';

 * sorting procedure

 proc sort;
   by  year time  sex;
 run;

 * General Linear Model Procedure for AHH

 proc glm;
   class time   ;
   model ahh= time ;
   means time / lsd lines;

 /*  OPTIONAL CONTRAST STATEMENTS
     contrast 'pre1 vs post1' time     -1  0  1  0  0  0  0  0  0;
     contrast 'spawn1 vs post1' time   -1  0  0  0  1  0  0  0  0;
     contrast 'post1 vs summer1' time  -1  0  0  0  0  0  1  0  0;
     contrast 'pre2 vs spawn2' time     0  0  0  1  0 -1  0  0  0;
     contrast 'pre2 vs post2' time      0 -1  0  1  0  0  0  0  0;
     contrast 'spawn2 vs post2' time    0 -1  0  0  0  1  0  0  0;
     contrast 'post2 vs summer2' time   0 -1  0  0  0  0  0  1  0;
 */
 run;

 * horizontal bar chart procedure

 proc chart ;
   hbar time / sumvar=bap type = mean;
 run;

 * General Linear Model Procedure for log (AHH)

 proc glm;
   class time ;
   model lahh= time ;
```

```
   means time / lsd lines;

 /*  OPTIONAL CONTRAST STATEMENTS
     contrast 'pre1 vs post1' time     -1  0  1  0  0  0  0  0  0;
     contrast 'spawn1 vs post1' time   -1  0  0  0  1  0  0  0  0;
     contrast 'post1 vs summer1' time  -1  0  0  0  0  0  1  0  0;
     contrast 'pre2 vs spawn2' time     0  0  0  1  0 -1  0  0  0;
     contrast 'pre2 vs post2' time      0 -1  0  1  0  0  0  0  0;
     contrast 'spawn2 vs post2' time    0 -1  0  0  0  1  0  0  0;
     contrast 'post2 vs summer2' time   0 -1  0  0  0  0  0  1  0;
 */
 run;

 * horizontal bar chart procedure

 proc chart ;
   hbar time / sumvar=lbap type = mean;
 run;


 * General Linear Model Procedure for EROD

 proc glm;
   class time  ;
   model erod= time ;
   means time / lsd lines;

 /*  OPTIONAL CONTRAST STATEMENTS
     contrast 'pre1 vs post1' time     -1  0  1  0  0  0  0  0  0;
     contrast 'spawn1 vs post1' time   -1  0  0  0  1  0  0  0  0;
     contrast 'post1 vs summer1' time  -1  0  0  0  0  0  1  0  0;
     contrast 'pre2 vs spawn2' time     0  0  0  1  0 -1  0  0  0;
     contrast 'pre2 vs post2' time      0 -1  0  1  0  0  0  0  0;
     contrast 'spawn2 vs post2' time    0 -1  0  0  0  1  0  0  0;
     contrast 'post2 vs summer2' time   0 -1  0  0  0  0  0  1  0;
 */
 run;

 * horizontal bar chart procedure

 proc chart ;
   hbar time / sumvar=erod type = mean;
 run;

 * General Linear Model Procedure for log(EROD)

 proc glm;
   class time  ;
   model lerod= time ;
   means time / lsd lines;

 /*  OPTIONAL CONTRAST STATEMENTS
     contrast 'pre1 vs post1' time     -1  0  1  0  0  0  0  0  0;
     contrast 'spawn1 vs post1' time   -1  0  0  0  1  0  0  0  0;
     contrast 'post1 vs summer1' time  -1  0  0  0  0  0  1  0  0;
     contrast 'pre2 vs spawn2' time     0  0  0  1  0 -1  0  0  0;
     contrast 'pre2 vs post2' time      0 -1  0  1  0  0  0  0  0;
     contrast 'spawn2 vs post2' time    0 -1  0  0  0  1  0  0  0;
     contrast 'post2 vs summer2' time   0 -1  0  0  0  0  0  1  0;
 */
```

```
run;

* horizontal bar chart procedure

proc chart ;
  hbar time / sumvar=lerod type = mean;
run;
```

SAS program using GLM for analysis of any significant difference between sexes for EROD and AHH activities for seasonal data.

```
libname rb '[boychuk]';
filename mfodata1 '[boychuk]data.dat';

goptions device=x40451 nodisplay chartype=37
         ftext=zapfb nocell gsflen=132 gsfmode=replace
         gsfname=analysis;

* input statements

data data1;
     infile mfodata1 recfm=fb lrecl=73;
     input sample 2-8 flength 10-12 wholewt 13-16 guttedwt 17-20
           liver 22-25 gonad 26-30 sex $ 31-32
           erod 34-38  ahh 40-44 cond $ 45-52 time $ 53-60
           conc 62-65 pcb 67-73;
     if sex='I?' then sex='I';
     if sex='M?' then sex='I';
     if sex='F?' then sex='I';
     if sex='I' then delete;
     if time='90' and cond='late win' then cond='a late win 90';
     if time='90' and cond='prespawn' then cond='b prespawn 90';
     if time='90' and cond='spawn' then cond='c spawn 90';
     if time='90' and cond='postspaw' then cond='d postspawn 90';
     if time='90' and cond='fall' then cond='f fall 90';
     if time='90' and cond='summer' then cond='e summer 90';
     if time='90' and cond='winter' then cond='g winter 90';
     if time='91' and cond='late win' then cond='h late win 91';
     if time='91' and cond='prespawn' then cond='i prespawn 91';
     if time='91' and cond='spawn' then cond='j spawn 91';
     if time='91' and cond='postspaw' then cond='k postspawn 91';
     if time='91' and cond='summer' then cond='l summer 91';
     if time='spring90' then delete;
     if time='spring91' then delete;
     if time='t fall91' then delete;
*     if time='90' then delete;
*     if time='91' then delete;

 keep time cond ahh erod sex conc;

run;

* sorting procedure

proc sort;
   by time cond ;
run;

* General Linear Model for EROD

proc glm;
    class time cond sex;
    model  erod= cond sex cond*sex;
run;

* General Linear Model for AHH
```

```
proc glm;
    class time cond sex;
    model  ahh= cond sex cond*sex;
run;
```

SAS program using ANCOVA to compare 1990 and 1991 cycles of seasonal activities for EROD and AHH and their logs. Example for female white suckers.

```
* input statements

libname rb '[boychuk]';
filename seamfo '[boychuk]season1.dat';
data mfosea;
     infile seamfo recfm=fb lrecl=48;
     input sample $ 1-10 sex $ 14 code 22-28
     erod 33-37 bap 42-46 year 48;
     if  sex='I' or sex='?' or sex='M' then delete;
     if time='fall' then delete;
     if erod=0.000 then erod=0.001;
     lbap=log10(bap); lerod=log10(erod);
     if sample='postspawn1' then period='postspawn';
     if sample='postspawn2' then period='postspawn';
     if sample='prespawn1' then period='prespawn';
     if sample='prespawn2' then period='prespawn';
     if sample='spawn1' then period='spawn';
     if sample='spawn2' then period='spawn';
     if sample='summer1' then period='summer';
     if sample='summer2' then period='summer';
     if sample='late wint' then delete;
     if sample='winter' then period='winter';

     keep period sex erod bap year lbap lerod;
run;

* sorting procedure

proc sort;
  by  year period sex;
run;

title1 'for female white sukers';

* ANCOVA for AHH

proc glm;
  class year period;
  model bap=year period year*period;
run;

* ANCOVA for log(AHH)

proc glm;
  class year period;
  model lbap=year period year*period;
run;

* ANCOVA for EROD

proc glm;
  class year period;
  model erod=year period year*period;
run;

* ANCOVA for log(EROD)
```

```
proc glm;
  class year period;
  model lerod=year period year*period;
run;
```

SAS program using contrasts in the GLM to determine any significant difference between injection concentrations for EROD and AHH enzymes and their logs. Example for Spring 1990.

```
libname rb '[boychuk]';
filename mfodata1 '[boychuk]data.dat';

goptions device=x40451 nodisplay chartype=37
         ftext=zapfb nocell gsflen=132 gsfmode=replace
         gsfname=analysis;

* input statements

data data1;
     infile mfodata1 recfm=fb lrecl=73;
     input sample 2-8 flength 10-12 wholewt 13-16
           guttedwt 17-20 liver 22-25 gonad 26-30
           sex $ 31-32 erod 34-38  ahh 40-44
           cond $ 45-52 time $ 53-60
           conc 62-65 pcb 67-73;
*     if conc='.1' then delete;
*     if conc='1' then delete;
*     if conc='30' then delete;
*     if conc='10' then delete;
*     if conc='100' then delete;
*     if conc='300' then delete;
      if conc='0' then conc=1;
*     if cond='E' then delete;
      if cond='C 10' then delete;
      if cond='CONT 10' then delete;
*     if time='spring90' then delete;
      if time='spring91' then delete;
      if time='t fall91' then delete;
      if time='90' then delete;
      if time='91' then delete;
      lconc=log10(conc);
 keep time cond ahh erod conc lconc;
run;

* sorting procedure

proc sort;
   by time conc ;
run;

* General Linear Model and contrasts for EROD

proc glm;
    class time conc;
    model  erod= conc time time*conc;
    means time*conc / lsd;
    contrast '0,10,30,100,&300 vs 1000'  conc -1 -1 -1 -1 -1  5;
    contrast '0 vs 10'                   conc -1  1  0  0  0  0;
    contrast '0 vs 30'                   conc -1  0  1  0  0  0;
    contrast '0 vs 100'                  conc -1  0  0  1  0  0;
    contrast '0 vs 300'                  conc -1  0  0  0  1  0;
    contrast '300 vs 1000'               conc  0  0  0  0 -1  1;
```

```
    contrast '100 vs 1000'                conc  0  0  0 -1  0  1;
run;

* General Linear Model and contrasts for EROD
* with log(injection concentration)

proc glm;
    class time lconc;
    model  erod= lconc time time*lconc;
    means time*lconc / lsd;
    contrast '0,10,30,100,&300 vs 1000'  lconc -1 -1 -1 -1 -1  5;
    contrast '0 vs 10'                   lconc -1  1  0  0  0  0;
    contrast '0 vs 30'                   lconc -1  0  1  0  0  0;
    contrast '0 vs 100'                  lconc -1  0  0  1  0  0;
    contrast '0 vs 300'                  lconc -1  0  0  0  1  0;
    contrast '300 vs 1000'               lconc  0  0  0  0 -1  1;
    contrast '100 vs 1000'               lconc  0  0  0 -1  0  1;
run;

* General Linear Model and contrasts for AHH

proc glm;
    class time conc;
    model  ahh= conc time time*conc;
    means time*conc / lsd;
    contrast '0,10,30,100,&300 vs 1000'  conc -1 -1 -1 -1 -1  5;
    contrast '0 vs 10'                   conc -1  1  0  0  0  0;
    contrast '0 vs 30'                   conc -1  0  1  0  0  0;
    contrast '0 vs 100'                  conc -1  0  0  1  0  0;
    contrast '0 vs 300'                  conc -1  0  0  0  1  0;
    contrast '300 vs 1000'               conc  0  0  0  0 -1  1;
    contrast '100 vs 1000'               conc  0  0  0 -1  0  1;


run;

* General Linear Model and contrasts for AHH
* with log(injection concentration)

proc glm;
    class time lconc;
    model  ahh= lconc time time*lconc;
    means time*lconc / lsd;
    contrast '0,10,30,100,&300 vs 1000'  lconc -1 -1 -1 -1 -1  5;
    contrast '0 vs 10'                   lconc -1  1  0  0  0  0;
    contrast '0 vs 30'                   lconc -1  0  1  0  0  0;
    contrast '0 vs 100'                  lconc -1  0  0  1  0  0;
    contrast '0 vs 300'                  lconc -1  0  0  0  1  0;
    contrast '300 vs 1000'               lconc  0  0  0  0 -1  1;
    contrast '100 vs 1000'               lconc  0  0  0 -1  0  1;


run;
```

SAS program using ANCOVA to compare the patterns of PCB congener 77 distribution in white sucker tissues.

```
filename gsasfile printer flag=no notify=yes
         passall=yes queue=rdps_print1;
goptions device=x4045L gaccess=gsasfile ftext=zapfb;

libname rb '[boychuk]';
filename oxidd '[boychuk]oxid.data';
data oxid;
     infile oxidd recfm=fb lrecl=46;
     input tissue $ 1-9 sample $ 11-17 code $ 22
     inject 28-31 conc 35-41 sex $ 46;
     conc=conc*1000;
     if code='E' then conc=conc/10;
     if inject=10 then delete;
      if inject=300 then conc=conc/3;
      if conc< .00001 then delete;
*     keep tissue code conc ;
run;

* sorting procedure

proc sort;
  by inject  tissue;
run;


* ANCOVA analysis

proc glm;
   class inject tissue;
   model  conc=tissue inject inject*tissue;
   means tissue / lsd;
   means tissue/pdiff;
run;
```

APPENDIX C. Raw Data

SAMPLE NUMBER	Fork Length	Whole Weight	Gutted Weight	Liver Weight	Gonad Weight	SEX	EROD	AHH	Sample Period	Sample Date	Sample Year
9008014	397	789	717	8.4	13.1	M	0.003	0.018	prespawn	17 may	90
9008001	378	780	698	8.5	14.8	M	0.018	0.145	prespawn	9 may	90
9008002	398	776	710	6.0	14.8	M	0.007	0.065	prespawn	9 may	90
9008004	409	902	800	19.1	23.9	M	0.004	0.021	prespawn	9 may	90
9008003	410	931	774	14.0	71.0	F	0.006	0.053	prespawn	9 may	90
9008005	441	1046	861	14.6	110.2	F	0.003	0.030	prespawn	9 may	90
9008006	422	992	825	15.0	85.7	F	0.003	0.028	prespawn	9 may	90
9008007	414	938	774	15.9	73.3	F	0.007	0.032	prespawn	10 may	90
9008008	426	1024	873	11.9	79.0	F	0.002	0.028	prespawn	11 may	90
9008009	424	978	805	16.7	99.7	F	0.006	0.031	prespawn	11 may	90
9008010	405	861	729	10.0	68.1	F	0.005	0.038	prespawn	11 may	90
9008011	439	1039	871	16.7	82.7	F	0.007	0.041	prespawn	11 may	90
9008012	428	1062	901	14.7	84.0	F	0.006	0.039	prespawn	11 may	90
9008013	407	980	792	19.3	80.0	F	0.009	0.031	prespawn	11 may	90
9008024	414	912	671	8.6	35.8	F	0.003	0.016	spawn	7 jun	90
9008025	432	1156	958	11.6	121.2	F	0.004	0.022	spawn	7 jun	90
9008026	433	890	800	10.0	10.4	F	0.007	0.030	spawn	7 jun	90
9008029	421	1018	822	6.6	82.8	F	0.002	0.016	spawn	7 jun	90
9008033	373	713	594	7.6	93.2	F	0.009	0.014	spawn	8 jun	90
9008027	355	545	450	4.9	13.0	M	0.015	0.066	spawn	8 jun	90
9008028	389	650	570	4.8	6.8	M	0.006	0.038	spawn	8 jun	90
9008030	354	568	521	5.6	10.8	M	0.022	0.059	spawn	8 jun	90
9008031	373	622	578	5.1	9.8	M	0.008	0.040	spawn	8 jun	90
9008032	379	739	672	6.1	13.2	M	0.012	0.036	spawn	8 jun	90
9008096	426	901	810			F	0.013	0.029	postspaw	19 jun	90
9008098	413	873	794			F	0.016	0.016	postspaw	19 jun	90
9008099	388	685	621			F	0.010	0.038	postspaw	19 jun	90
9008100	421	872	774			F	0.004	0.011	postspaw	19 jun	90
9008101	425	938	807			F	0.007	0.045	postspaw	19 jun	90
9008105	433	901	822			F	0.024	0.040	postspaw	19 jun	90
9008097	361	595	540			M	0.027	0.071	postspaw	19 jun	90
9008102	376	653	589			M	0.025	0.061	postspaw	19 jun	90
9008103	374	672	614			M	0.015	0.081	postspaw	19 jun	90
9008113	432	937	837	6.7	14.5	F	0.016	0.050	summer	24 jul	90
9008114	368	680	616	5.9	10.5	F	0.009	0.044	summer	24 jul	90
9008108	363	621	555	7.4	8.2	F	0.019	0.051	summer	24 jul	90
9008110	375	654	593	5.8	10.8	F	0.021	0.052	summer	24 jul	90
9008106	379	672	614	4.9	5.2	M	0.032	0.058	summer	24 jul	90
9008107	289	761	655	7.7	7.2	M	0.026	0.050	summer	24 jul	90
9008109	390	733	673	10.0	4.2	M	0.013	0.029	summer	24 jul	90
9008111	383	673	616	6.2	2.3	M	0.014	0.063	summer	24 jul	90
9008117	357	609	530	7.2	4.2	M	0.039	0.150	summer	24 jul	90
9008145	379	668	580	15.6	28.9	F	0.020	0.054	winter	12 dec	90
9008143	423	906	789	15.6	35.8	F	0.007	0.030	winter	12 dec	90
9008142	421	919	783	15.3	49.9	F	0.009	0.029	winter	12 dec	90
9008149	430	1058	936	14.3	48.1	F	0.008	0.048	winter	13 dec	90
9008147	347	545	486	7.9	14.5	M	0.019	0.073	winter	12 dec	90
9008144	364	648	578	7.6	13.1	M	0.014	0.072	winter	12 dec	90
9008151	406	902	775	14.4	50.8	F	0.012	0.038	late win	5 april	91
9008155	337	492	441	9.8	2.0	M	0.032	0.105	late win	5 april	91
9008159	383	712	628		24.3	M	0.052	0.144	prespawn	9 may	91
9008161	396	763	692		14.6	M	0.036	0.147	prespawn	9 may	91
9008162	382	720	652		28.8	M	0.038	0.142	prespawn	9 may	91
9008168	404	908	839		57.2	M	0.018	0.077	prespawn	9 may	91
9008169	373	722	658		14.2	M	0.037	0.137	prespawn	9 may	91
9008164	367	610	550		18.3	M	0.014	0.092	prespawn	9 may	91

SAMPLE NUMBER	Fork Length	Whole Weight	Gutted Weight	Liver Weight	Gonad Weight	SEX	EROD	AHH	Sample Period	Sample Date	Sample Year
9008166	374	704	630		19.8	M	0.085	0.198	prespawn	9 may	91
9008157	406	844	732		58.2	F	0.002	0.026	prespawn	9 may	91
9008160	420	982	940		70.2	F	0.002	0.039	prespawn	9 may	91
9008163	404	908	839		57.2	F	0.009	0.042	prespawn	9 may	91
9008170	432	1209	983		129.7	F	0.010	0.059	prespawn	9 may	91
9008165	401	786	652		64.0	F	0.001	0.035	prespawn	9 may	91
9008167	405	914	72		77.0	F	0.008	0.065	prespawn	9 may	91
9008171	401	729	676			M	0.002	0.012	spawn	31 may	91
9008173	377	670	620			M	0.004	0.043	spawn	31 may	91
9008175	386	760	685			M	0.002	0.030	spawn	31 may	91
9008177	378	644	600			M	0.001	0.037	spawn	31 may	91
9008179	386	743	675			M	0.004	0.068	spawn	31 may	91
9008172	427	850	722			F	0.001	0.032	spawn	31 may	91
9008174	399	866	692			F	0.000	0.024	spawn	31 may	91
9008176	436	1038	917			F	0.002	0.037	spawn	31 may	91
9008178	431	1015	865			F	0.001	0.034	spawn	31 may	91
9008180	415	975	799			F	0.000	0.016	spawn	31 may	91
9008186	366	645	610			M	0.036	0.100	postspaw	19 jun	91
9008185	366	645	610			M	0.019	0.069	postspaw	19 jun	91
9008188	366	645	610			M	0.010	0.078	postspaw	19 jun	91
9008183	363	648	591	5.1		F	0.081	0.144	postspaw	19 jun	91
9008184	371	727	667			F	0.013	0.054	postspaw	19 jun	91
9008181	395	689	629	5.7		F	0.014	0.059	postspaw	19 jun	91
9008187	355	589	543			F	0.008	0.053	postspaw	19 jun	91
9008189	342	544	504			F	0.033	0.129	postspaw	19 jun	91
9008279	346	509	461	4.4	10.6	M	0.019	0.107	summer	20 aug	91
9008276	358	674	597	4.5	28.5	M	0.004	0.066	summer	20 aug	91
9008272	360	670	596	4.5	22.2	M	0.021	0.090	summer	20 aug	91
9008270	394	748	683	7.9	13.6	F	0.007	0.064	summer	20 aug	91
9008271	380	700	630	4.1	10.7	F	0.015	0.096	summer	20 aug	91
9008274	400	678	623	3.8	2.9	F	0.009	0.088	summer	20 aug	91
9008275	365	655	593	5.5	11.5	F	0.003	0.061	summer	20 aug	91
9008277	389	832	752	7.0	19.2	F	0.011	0.075	summer	20 aug	91
9008278	359	606	543	5.5	4.4	F	0.012	0.101	summer	20 aug	91

SAMPLE NUMBER	Fork Length	Whole Weight	Gutted Weight	Liver Weight	Gonad Weight	Sex	EROD	AHH	Injection Code	Injection period	Injection Conc. ug/kg	ugPCB/g wet wt
9008049	361	582	490	5.1	41.0	F	0.001	0.020	CONTROL	spring 90	0	
9008045	415	909	760	6.4	83.2	F	0.006	0.032	CONTROL	spring 90	0	
9008053	405	774	651	6.3	40.7	F	0.004	0.021	CONTROL	spring 90	0	
9008086	335	437	393	4.0	1.1	F	0.002	0.013	CONTROL	spring 90	0	
9008071	386	765	657	5.2	22.0	F	0.039	0.126	CONTROL	spring 90	0	
9008087	394	696	613	4.5	8.5	M	0.011	0.055	CONTROL	spring 90	0	
9008091	378	717	611	6.2	31.5	M	0.007	0.052	CONTROL	spring 90	0	
9008052	360	565	500	3.5	16.3	M	0.013	0.077	CONTROL	spring 90	0	
9008044	400	725	657	4.3	27.0	M	0.002	0.004	CONTROL	spring 90	0	
9008066	376	644	550	4.3	23.6	M	0.010	0.037	CONTROL	spring 90	0	
9008038	405	863	704	6.2	80.8	F	0.005	0.028	A	spring 90	0.1	
9008057	415	845	697	6.5	62.4	F	0.002	0.021	B	spring 90	1	
9008080	396	721	624	5.1	36.0	F	0.008	0.039	C	spring 90	10	0.01416
9008056	395	791	655	5.1	71.2	F	0.012	0.059	D	spring 90	100	0.24949
9008036	394	765	628	6.8	90.2	F	0.008	0.038	D	spring 90	100	0.05269
9008090	375	672	558	4.9	52.9	F	0.011	0.042	D	spring 90	100	0.01229
9008068	422	833	648	6.6	77.3	F	0.004	0.029	D	spring 90	100	0.09433
9008083	366	671	521	4.4	19.6	F	0.025	0.082	D	spring 90	100	0.18767
9008083	366	671	521	4.4	19.6	F	0.034	0.106	D	spring 90	100	0.18767
9008075	387	744	618	5.3	83.7	M	0.004	0.020	D	spring 90	100	
9008061	360	641	577	4.6	16.2	M	0.018	0.080	D	spring 90	100	0.17114
9008035	360	548	510	4.3	11.7	M	0.012	0.053	D	spring 90	100	0.24038
9008077	396	715	655	5.2	64.3	M	0.031	0.080	D	spring 90	100	0.08875
9008062	393	757	662	5.5	14.1	M	0.059	0.127	D	spring 90	100	0.09851
9008082	370	701	613	5.3	24.9	M	0.012	0.035	D	spring 90	100	0.06055
9008048	394	701	587	4.9	52.9	F	0.532	0.373	E	spring 90	1000	1.71719
9008048	394	701	587	4.9	52.9	F	0.544	0.350	E	spring 90	1000	1.71719
9008042	432	956	810	6.1	75.9	F	0.176	0.250	E	spring 90	1000	1.85863
9008073	416	785	687	6.1	10.8	F	0.110	0.145	E	spring 90	1000	0.06301
9008093	369	693	569	4.9	61.2	F	0.423	0.362	E	spring 90	1000	0.93727
9008054	410	867	722	7.1	72.3	F	0.132	0.202	E	spring 90	1000	1.54218
9008039	367	586	528	4.4	15.3	M	0.716	0.467	E	spring 90	1000	1.19953
9008088	393	739	690	5.5	14.3	M	0.212	0.274	E	spring 90	1000	0.76399
9008070	382	681	613	4.6	11.6	M	0.465	0.404	E	spring 90	1000	0.12651
9008067	417	817	711	7.0	7.7	M	0.496	0.431	E	spring 90	1000	0.44031
9008055	385	664	597	4.3	18.1	M	0.327	0.311	E	spring 90	1000	
9008221	379	663	630			M	0.023	0.104	CONTROL	spring 91	0	
9008227	377	612	575			M	0.007	0.068	CONTROL	spring 91	0	
9008206	395	720	685			M	0.007	0.076	CONTROL	spring 91	0	
9008217	420	963	811			F	0.003	0.026	CONTROL	spring 91	0	
9008224	419	917	876			F	0.019	0.076	CONTROL	spring 91	0	
9008217	420	963	811			F	0.003	0.026	CONTROL	spring 91	0	
9008243	420	880	790			F	0.008	0.034	CONTROL	spring 91	0	
9008202	421	837	761			F	0.003	0.044	A	spring 91	10	
9008203	360	581	536			M	0.007	0.084	A	spring 91	10	
9008207	425	843	785			F	0.000	0.025	A	spring 91	10	
9008208	372	597	561			F	0.011	0.102	A	spring 91	10	
9008205	366	606	574			F	0.014	0.064	B	spring 91	30	
9008210	414	765	655			F	0.008	0.074	B	spring 91	30	
9008229	407	853	741			F	0.022	0.073	C	spring 91	100	
9008209	407	813	750			M	0.020	0.137	C	spring 91	100	
9008201	391	590	549			M	0.006	0.050	C	spring 91	100	0.02137
9008215	375	588	550			M	0.082	0.194	C	spring 91	100	0.05979

SAMPLE NUMBER	Fork Length	Whole Weight	Gutted Weight	Liver Weight	Gonad Weight	Sex	EROD	AHH	Injection Code	Injection period	Injection Conc. ug/kg	ugPCB/g wet wt
9008216	392	781	716			M	0.033	0.143	D	spring 91	300	0.95480
9008232	389	721	659			M	0.128	0.249	D	spring 91	300	0.96066
9008200	417	859	741	7.0	83.0	F	0.016	0.094	D	spring 91	300	
9008212	428	845	783			F	0.008	0.095	D	spring 91	300	0.05978
9008226	411	881	747			F	0.028	0.134	D	spring 91	300	0.15756
9008233	373	596	560			F	0.002	0.020	D	spring 91	300	0.13462
9008234	415	909	779			F	0.004	0.034	D	spring 91	300	1.00975
9008204	339	475	444			M	0.093	0.145	E	spring 91	1000	1.77586
9008220	355	549	518			M	0.409	0.401	E	spring 91	1000	0.14529
9008228	358	525	488			M	0.174	0.324	E	spring 91	1000	2.31882
9008230	421	878	767			F	0.138	0.189	E	spring 91	1000	0.42421
9008213	394	731	676			F	0.617	0.678	E	spring 91	1000	0.78117
9008211	415	860	736			F	0.072	0.162	E	spring 91	1000	0.86638
9008218	425	936	804			F	0.431	0.447	E	spring 91	1000	1.33652
9008248	403	725	670			M	0.020	0.096	10 DAY CTR	spring 91	0	
9008249	396	722	671			M	0.006	0.049	10 DAY CTR	spring 91	0	
9008253	366	590	542			M	0.009	0.055	10 DAY CTR	spring 91	0	
9008257	410	724	684			M	0.010	0.048	10 DAY CTR	spring 91	0	
9008263	390	607	568			M	0.004	0.039	10 DAY CTR	spring 91	0	
9008251	422	990	860			F	0.005	0.049	10 DAY CTR	spring 91	0	
9008252	388	717	612			F	0.003	0.022	10 DAY CTR	spring 91	0	
9008261	435	1019	898			F	0.002	0.038	10 DAY CTR	spring 91	0	
9008256	425	921	800			F	0.006	0.030	10 DAY CTR	spring 91	0	
9008254	369	583	535			M	0.018	0.055	10 DAY INJ	spring 91	100	
9008247	344	479	441			M	0.028	0.102	10 DAY INJ	spring 91	100	
9008250	392	690	635			M	0.015	0.094	10 DAY INJ	spring 91	100	
9008259	400	835	766			M	0.017	0.076	10 DAY INJ	spring 91	100	
9008246	452	1070	915			F	0.007	0.049	10 DAY INJ	spring 91	100	
9008255	413	867	758			F	0.013	0.083	10 DAY INJ	spring 91	100	
9008258	414	839	694			F	0.038	0.116	10 DAY INJ	spring 91	100	
9008260	386	635	586			M	0.039	0.125	10 DAY INJ	spring 91	100	
9008262	435	1077	901			F	0.009	0.039	10 DAY INJ	spring 91	100	
9008310	360	639	586	5.9	22.2	M	0.001	0.036	CONTROL	fall 91	0	
9008309	419	922	820	7.5	32.2	F	0.007	0.045	CONTROL	fall 91	0	
9008303	445	1037	937		39.1	F	0.004	0.037	CONTROL	fall 91	0	
9008321	341	570	508	4.0	17.0	M	0.007	0.106	A	fall 91	10	
9008319	360	610	551	5.1	18.3	M	0.014	0.074	A	fall 91	10	
9008302	346	529	480	4.2	12.4	M	0.004	0.053	B	fall 91	30	
9008322	396	838	742	6.0	39.0	M	0.002	0.062	B	fall 91	30	
9008304	357	578	517	3.7	17.2	M	0.008	0.057	C	fall 91	100	
9008305	338	477	437	3.7	0.0	I	0.014	0.120	C	fall 91	100	
9008312	383	736	670	6.8		F	0.007	0.065	C	fall 91	100	
9008324	363	725	675	8.2	29.2	F	0.006	0.061	C	fall 91	100	
9008306	335	544	506	4.0	0.0	I	0.004	0.057	D	fall 91	300	0.47746
9008311	395	743	671	8.6	7.4	M	0.002	0.032	D	fall 91	300	0.38383
9008301	384	713	646	6.1	21.8	F	0.008	0.078	D	fall 91	300	0.44993
9008323	392	906	808	9.2	33.0	F	0.051	0.131	D	fall 91	300	0.04878
9008325	399	826	751	8.5	27.9	F	0.013	0.077	D	fall 91	300	0.52184
9008318	398	796	713	7.3	34.1	F	0.020	0.073	D	fall 91	300	0.04226
9008307	370	620	559	3.9	15.9	F	0.044	0.177	E	fall 91	1000	1.38496
9008314	420	831	758	6.8	28.0	F	0.058	0.163	E	fall 91	1000	2.47881
9008316	403	651	602	3.8		F	0.067	0.177	E	fall 91	1000	1.64466
9008317	382	721	634	8.3	25.0	F	0.312	0.296	E	fall 91	1000	0.58666

SAMPLE NUMBER	Fork Length	Whole Weight	Gutted Weight	Liver Weight	Gonad Weight	Sex	EROD	AHH	Injection Code	Injection period	Injection Conc. ug/kg	ugPCB/g wet wt
9008320	404	764	675	6.5	25.7	F	0.308	0.364	E	fall 91	1000	0.32080