THE EFFECTS OF SMOLTIFICATION AND ENVIRONMENTAL SALINITY ON THE UPTAKE, DISTRIBUTION, AND METABOLISM OF BENZO[A]PYRENE IN JUVENILE COHO SALMON (*ONCORHYNCHUS KISUTCH*)

by

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ABSTRACT

This study examined the effects of parr-smolt transformation on the uptake, distribution, and metabolism of benzo[a]pyrene (BaP) in juvenile coho salmon (*Oncorhynchus kisutch*). In addition, the effects of environmental salinity on the uptake, distribution, and metabolism of BaP were investigated in juvenile coho salmon during smoltification. The rates of BaP uptake at an initial concentration of 5 μg/L increased during smoltification which corresponded to an increase in oxygen consumption rates. During the initial sampling period (February) the highest levels of BaP or its metabolites were found in the skin and, in the following months, in the liver (March) and the bile (May and June). The fish metabolized BaP to both Phase I and Phase II metabolites, however, the proportions of each metabolite class were not affected by the parr-smolt transformation. HPLC analysis of organic-soluble metabolites indicated that the levels of 7,8-dihydrodiol BaP decreased and the levels of 3-OH and 9-OH increased during smoltification. These changes corresponded with a decrease in the amount of BaP-DNA adducts formed in the liver as smoltification progressed.

Uptake rates of BaP decreased with an increase in environmental salinity during smoltification but the rates of oxygen consumption did not show the same pattern. The levels of BaP or its metabolites in the skin, gill and intestine decreased with an increase in salinity. However, the levels of BaP or its metabolites in the bile increased with an increase in salinity. Metabolic patterns of BaP were not affected by environmental salinity. The proportions of Phase I and Phase II metabolites did not show significant differences between salinity treatments and only the proportion of 9,10-dihydrodiol of all organic-soluble metabolites was altered by environmental salinity. In conclusion, the toxicokinetics of xenobiotics such as BaP can be modulated by an organism's developmental stage (smoltification) and its environment (salinity).

DEDICATION

This thesis is dedicated to my mother, Janina

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LIST OF ABBREVIATIONS

BaP	benzo[a]pyrene
Ci	curie
dpm	disintegrations per minute
EDTA	ethylenediamine-tetraacetic acid
g	acceleration of gravity
g	gram
h	hour
HPLC	high performance liquid chromatography
Hg	mercury
i.p.	intraperitoneal
L	litre
LSC	liquid scintillation counting
M	molar
min	minute
mm	millimeter
N	normal
nm	nanometer
O_2	oxygen
PAH	polycyclic aromatic hydrocarbons
pg	picogram
S.E.	standard error
U	unit
v/v	volume to volume
w/w	weight to weight

GENERAL INTRODUCTION

Many species of Pacific salmon and trout (genus *Oncorhynchus*) including coho salmon are well known for their anadromous life cycles. Born in freshwater, they spend most of their lives in the ocean, then they return to their natal streams to spawn and die. They are adapted through an evolutionary history to both freshwater and the marine environment. During early development from egg to fry, freshwater ecosystems provide a relatively protected environment but nutritional limitations force the fish to migrate to nutrient-rich oceanic ecosystems (Pearcy, 1992). In the marine system, fish grow fast and mature rapidly, eventually returning to their freshwater streams to spawn.

Salmonids have been an important food source to the inhabitants of the Pacific rim countries and an important part of freshwater and marine ecosystems (Pearcy, 1992). In addition, for the past century, they have supported vast commercial and recreational fisheries becoming an important economic determinant in the North Pacific region. However, due to intensive human activities in recent years including overfishing, habitat alteration, and water pollution, the numbers of returning populations of salmonids have decreased and even disappeared (Pearcy, 1992).

It has been known that freshwater and marine environments act as ultimate sinks for natural and anthropogenic chemical pollution (Ramade, 1987). Geochemical phenomena, soil erosion, and particulate precipitation

result in the majority of natural and man-made pollutants entering the hydrosphere. Anthropogenic sources of water pollution include untreated or inadequately treated sewage, excessive nutrient loads, suspended solids, elevated temperatures, and a variety of toxic chemicals particularly metals, chlorine, cyanides, pesticides, polychlorinated biphenyls and petroleum hydrocarbons (Heath, 1995).

A small fraction of petroleum hydrocarbons is represented by the polycyclic aromatic hydrocarbons (PAHs) which are released into the environment by natural processes such as forest and grass fires as well as from a variety of anthropogenic activities including burning of fossil fuels (Klaassen, 1986). Incomplete combustion of carbonaceous materials results in formation of 4 to 6 ring large aromatic hydrocarbons which are highly hydrophobic and are accumulated by the aquatic organisms rapidly in direct relationship to the number of aromatic rings present (Anderson, 1979). These compounds are also extensively biotransformed by the tissues of many vertebrates, particularly in the liver (Gelboin, 1980) to render them more water soluble and thus easier to excrete. Some of the high molecular weight aromatic hydrocarbons can be bioactivated during the enzymatic biotransformation and their metabolites have shown a carcinogenic potential in mammals and in fish (Klaassen, 1986, Varanasi et al., 1989). One of these compounds, benzo[a]pyrene (BaP), has been linked to the formation of hepatic lesions and tumors in benthic fish such as English sole (Varanasi et al., 1989) and has been extensively studied in a variety of freshwater and marine fish. The vast amount of information on the

uptake, distribution, metabolism and excretion of BaP available in the literature makes BaP an excellent model compound for studying the fate and effects of chemicals on fish.

The toxicity of a chemical to an organism is primarily dependent on the exposure concentration, exposure duration, the type of chemical in question and the final concentration at the target site (Klaassen, 1986). The concentration reaching target site depends on chemical toxicokinetics which includes its absorption, distribution, metabolism, and excretion. The process of absorption for hydrophobic compounds in aquatic organisms has been linked to their passive diffusion based on partition coefficients, diffusional resistance of unstirred layers of water associated with the respiratory epithelium in fish, and the size and shape of the molecule (Kennedy, 1995). Following absorption, the chemical may be distributed to the site of toxic action, be transferred to a storage depot, or transported to organs that will detoxify, activate or eliminate the chemical (Riviere, 1994). The process of distribution takes place via convection systems such as the blood and is determined by the blood flow through an organ and the lipid solubility of the chemical. Some chemicals, including BaP and its metabolites, may be associated with plasma proteins such as albumin and lipoproteins (Heath, 1995) to facilitate their transport in an aqueous medium.

The enzymatic conversions of a chemical into another form may also take place in an aquatic organism and are termed biotransformation. The rates at which a compound is biotransformed will affect the bioaccumulation,

persistence, and toxicity of that chemical to fish (Kennedy, 1995) as biotransformation alters the concentration of the chemical in the blood and other tissues, as well as it affects chemical half-life and toxicity. The metabolism of xenobiotics usually occurs via Phase I and Phase II enzymatic reactions. The former introduces or unmasks polar groups onto a molecule by chemical oxidation, reduction or hydrolysis, and the latter results in formation of highly water-soluble conjugates coupled with endogenous compounds including sulfate, glucuronic acid, glutathione, or a variety of amino acids (Bend and James, 1978; Hodgson and Levi, 1994).

Elimination of a chemical can take place via urine, skin, bile and across the gills in aquatic species (Bend and James, 1978; Heath, 1995). The chemical can be excreted as a parent compound or as metabolites depending on its physicochemical properties, physiological constraints and environmental variables (Kennedy, 1995). Smaller water-soluble compounds are usually excreted by the kidney in addition to a variety of water-soluble metabolites and larger lipid-soluble compounds and polar metabolites are eliminated in the bile as reported for many species of fish (Bend and James, 1978). The elimination via the gill is limited and has been shown mostly for weakly basic lipid-soluble compounds such as the anaesthetic ethyl *m*-aminobenzoate (MS-222) (Bend and James, 1978).

A variety of factors may affect the toxicokinetics of xenobiotics in fish which include environmental factors such as temperature, oxygen availability water pH, or salinity (Heath, 1995). It has been shown that the rates of uptake,

metabolism and elimination of BaP by bluegill sunfish (*Lepomis macrochirus*) increased with an increase in temperature (Jimenez et al., 1987) as did the activity of mixed-function oxidase system (MFO) in bluegill sunfish, (*Lepomis macrochirus*) (Jimenez and Burtis, 1989) Black et al. (1991) reported that, as the experimental temperature was lowered, oxygen and BaP uptake efficiencies decreased in rainbow trout (*Oncorhynchus mykiss*). Thomas and Rice (1986) have found that metabolism of toluene increased in Dolly Varden char (*Salvelinus malma*) with temperature and resulted in a smaller body burden of several tissues. In addition, the elimination of BaP and its metabolites to the bile increased with an increase in acclimation temperature in gulf toadfish, (*Opsanus beta*) (Kennedy et al., 1989a).

Dissolved oxygen levels and pH have also been implicated as factors contributing to changes in toxicokinetics of chemicals. It was reported by McKim and Goeden (1982) that the uptake of endrin across the gills of brook trout (*Salvelinus fontinalis*) increased as the water oxygen concentrations decreased. A decrease in oxygen saturation increased the uptake and toxicity of pulp mill effluent to juvenile salmonids as reported by Alderdice and Brett (1957). The uptake of 3-trifluoromethyl-4-nitrophenol (TFM) by rainbow trout (*Oncorhynchus mykiss*) decreased with an increase in pH, at which more TFM was ionized (Hunn and Allen, 1974). A similar relationship has been shown by Saarikoski et al. (1986) for a variety of phenols and carboxylic acids in the guppy (*Poecilia reticulata*) at different water pH.

Environmental salinity may also modulate the toxicokinetics of chemicals. For example, it has been shown that the accumulation and elimination of pentachlorophenol (PCP) in killifish (*Fundulus heteroclitus*) has been altered by seawater exposure (Tachikawa et al. 1991). Thomas and Rice (1981) reported slower metabolism and an increased accumulation of naphthalene in Dolly Varden char (*Salvelinus malma*) in seawater than in freshwater. Killifish (*Fundulus heteroclitus*) exposed to naphthalene at higher salinities showed an increase in the rates of uptake of the hydrocarbon caused by osmoregulatory dysfunction (Levitan and Taylor, 1979).

Similarly, the general health status, energy requirements and developmental stage of fish may affect the susceptibility of organisms to stress caused by variety of factors including toxicants (Fagerlund et al., 1995). Sprague (1985) reported an increase in toxicity of pesticides such as chlordane when protein content of fish food decreased. Life stage and size of fish appear to have an effect on toxicokinetics of chemicals in aquatic organisms. Stegeman et al. (1984) have shown that BaP was metabolized more efficiently by killifish adult (*Fundulus heteroclitus*) than embryos which also produced significant amounts of proximate mutagen and carcinogen (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (7,8-dihyrodiol BaP). Kumaraguru and Beamish (1981) observed that the uptake and toxicity of Permethrin to rainbow trout (*Oncorhynchus mykiss*) was greater in juveniles than in adult fish which was explained by a higher metabolic rate in smaller fish which led to a higher uptake of the pesticide and higher efficiency of detoxification enzymes such as

MFO enzymes in adult fish. The investigations on the effects of toluene, naphthalene, and water-soluble fractions of Cook Inlet crude oil on pink salmon fry (*Oncorhynchus gorbuscha*) indicated that the fish increased their oxygen consumption rates and metabolic rate to metabolize and excrete the aromatic hydrocarbons which resulted in decreased feeding and growth (Thomas and Rice, 1979; Moles and Rice, 1983). In addition, it has been implicated that the process of smoltification, encompassing a variety of morphological, physiological and biochemical changes which adapt the freshwater fish to marine environment, is a factor altering the toxicity of metals such as copper in juvenile salmonids (Lorz and McPherson, 1976; Beckman and Zaugg, 1988). Specifically, the activity of the osmoregulatory enzyme Na^+,K^+-ATPase in the gill of coho salmon (*Oncorhynchus kisutch*) decreased in seawater-migrating smolts as compared to freshwater parr. In salmonids, seawater-acclimated outmigrants of pink salmon, sockeye salmon and Dolly Varden char (*Salvelinus malma*) were more sensitive to the water soluble fraction of Prudhoe Bay crude oil than freshwater-acclimated outmigrants (Moles et al., 1979) possibly due to differential distribution and accumulation of these compounds.

The objective of this study was to determine the effects of developmental modulator such as smoltification and the environmental factor salinity on the uptake, distribution, and metabolism of a model xenobiotic benzo[a]pyrene in juvenile coho salmon.

PART I. The effects of smoltification on the toxicokinetics of benzo[a]pyrene

INTRODUCTION

Many members of the genus *Oncorhynchus* and *Salmo* are anadromous species which spend the early part of their life cycle in freshwater rivers and streams. After hatching, the development of young salmonids from the fry to freshwater parr stage may take from two months to two years, depending on the species and the location of the parent stream. Following that, the fish undergo various morphological, physiological, and behavioural changes which occur simultaneously and adapt them for residence in the marine environment. The transformation of stream-dwelling coho parr to seaward-migrating smolts usually occurs in spring and the fish begin their migration by June (Folmar and Dickhoff, 1980; Boeuf, 1992).

Characteristic changes in body shape and coloration have been observed during the transformation of parr to smolts, also known as smoltification (Fessler and Wagner, 1969; Folmar and Dickhoff, 1980). Darkly pigmented bars on the lateral surface of parr become less visible as smoltification progresses. Due to increased growth and a decrease in condition factor (a ratio of weight to fork length), silvery smolts are more slender and streamlined in appearance than parr (Boeuf, 1992). The appearance of teeth and other changes in morphological characteristics such as alterations in fin

coloration, head shape, scale attachment, and reduction in skin mucus have also been observed (Hoar, 1976). Behavioural changes include a decrease in territorial behaviour and aggregation into schools, reduced swimming ability, reduced feeding, an increase in salinity preference and migration to sea water (Kutty and Saunders, 1973; Folmar and Dickhoff, 1980). These fish migrate downstream passively relying on river and tidal currents, and depending upon phylogeny, preferentially at night.

Apart from these general morphological and behavioural aspects, the parr-smolt transformation involves many physiological changes as well. Many studies have shown that during smoltification the metabolic rate of smolts increases as compared to that of parr and energy utilization by the fish is enhanced (Higgins, 1985; Maxime et al., 1989; Wiggs et al.,1989). McKeown (1984) and Maxime et al. (1989) have linked the increase in metabolic rate to an increase in oxygen consumption rate in smolts due to greater respiratory enzyme activity. Specifically, the activities of enzymes of the electron transport chain such as succinic dehydrogenase, citrate synthase, and cytochrome-c oxidase are increased during the transformation (Sargent et al., 1975).

It has been reported that, due to a changing internal environment, smolts reduce their feeding rates and their metabolism shifts to a catabolic pattern (McKeown, 1984). Malicova's (1959) study on Baltic salmon has found that a sharp decrease in body fat content (up to 60 %) is observed during the transition of parr to smolts. Depletion of total body lipids and glycogen in the liver and muscle during smoltification has been reported in a number of

salmonid species (Fessler and Wagner, 1969; McKeown, 1984). There is an extensive alteration in lipid metabolism accompanying smoltification (Sheridan et al. 1985; Sheridan, 1989). Total lipid concentration of various tissues (the mesenteric fat, red muscle, white muscle, and liver) decreases significantly, together with the total mass of lipids in the adipose tissue. In addition, an analysis of the fatty acid composition of several lipid classes indicated that freshwater parr contained a higher proportion of saturated fatty acids and a low proportion of unsaturated fatty acids. This pattern is reversed in smolts in which the long-chain polyunsaturated fatty acids increase in dominance (Sheridan, 1989, Sweeting, 1989). McKeown (1984) suggested that lipids may be involved in growth and protein synthesis or may be used as fuel for increased activity during migration.

Changes in protein metabolism during smoltification include a decrease in total body protein by approximately 10 % (Fessler and Wagner, 1969), a decrease in serum proteins by approximately 15 % (Woo et al. 1978), and an increase in creatine content in the muscle (Cowey and Parry, 1963). Moreover, moisture content and mineral composition is also altered in smolts (Malikova, 1959) as a consequence of ionic changes.

The transition from freshwater to seawater requires the reversal of a net ion influx to a net ion efflux which is regulated primarily by the gills but also involves the kidney, gastrointestinal tract and urinary bladder (Folmar and Dickhoff, 1980). In several salmonid species, an increase in gill Na^+,K^+-ATPase activity occurs prior to seawater entry (McCormick et al., 1987). This enzyme is

responsible for generating the ionic and electrical gradients necessary for an increased net ion efflux across the gills in seawater. Mitochondria-rich chloride cells of the gill opercular epithelium, which are responsible for ion transport including active chloride secretion, increase in size, number, and activity during smoltification (Loretz et al., 1982).

Urine flow and water excretory rates of some smolts in freshwater decrease relative to those in parr and are due to a reduction in glomerular filtration rate by as much as 48 % (McCormick and Saunders, 1987). The urine is concentrated and Na^+ and Cl^- absorption in the urinary bladder is abolished (Loretz et al., 1982). In addition, intestinal osmoregulatory function assumes the seawater pattern of water re-absorption in smolts as compared to parr.

Other tissues undergo adaptive alterations during the parr-smolt transformation as well. For example, there are several histological changes in the size and activity of hepatic parenchymal cells (Bradley, 1989; Robertson and Bradley, 1991). Liver parenchymal cells of smolts exhibited a shift from a role in glycogen storage to glycogen utilization and became more active as compared to parr cells. As a result of a decrease in parenchymal cell volume, diffusion distances were reduced and volume of organelles such as Golgi bodies and smooth and rough endoplasmic reticulum have increased.

All the morphlogical, behavioural and physiological changes associated with smolting are influenced by environmental factors, particularly photoperiod and temperature, which act to synchronize both the nervous and endocrine systems of the fish (McKeown, 1984). These environmental factors stimulate

the release of growth hormone, prolactin, and thyroid hormones by the pituitary-thyroid axis which appears to be directly involved in controlling smoltification (Barron, 1986).

Knowledge of the smoltification process has its importance not only in basic fish physiology and fisheries management but also in other areas of science such as aquatic toxicology since the migration phenomenon may expose juvenile salmonids to common water pollutants such as polycyclic aromatic hydrocarbons (PAH). Moles et al. (1979) have reported an increased sensitivity of sockeye salmon, pink salmon and Dolly Varden smolts to water-soluble fraction of Prudhoe Bay crude oil and benzene as compared to parr. Moreover, the 96-h LC_{50} of copper for coho salmon smolts was lower (74 μg/L) than the 96-h LC_{50} of copper for coho salmon parr (60 μg/L) as reported in the study of Lorz and McPherson (1976). In addition, the same study indicated that acute and chronic exposures of coho salmon smolts to sublethal concentrations of copper shortly before migration had deleterious effects on the downstream movement of the fish and their survival in the seawater. The levels of Na^+,K^+-ATPase activity were decreased by the chronic exposure to copper more significantly in smolts than in parr. Similar results have been found by Beckman and Zaugg (1988) in chinook salmon smolts which have shown a decline in activity of the same enzyme of greater than 50 % within 24 h, as compared to parr. It was not determined, however, whether the change in the sensitivity of smolts to the toxicants is caused directly by changes in their uptake, metabolism

and excretion or is caused by the required changes in ionic and osmotic or other regulatory processes the fish must undergo to adapt to seawater.

Several changes that occur during the parr-smolt transformation may influence the toxicokinetics (absorption, distribution, metabolism, and excretion) of xenobiotics and increase the sensitivity of juvenile salmonids to pollutants. For example, higher oxygen consumption rates observed in smolts may affect the rates of xenobiotic uptake since the uptake mechanism of organic xenobiotics across fish gills is closely related to that of oxygen (Murphy and Murphy, 1971; Pärt, 1989). Changes in the composition of lipids in the cell and cell membranes in smolts as compared to parr from the predominance of saturated fatty acids to polyunsaturated fatty acids may alter membrane permeability and, consequently, affect the rates of diffusion of organic chemicals into, throughout and out of cells. In addition, a shift to a catabolic pattern of metabolism and the depletion of body lipids and glycogen in the liver and muscle during smoltification may alter the distribution and the amount of accumulated xenobiotics in various tissues. The reported increase in metabolic rate of smolts may also alter the biotransformation rates of contaminants in the fish. Specifically, the changes may be reflected in the type and the amounts of different metabolite classes such as Phase I and Phase II metabolites produced in the liver and other tissues. These metabolic alterations may include changes in the formation of specific reactive metabolites and subsequent DNA-adduct formation. Osmoregulatory changes in the gill, intestine and the reduction in the

glomerular filtration rates of the kidney may also affect xenobiotic excretion rates and half-life.

The first objective of this study was to examine the effects of smoltification on the uptake, distribution and metabolism of benzo[a]pyrene in juvenile coho salmon. The second objective was to determine whether any alterations in oxygen consumption rate occurred during smoltification and whether these changes were associated with corresponding changes in the uptake of BaP. The third objective was to examine alterations in BaP-DNA adduct formation in the liver during the parr-smolt transformation.

MATERIALS AND METHODS

General

Fish

Juvenile coho salmon (*Oncorhynchus kisutch,* 0+) were obtained on 12 separate dates from the Capilano Hatchery, North Vancouver, British Columbia during the period from February to June, 1994. Fish were maintained in flow-through, filtrated, dechlorinated municipal water of pH 6.7, O_2 saturation >95%, and hardness 5.2 to 6.0 mg/L $CaCO_3$. Fish were kept under a natural photoperiod and natural temperature regime (5-12°C) for 1 week prior to an experiment. During that time, fish were fed commercial salmon pellets (Biodiet, Warrenton, OR, USA) until 2 days before an experiment.

Chemicals

[1,3,6-^{3}H] Benzo[*a*]pyrene (BaP) (specific activity of 52 Ci/mmol) was purchased from NEN Research Products (Boston, MA, USA). Unlabelled BaP (>99% purity) was purchased from Sigma Chemical Company (St. Louis, MO, USA). BaP metabolite standards were obtained from the National Cancer Institute Chemical Repository, Midwest Research Institute (Kansas City, MO, USA). ß-glucuronidase (with no sulfatase activity) and sulfatase were purchased from Sigma Chemical Company (St. Louis, MO, USA). Sodium citrate, HPLC-grade methanol and ethyl acetate were purchased from BDH Inc. (Toronto, ON, Canada). Sulfuric acid (1 N H_2SO_4) and 1 N sodium hydroxide

(NaOH) were obtained from Fisher Scientific (Napean, ON, Canada). Tricaine methane sulfonate (MS-222) was purchased from Syndel Laboratories Ltd. (Vancouver, B.C., Canada). Sodium bicarbonate ($NaHCO_3$) and sodium chloride (NaCl) were purchased from Sigma Chemical Company (St. Louis, MO, USA).

A. Uptake of BaP

This experiment was performed to determine the effects of smolt status on benzo[a]pyrene (BaP) uptake in coho salmon as the fish undergo the parr-smolt transformation. The fish were collected on 12 different days from February to June and exposed to radiolabelled BaP as follows.

Exposure to BaP

Individual fish at each sampling time (February to June) were placed in 1-liter aerated Erlenmeyer flasks which were submerged in a waterbath to maintain a constant water temperature. The water temperature was adjusted for seasonal changes in the ambient water temperature registered at the hatchery. [^{3}H]BaP, dissolved in 1-2 drops of Mulgosen EL-719 (GAS Ltd., Manchester, United Kingdom) and 20 ml of water, was added to the water in the flasks via a glass syringe 2 hours after the addition of the fish. The initial concentration of BaP in the flask was 5 µg BaP/L (1 µCi [^{3}H]BaP/L) . All flasks, including the

control flasks, were placed behind a dark screen to reduce disturbance of the fish. No fish were added to the control flasks.

Sampling and analysis

BaP uptake rates were estimated as described by Kennedy et al. (1989a) as the inverse rate of disappearance of BaP-derived radioactivity from the water. Water samples (1.0 ml) were collected at 0, 0.5, 1, 2, 4, 5, 6, 8, and 24 hours following the addition of the chemical to the experimental flasks without disturbing the fish. A Biodegradable Counting Scintillant (BCS) (Amersham, Arlington Heights, IL, USA) was added to each water sample which was then dark adapted for at least 24 h and counted for total [^{3}H] radioactivity using a Beckman LS 3801 liquid scintillation counter (Beckman Instruments, Irvine, CA, USA) with a sample channel correction for quench. To ensure that no BaP metabolites had been excreted into the water, an additional water sample (15 ml) was collected at the end of each experiment and analyzed for BaP metabolites as follows: water samples were hydrolyzed by the addition of 1 N H_2SO_4 to pH 2.0 and incubated for 24 h in an 80°C water bath. Following the incubation, the samples were extracted 3 times with 15 ml of ethyl acetate. The combined extracts were evaporated under nitrogen and resuspended in 1 ml of methanol. BaP and its metabolites were separated by reverse-phase HPLC using a Hewlett Packard 1050 Series liquid chromatograph (Avondale, PA, USA) and a Perkin-Elmer Analytical PAH HC-ODS 0089-0716 column (25 cm x 0.5 cm; Norwalk, CT, USA), Hewlett Packard Programmable Fluorescence

Detector HP 1046 A (Excitation = 380 nm; Emission = 430 nm), and Hewlett Packard HP 3396 Series II Integrator. The method of Elnenaey and Schoor (1981) was followed in setting up the solvent system, the flow rates and concave gradient solvent delivery. Briefly, the gradient system consisted of a solvent A (90/10 methanol/water) and a solvent B (30/70 methanol/water). The flow rate was 0.6 ml/min and the concave gradient was calculated using the formula:

$$P(t) = P(i) + [\, P(f) - P(i) \,] \, (t / T)^N$$

where:

P(t) = A / A(A + B)% which is the percent of solvent A

P(i) is the initial percent of solvent A

P(f) is the final percent of solvent A

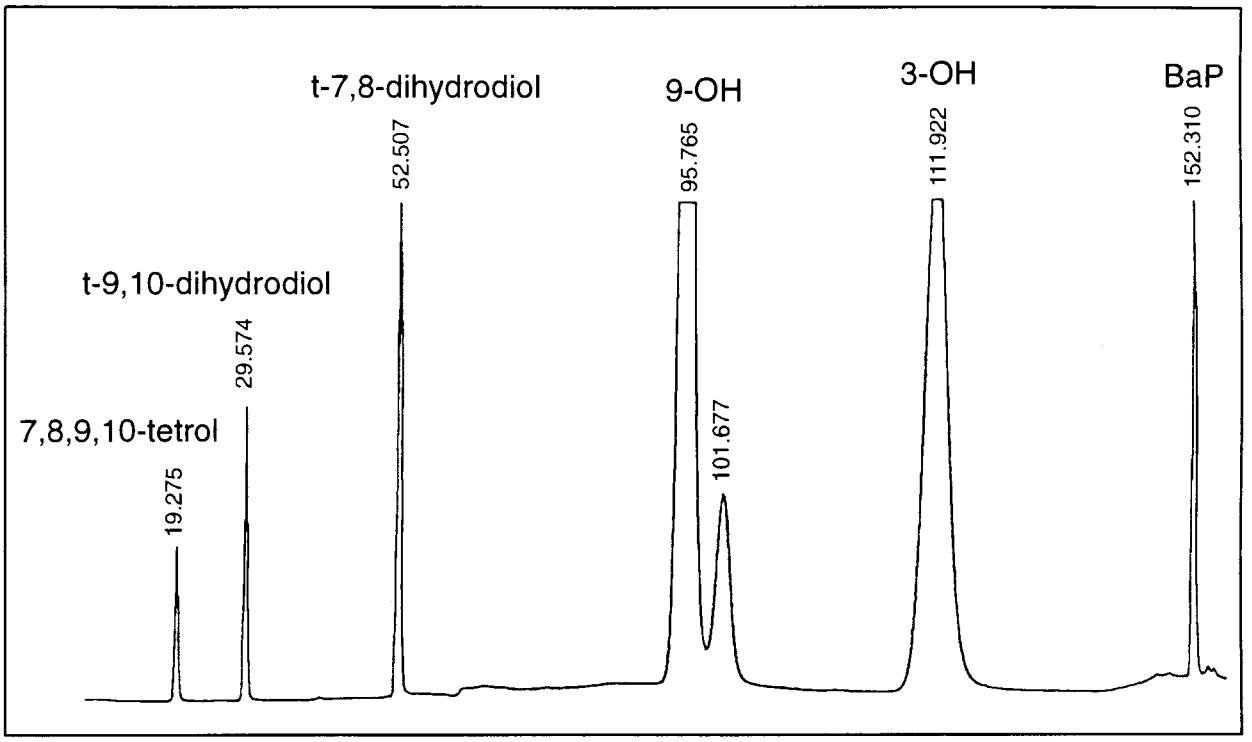
t is the elapsed time in the run (min)

T is the length of each time segment (min)

N is the gradient curvature or the degree of increase in solvent A

The gradient was changed as follows: solvent B was used to equilibrate the column for 20 min before each injection, followed by an increase in solvent A from 1-15% in 10 min of concave gradient (N=5), followed by 15-25% in a 15 min concave gradient (N=5), 30% for 15 min, 45% for 15 min, and 60% for 100 min. Total run time was 155 min. The identity of BaP or its metabolites was confirmed by comparing the retention times of recorded peaks with the retention times of known standards as shown in Figure 1.1.

Figure 1.1. A typical high performance liquid chromatograph of BaP and BaP metabolites separated using the method of Elnenaey and Schoor (1981).


7,8,9,10-tetrol
19.275
t-9,10-dihydrodiol
29.574
t-7,8-dihydrodiol
52.507
9-OH
95.765
101.677
3-OH
111.922
BaP
152.310

Calculations and statistical analysis

Uptake rates of BaP (µg BaP/g/h) by juvenile coho were calculated as described by Kennedy et al. (1989a) as the inverse of the disappearance of radioactivity from the water. The concentration of BaP in the water was plotted against time and fitted to an exponential curve using the CA - Cricket Graph III computer program (Computer Associates International Inc., Islandia, NY, USA). Uptake rates at a given time were estimated as the inverse of the first derivative of the fitted exponential curve with respect to the decreasing chemical concentration using the volume of the flask, specific activity of BaP and the weight of the fish. Uptake rates of BaP at the inital chemical concentration of 5 µg/L were calculated and compared statistically.

A one-way analysis of variance (ANOVA) and Student-Newman-Kuels multiple range comparison test (Zar, 1984) were used to test for significant differences in initial BaP uptake rates in fish sampled from February to June. Differences in BaP uptake rates between different sampling times were considered significant at $p < 0.05$.

B. Distribution of BaP

The objective of this experiment was to investigate possible changes in the distribution patterns of benzo[a]pyrene (BaP) in tissues as the fish undergo

the parr-smolt transformation. The fish were collected on 12 different days from February to June and exposed to radiolabelled BaP as described in section A.

Sampling and analysis

Tissue samples

Following a 24 h exposure to [^{3}H]BaP, fish were anaesthetized in 0.2 g/L MS-222 buffered with the same concentration of $NaHCO_3$ and sacrificed by cephalic blow, weighed and dissected. Whole organs including the liver, stomach, intestine, kidney, gills, brain and visceral fat were dissected and weighed. All tissues were homogenized using a Glas-Col homogenizer (Terre Haute, IL, USA) in 1 ml of 0.9% saline. In addition, a subsample of the skin taken between the pectoral and pelvic fins on one side of the body was dissected, rinsed and descaled before weighing and homogenizing in 1 ml of 0.9% NaCl. A large subsample of skeletal muscle taken from the same location was dissected, weighed and homogenized in 5 ml of 0.9% NaCl. Total skin and skeletal muscle mass of the fish was estimated using the method of Kennedy et al. (1989a) which involved stripping the skin and muscle of several carcasses, weighing them and comparing the tissue weight to the total body weight. This allowed for the calculation of the proportion of the total body weight which could be attributed to the skin and skeletal muscle. These values were used further in the estimation of the BaP-derived radioactivity content of these tissues. Aliquots of the tissue homogenates were oxidized in an OX 300 Biological Oxidizer followed by the addition of OX-162 Tritium Cocktail (R.J. Harvey Instruments

Corp., Hillsdale, NJ, USA) and counted for [^{3}H] radioactivity after dark-adapting the oxidized samples for 24 h.

Calculations and statistical analysis

A one-way analysis of variance (ANOVA) and Student-Newman-Kuels multiple range comparison test (Zar, 1984) were used to test for significant differences in the percent body burden of radioactivity in the tissues and in the [^{3}H]BaP-derived radioactivity content of examined tissues between sampling times from February to June. Percent data were arcsin transformed before statistical analysis was performed. Since the amount of radioactivity in each tissue could be attributed to both BaP and BaP metabolites, [^{3}H]BaP-derived radioactivity content was represented as μg BaP-equivalent / g of tissue using the specific activity of [^{3}H]BaP, and the amount of [^{3}H]BaP and BaP added. Differences in percent body burden and [^{3}H]BaP-derived radioactivity content in tissues between different sampling times were considered significant at $p < 0.05$. In all figures, standard error values smaller than 5 % of the mean could not be distinguished graphically.

C. Metabolism of BaP

The objective of this experiment was to investigate possible alterations in the metabolism of benzo[a]pyrene (BaP) during smoltification, specifically, the ability of fish to metabolize BaP via Phase I and Phase II biotransformation

reactions. The fish were collected on 12 different days from February to June and exposed to radiolabelled BaP as described in section A.

Sampling and analysis

Bile analysis

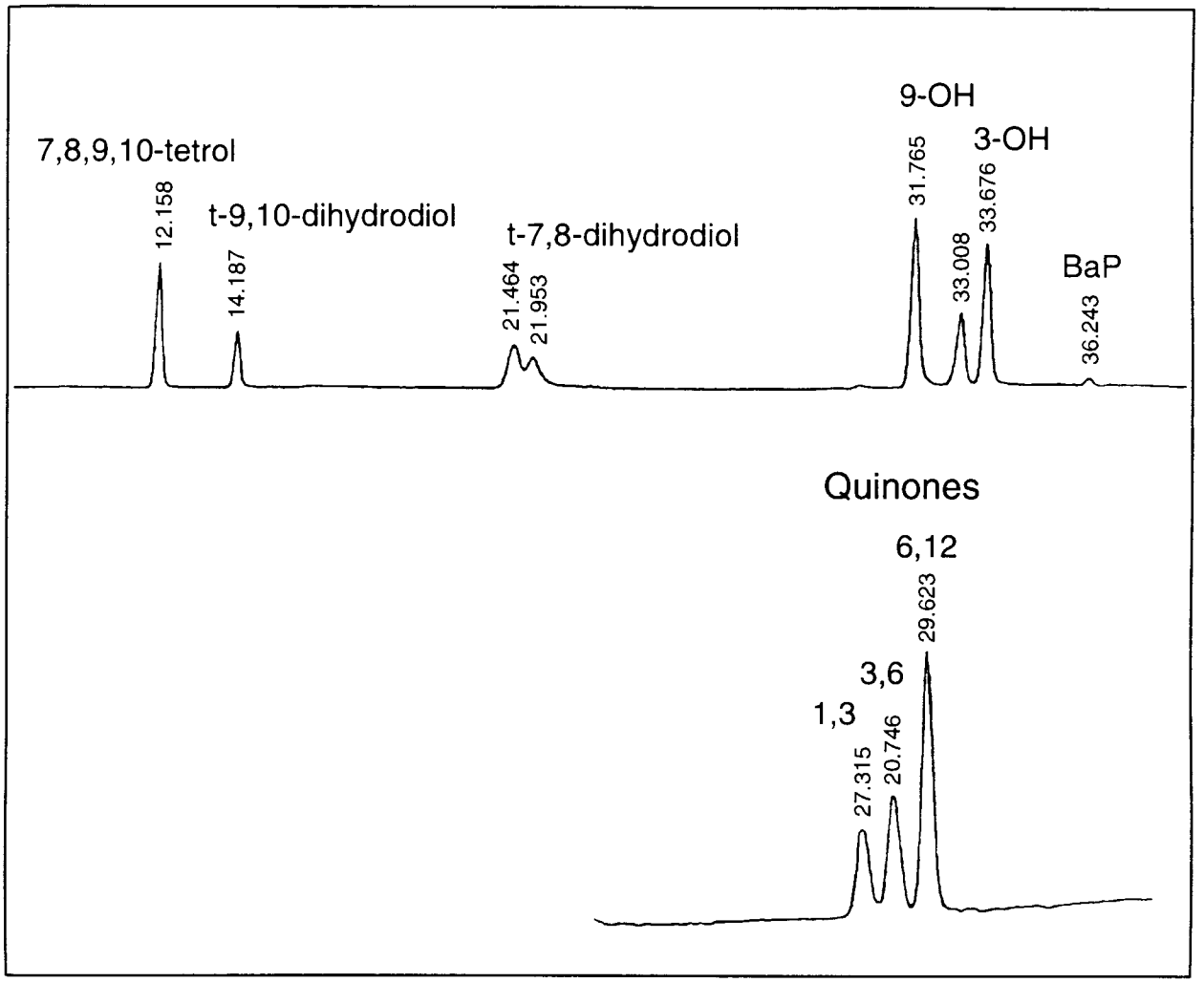
Following the 24 h exposure to [^{3}H]BaP, fish were anaesthesized and sacrificed as described in section B. Fish were weighed, and the gall bladder was removed and the bile released by perforation into a centrifuge tube followed by the addition of 1 ml 0.01 M sodium citrate buffer (pH 7.0). An aliquot of this bile solution was removed, mixed with 10 ml of BCS, and the vial dark adapted for 24 h before being counted for total [^{3}H] radioactivity. The remaining bile solution was extracted three times with 5 ml of ethyl acetate. Extraction included vortexing and shaking for 30 min on a reciprocating shaker followed by centrifugation for 5 min at 1500xg to separate the organic and aqueous layers. The extracted organic layers were combined and evaporated under a gentle stream of nitrogen, and resuspended in 1 ml of methanol for further analysis for BaP and Phase I metabolites by reverse-phase HPLC. A small aliquot was removed, mixed with 10 ml BCS, dark adapted for 24 h and counted for total [^{3}H] radioactivity. The remaining aqueous layer following ethyl acetate extraction was adjusted to pH 7.5 and incubated for 24 h at 37°C with 30 µl (2.5 U) of ß-glucuronidase to hydrolyze glucuronide conjugates. The solution was then extracted three times with 5 ml of ethyl acetate, mixed, shaken and centrifuged as described previously for Phase I metabolites. The organic

extracts, containing Phase I metabolites conjugated previously to glucuronic acid, were combined and evaporated under nitrogen, then resuspended in 1 ml of methanol. A small aliquot was removed, mixed with 10 ml of BCS, dark adapted for 24 h, and counted for total [^{3}H] radioactivity. The remaining aqueous layer was then adjusted to pH 7.0 and incubated for 24 h at 37°C with 50 µl (0.7 U) of sulfatase to hydrolyze sulfate conjugates. Following incubation, the solution was extracted three times with 5 ml of ethyl acetate and the organic and aqueous layers were separated by mixing, shaking and centrifuging the vials as described above. The organic extracts, containing Phase I metabolites conjugated previously to sulfate, were combined, evaporated under nitrogen, and resuspended in 1 ml of methanol. An aliquot was then removed from the solution, mixed with 10 ml of BCS, dark adapted for 24 h, and counted for total [^{3}H] radioactivity. The last step in the method was the acid hydrolysis of the remaining aqueous layer which was adjusted to pH 2.0 with 1 N H_2SO_4 and incubated for 24 h at 80°C. Following the incubation, the solution was extracted three times with ethyl acetate as described above. The resulting organic extracts were combined, evaporated under nitrogen, and resuspended in 1 ml of methanol. A small aliquot was removed, dark adapted for 24 h, and counted for total [^{3}H] radioactivity to quantify the remaining conjugates of BaP. The aqueous layer, containing unknown residues of BaP, was neutralized to pH 7.0 with 1 N NaOH to eliminate low pH effect on the scintillation counting process before being mixed with 10 ml of BCS, dark adapted for 24 h, and counted for total [^{3}H] radioactivity.

HPLC analysis of Phase I metabolites

Organic extracts collected after the first extraction of the bile which were resuspended in 1 ml of methanol were separated by reverse-phase HPLC using a Hewlett Packard 1050 Series liquid chromatograph (Avondale, PA, USA) equipped with a Phenomenex Prime Sphere MC 300A C-18 column (25 cm x 0.46 cm; Phenomenex, Torrance, CA, USA), Hewlett Packard Programmable Fluorescence Detector HP 1046 A (Excitation = 380 nm; Emission = 430 nm), Hewlett Packard HP 3396 Series II Integrator, and BioRad model 2110 Fraction Collector (BioRad Laboratories, Mississauga, ON, Canada). To shorten the time of separation of metabolite groups and the total length of the run, the linear gradient method of Gmur and Varanasi (1982) was followed and slightly modified in set up of the solvent system, the flow rates (1ml/min) and gradient solvent delivery. Starting at 80% solvent A (acetic acid:water, 0.5:99.5, v/v) and 20% solvent B (methanol), the gradient was changed from 20 to 60% B in 12.5 min, from 60 to 70% B in 12 min, and from 70 to 85% B in 10 min, form 85 to 90% B in 8 min, and from 90 to 100% B in 4.5 min. The final conditions were held for 8 min. The identity of BaP or its metabolites was confirmed by comparing recorded retention times with the retention times of known standards as shown in Figure 1.2. To determine the presence and retention times of BaP quinones which absorbance is maximized in the visible range of the light spectrum, the standards were run using a

Figure 1.2. A typical high performance liquid chromatograph of BaP and BaP metabolites separated using the method of Gmur and Varanasi (1982).


7,8,9,10-tetrol
12.158
t-9,10-dihydrodiol
14.187
t-7,8-dihydrodiol
21.464
21.953
9-OH
31.765
3-OH
33.008
33.676
BaP
36.243
Quinones
6,12
29.623
3,6
1,3
27.315
20.746

fluorescence detector set at 430 nm for excitation and 480 nm for emission and running the same solvent gradient. The amount of BaP or its metabolites was estimated by collecting 1 min interval fractions and counting [^{3}H] radioactivity in the fractions by adding 5 ml of BCS and dark adapting for 24 h.

Calculations and statistical analysis

A one-way analysis of variance (ANOVA) and Student-Newman-Kuels multiple range comparison test (Zar, 1984) were used to test for significant differences in the proportions of several metabolite groups as a percent of the total bile metabolites and in individually identified and separated Phase I metabolites between the sampling times from February to June. Percent data were arcsin transformed before statistical analysis was performed. Results were considered significant at $p < 0.05$.

In all figures, standard error values smaller than 5 % of the mean could not be distinguished graphically.

D. BaP-DNA adduct formation

This experiment was performed to determine the potential of benzo[a]pyrene (BaP) to form DNA adducts in the liver of juvenile salmonids and to determine if alterations in adduct formation occurr when fish undergo the parr-smolt transformation.

Fish

Juvenile coho salmon (*Oncorhynchus kisutch,* 0+) were obtained biweekly from the Capilano Hatchery, North Vancouver, British Columbia during the period from February to June, 1996. Fish were maintained in flow-through, filtrated, dechlorinated municipal water of pH 6.7, O_2 saturation >95%, and hardness 5.1 to 5.9 mg/L $CaCO_3$. Fish were kept under a natural photoperiod and natural temperature regime (5-13°C) for 1 week prior to an experiment. During that time, fish were fed commercial salmon pellets (Biodiet, Warrenton, OR, USA) until 2 days before an experiment.

Chemicals

[1,3,6-^{3}H] Benzo[*a*]pyrene (specific activity of 52 Ci/mmol) was purchased from NEN Research Products (Boston, MA, USA). Unlabelled BaP (>99% purity) was purchased from Sigma Chemical Company (St. Louis, MO, USA). Sodium chloride (NaCl), Tris-Cl, sodium bicarbonate ($NaHCO_3$), sodium dodecyl sulfate (SDS), Proteinase K, RNase A, and the phenol:chloroform:isoamyl alcohol (25:24:1) mixture were purchased from Sigma Chemical Company (St. Louis, MO, USA). Disodium ethylenediamine tetraacetate (Na_2EDTA,) was purchased from Fisher Scientific (Nepean, ON, Canada). Tricaine methane sulfonate (MS-222) was purchased from Syndel Laboratories Ltd. (Vancouver, B.C., Canada).

Exposure to BaP

At each sampling time, individual fish were anaesthetized in 0.1 g/L MS-222 buffered with the same concentration of $NaHCO_3$ and were injected intraperitoneally (i.p.) with 10 mg/kg (1μCi/fish) [^{3}H]BaP. The stock solution contained 8 mg of BaP dissolved in 1-2 drops of Mulgosen EL-719 (GAS Ltd., Manchester, United Kingdom) and 8 ml of 0.9% NaCl. The fish were injected with 0.1 ml / 10 g wet weight of fish so that each fish in this experiment received the same dose. The i.p. mode of injection was chosen to eliminate possible effects of smoltification on the uptake of BaP by the gills. Control fish were injected i.p. with 0.1 ml / 10 g of wet weight of a solution containing 0.9% NaCl and Mulgosen EL-719. Injected fish were placed in large, aerated aquariums supplied with flow-through dechlorinated water for 48 h and placed behind a dark screen to reduce any disturbance to the fish.

Sampling and analysis

Following exposure, fish were sacrificed, weighed, and the livers removed and weighed. The hepatic DNA and BaP-DNA adducts were extracted and quantified following the method of Sambrook et al. (1989) with some modifications: the livers were homogenized individually by 3-4 hand strokes in 5 ml of a homogenization buffer (0.15 M NaCl, 10 mM Tris-Cl, 50 mM Na_2EDTA, pH 8.0) using a glass homogenization tube placed on ice. To remove proteins associated with the nucleic acids, SDS and Proteinase K solutions were added to the homogenates to a final concentration of 10 mg

SDS/ml of homogenate and 100 μg Proteinase K/ml of homogenate respectively. The homogenates were then incubated for 2 h at 65°C in a shaking water bath. To further denature the proteins and purify the nucleic acids, the samples were extracted three times with phenol:chloroform:isoamyl alcohol (25:24:1; v/v) until the aqueous layers were clean of debris. To remove RNA associated with the DNA, RNase A solution was added to the extracts to a final concentration in a sample of 50 μg/ml and the extracts were further incubated for 1 h at 37°C in a shaking water bath. Following the incubation, 100 μl of 15 M sodium acetate was added and the DNA was precipitated by the addition of 10 ml 100% ice cold ethanol. DNA was spooled onto a glass hook, rinsed with 70 % ethanol and air-dried at room temperature. The DNA was then resuspended in 1 ml of 10 mM Tris and 1 mM Na_2EDTA buffer (pH 8.0) and the amount of DNA extracted was quantified using a spectrophotometric method of Sambrook et al. (1989) using Beckman spectrophotometer model DU 650 (Beckman Instruments, Fullerton, CA, USA). The absorbance of a small aliquot containing the dissolved DNA was measured at 260 nm and 280 nm and the A_{260}/A_{280} ratio was calculated to assess the purity of DNA solution. Optical absorbance (OD) of 1 at 260 nm was equivalent to 50 μg/ml of DNA (Sambrook et al., 1989). The A_{260}/A_{280} ratio of 1.7 to 2.0 indicated DNA free of proteins and RNA (Sambrook et al., 1989). The amount of BAP-DNA adducts in the DNA was quantified by counting the [^{3}H] radioactivity in the remaining DNA solution following the addition of 10 ml BCS and dark adapting the samples for 24 h.

Calculations and statistical analysis

Significant differences in the levels of BaP-DNA adducts (pg BaP/μg hepatic DNA) in the livers of coho salmon between the sampling times were compared by a one-way analysis of variance (ANOVA) and Student-Newman-Kuels multiple range comparison test (Zar, 1984). Results were considered significant at $p < 0.05$.

E. The effects of smoltification on oxygen consumption rate

The objective of this experiment was to investigate the effects of changes in smolt status on oxygen consumption rate as the fish undergo smoltification. In addition, this study intended to correlate any changes in oxygen uptake with changes in the uptake of chemicals such as benzo[a]pyrene (BaP) by the fish during the same period of time.

Fish

Juvenile coho salmon (*Oncorhynchus kisutch,* 0+) were obtained on a monthly basis from the Capilano Hatchery, North Vancouver, British Columbia during the period from February to June, 1995. Fish were maintained in flow-through, filtrated, dechlorinated municipal water of pH 6.7, O_2 saturation >95%, and hardness 5.3 to 6.1 mg/L $CaCO_3$. Fish were kept under a natural photoperiod and natural temperature regime (4-11°C) for 1 week prior to an

experiment. During that time, fish were fed commercial salmon pellets (Biodiet, Warrenton, OR, USA) until 2 days before an experiment.

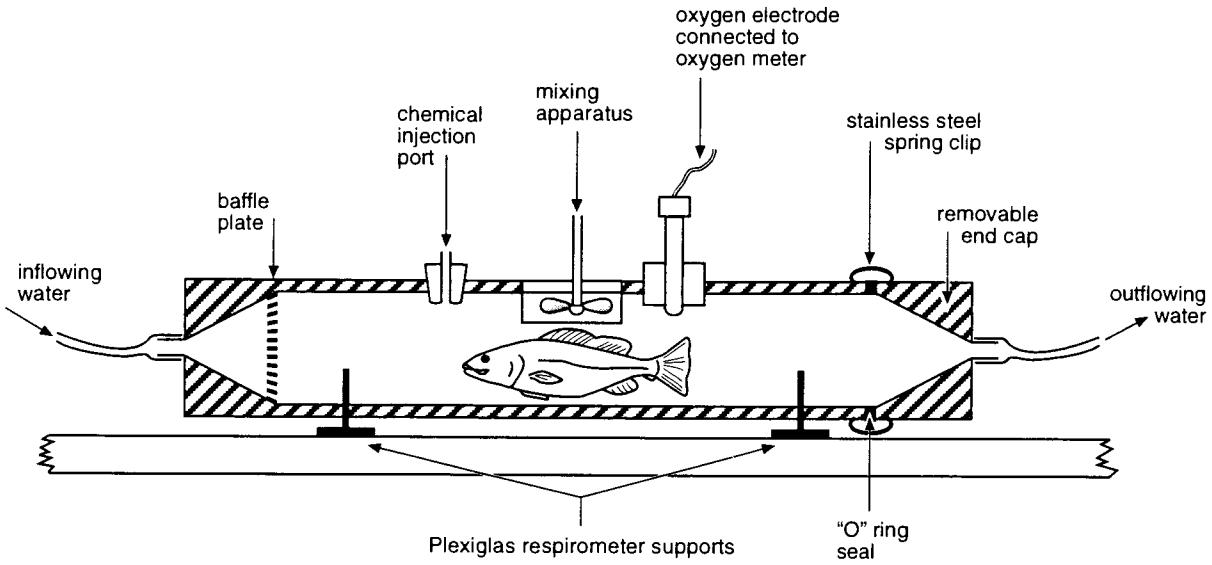
Chemicals

Benzo[a]pyrene (>99% purity) and 1,2 benz[a]anthracene (>99% purity) were purchased from Sigma Chemical Company (St. Louis, MO, USA). [1,3,6-^{3}H] Benzo[*a*]pyrene (BaP) (specific activity of 52 Ci/mmol) was purchased from NEN Research Products (Boston, MA, USA). HPLC-grade methanol, ethyl acetate and acetonitrile were purchased from BDH Inc. (Toronto, ON, Canada). HPLC-grade pentane and dichloromethane were obtained from Anachemia (Montreal, PQ, Canada). Potassium hydroxide (KOH) pellets and sodium chloride (NaCl) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Hydrochloric acid (12 N HCl) was purchased from BDH Inc. (Toronto, ON, Canada). Sulfuric acid (1 N H_2SO_4) was obtained from Fisher Scientific (Napean, ON, Canada).

Oxygen consumption measurements and exposure to BaP

At each sampling time (February 16, March 2, April 4, May 2, and June 9, 1995), 5 fish were placed in a 9-liter glass respirometer (Figure 1.3) which was submerged in a waterbath to maintain a constant water temperature. The respirometer was placed behind a dark screen to reduce disturbance of the fish. The water temperature in the water bath was adjusted to seasonal temperatures registered at the hatchery. The fish were kept in the respirometer for two days

Figure 1.3. A schematic representation of a respirometer used in determination of oxygen consumption rates and BaP uptake by the fish.


oxygen electrode
connected to
oxygen meter
mixing
apparatus
chemical
injection
port
stainless steel
spring clip
baffle
plate
removable
end cap
inflowing
water
outflowing
water
Plexiglas respirometer supports
"O" ring
seal

supplied with flow-through water prior to the addition of BaP. When oxygen consumption measurements were made, the water flow was stopped and BaP, dissolved 1-2 drops of in Mulgosen EL-719 (GAS Ltd., Manchester, United Kingdom) and water, was added to the chamber under constant pressure through a syringe. The initial concentration of the chemical in the chamber was 5 µg BaP/L. No chemical was added to the control run. As an additional control, bacterial oxygen consumption and any pressure changes unrelated to fish activity were estimated by measuring oxygen partial pressure in the chamber without fish in it. After the water flow through the respirometer was stopped and BaP was added to the water, the partial pressure of oxygen was measured continuously for 1 h using YSI model 58 oxygen electrode (Yellow Springs Inc., Yellow Springs, OH, USA) connected to a chart recorder (OmniScribe Series D5000 recorder, Houston Instruments, Houston, TX, USA). After 1 h, the measurements were stopped and fish were removed from the respirometer both to prevent the fish from entering hypoxia and to analyze BaP tissue content of the fish.

Sampling and analysis

Tissue BaP determination

To determine the tissue content of BaP, the fish were sacrificed by cephalic blow following the 1 h exposure, weighed and homogenized using a Glas-Col homogenizer (Terre Haute, IL, USA) in an appropriate amount of 0.9% NaCl solution (2 ml of NaCl / 1 g of tissue). Due to a low exposure

concentration of BaP, the sensitivity of BaP quantitiation was enhanced by a modified saponification procedure described by Lebo et al. (1991). In order to break down the lipids contained in the cells to free fatty acids and glycerol and to enhance the release of a lipid-associated BaP, homogenized fish were divided into approximately 5 g subsamples, spiked with the internal standard of 1,2 benz[a]anthracene (100 ng / g of homogenate) and were saponified with 3.0 ml concentrated KOH (50% w/w) and 10 ml methanol. The subsamples were incubated for 1.5 h at 80°C in a water bath and mixed every 30 min. The subsamples were then acidified to pH 5.9-6.1 using concentrated HCL, and incubated again for 1 h at 80°C. Following saponification, the homogenates were extracted three times with 20 ml of pentane to separate BaP from the formed soaps. A small amount of non-volatile isooctane (0.5 ml) was added to the combined organic extracts before the extracts were evaporated under nitrogen to prevent loss of BaP during evaporation. To remove long chain fatty acids and other compounds coextracted with BaP and avoid their interference during HPLC analysis, the resulting 0.5 ml samples were run through a silica gel column (230-400 mesh size, Sigma, St. Louis, MO, USA) filled up to 1.5 ml bed volume with a glass wool stopper. The samples were eluted twice with 0.5 ml pentane and three times with 0.5 ml dichloromethane, and each eluted 0.5 ml fraction was collected and evaporated under nitrogen. To determine which fractions contained eluted BaP, a sample of 0.5 ml containing 100 µl [^{3}H]BaP (0.01 µCi) and 400 µl isooctane was added to a test column and eluted in the same way by collecting 0.5 ml fractions, evaporating them under nitrogen,

resuspending in 1 ml of methanol, mixing with 5 ml of BCS, dark adapting for 24 h and counting for [^{3}H] radioactivity. Following that procedure, it was found that the last two dichloromethane fractions contained majority of [^{3}H]BaP (83% efficiency) and were thus collected from the samples and resuspended in 1 ml of methanol. All extracted and eluted 5 g subsamples from each fish were recombined into one, evaporated under nitrogen and resuspended in 1 ml of methanol and separated to determine BaP tissue content by HPLC using a Hewlett Packard 1050 Series liquid chromatograph (Avondale, PA, USA) equipped with a Phenomenex Prime Sphere MC 300A C-18 column (25 cm x 0.46 cm; Phenomenex, Torrance, CA, USA), Hewlett Packard Programmable Fluorescence Detector HP 1046 A (Excitation = 255 nm; Emission = 315 nm), and Hewlett Packard HP 3396 Series II Integrator. The samples were run using 90/10 acetonitrile/water as a solvent system at a constant flow of 0.5 ml/min for 10 minutes and an internal standard of 1,2 benz[a]anthracene.

Calculations

Recorded partial pressures of oxygen were converted into oxygen concentrations (mg O_2/L) using the formulas listed by Cech (1990) and oxygen solubility tables and water vapour pressure tables (Green and Carritt, 1967) using the following equations:

$$CO_2(ws) = [\ PO_2(ws) \times C\,O_2(as)\ ] / P\,O_2(as)$$

where:

$CO_2(ws)$ = O_2 concentration (ml O_2/L) in the water sample,

$PO_2(ws)$ = O_2 partial pressure (mm Hg) in the water sample,

$C\ O_2(as)$ = O_2 concentration in water at air saturation (from oxygen solubility table by Green and Carritt, 1967), and

$P\ O_2(as)$ = O_2 partial pressure at air saturation, calculated from equation:

$$P\ O_2(as) = 0.2094\ (\ Pb - Pwv\ )$$

where:

0.2094 = volumetric fraction of O_2 in the atmosphere,

Pb = total barometric pressure (mm Hg) (recorded each day of the experiment), and

Pwv = water vapour pressure at the experimental temperature (mm Hg) (from water vapour pressure table by Green and Carritt, 1967).

Oxygen concentrations in ml O_2/L were converted to mg O_2/L using equation of Cech (1990):

$$mg\ O_2/L = 1.428 \times ml\ O_2/L$$

Because water oxygen concentrations decreased initially (5-10 min) in a curvilinear fashion due to possible pressure changes in the chamber after closing the system (Morgan and Iwama, 1991), oxygen consumption rates were estimated over the linear portion of the measurements (40-50 min) as suggested by the same authors and by fitting a regression line through the recorded oxygen partial pressures. The regression slope was used as an estimate of oxygen consumption rates and was expressed in terms of wet weight of fish over a 1 h period (mg O_2/kg/h) after converting oxygen partial pressures (mm Hg) into oxygen concentrations (mg O_2/L) as described above.

The rates of bacterial oxygen consumption were subtracted to estimate the net oxygen consumption by the fish. These rates represented total oxygen consumption by the 5 fish in a chamber. Due to a small size of replicates, statistical analysis of the data was not performed.

RESULTS

A. Uptake of BaP

In investigating the effects of the parr-smolt transformation on the uptake of BaP by juvenile coho salmon, an experimental procedure following that of Kennedy et al. (1989a) was used. In their experiments, this procedure was used to determine the effects of temperature on the uptake, distribution, and metabolism of BaP in the gulf toadfish (*Opsanus beta*). By following the disappearance of the radioactivity from the water, it was confirmed in the present study that less than 5 % of the [^{3}H]BaP added to the control flasks was lost due to adsorption onto the glass walls or by volatilization (Figure 1.4). HPLC analysis of the 15 ml water samples, taken at the end of each experiment, revealed no BaP metabolites in the water indicating that no excretion of BaP metabolites by the fish had occurred eliminating the fish's possible re-exposure to the contaminant. These two results indicate that the disappearance of BaP from the water reflected the amount taken up into the fish which remained in the fish for the duration of the experiment.

The disappearance of radioactivity from the water displayed an exponential decline in all cases (Figure 1.4). Uptake rates of BaP appear to increase with time from February to June (Figure 1.5). At the initial chemical concentration of 5 µg BaP/L, the uptake rates of BaP on February 8, 22 and March 8 were significantly lower than the uptake rates at any other sampling time from March 22 through to June 21 (Figure 1.5). The rates of BaP uptake on

Figure 1.4. Typical time course of [^{3}H] radioactivity disappearance from the flasks containing coho salmon exposed for 24 h to [^{3}H]BaP (5 µg/L) (□). Control chambers (o) contained [^{3}H]BaP but no fish.

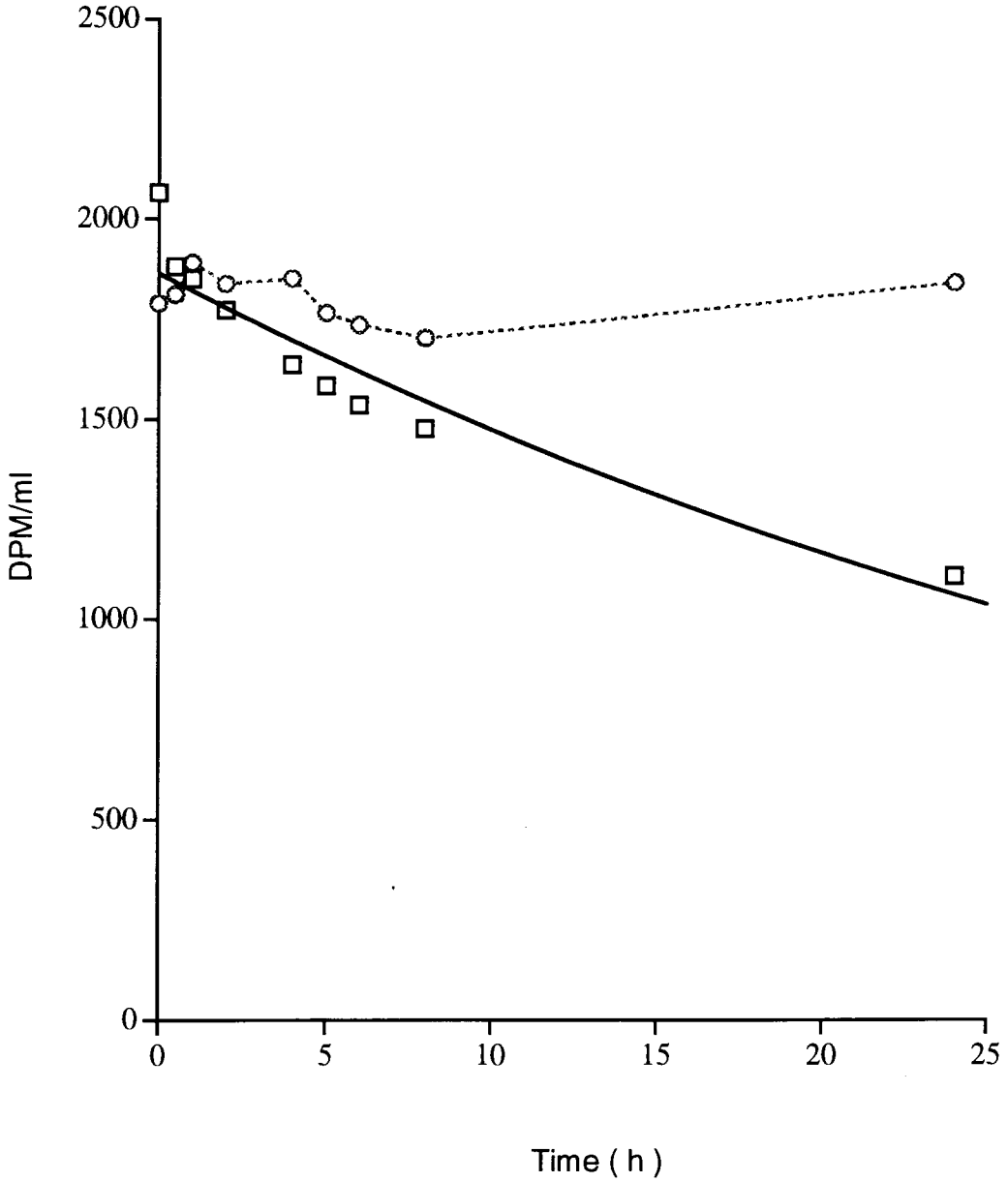
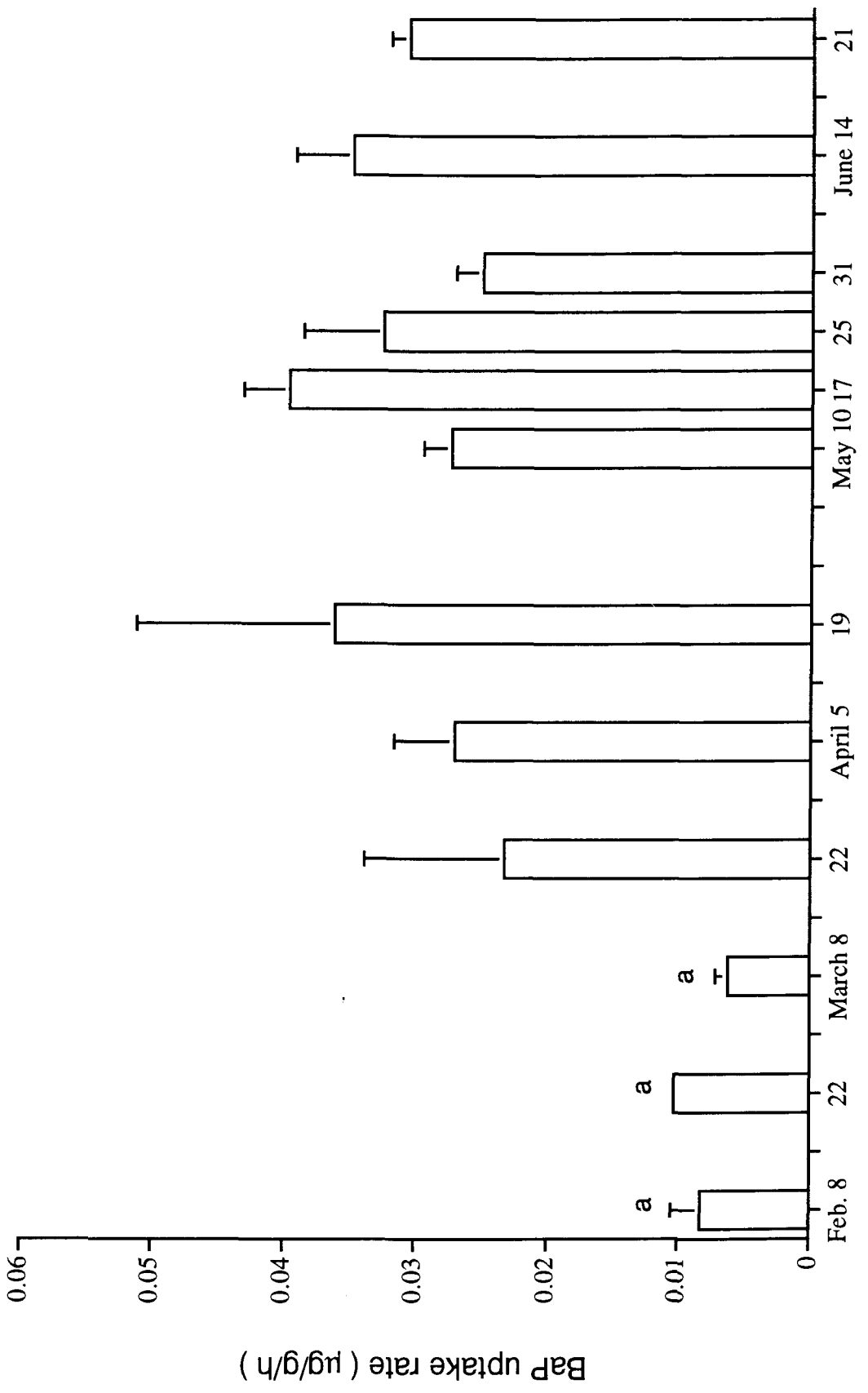

2500
2000
1500
1000
500
0
DPM/ml
0
5
10
15
20
25
Time (h)

Figure 1.5. Uptake rates of [^{3}H]BaP by coho salmon during the parr-smolt transformation from February to June at an initial BaP concentration of 5 μg (1 μCi)/L. Values are means ±SE for three fish. Values with common symbols are not significantly different between the sampling times at p <0.05.


BaP uptake rate (μg/g/h)
0.06
0.05
0.04
0.03
0.02
0.01
0
a
a
a
Feb. 8
22
March 8
22
April 5
19
May 10
17
25
31
June 14
21
Date

April 19 and May 17 were three to four fold higher than the rates of BaP uptake on February 8, 22 and March 8.

B. Distribution of BaP

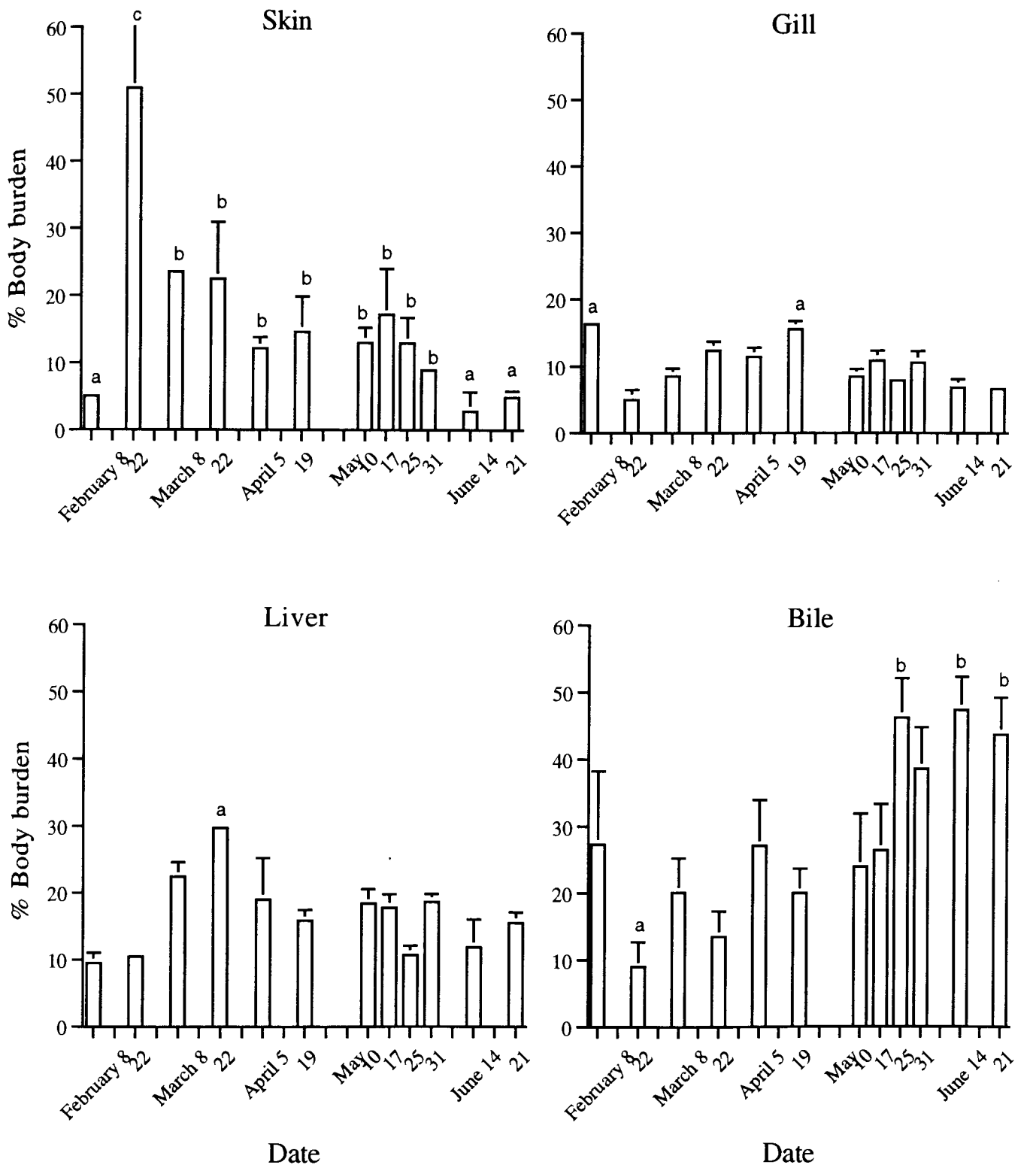
The analysis for BaP in the tissues of juvenile coho salmon during the parr-smolt transformation revealed that BaP was rapidly distributed after the initial uptake of the chemical. The bile had typically the highest percent body burden of [^{3}H]BaP-derived radioactivity (15 - 47 %) in the fish and the brain typically contained the lowest (0 - 0.9 %) The percent body burden of [^{3}H]BaP-derived radioactivity in tissues such as the brain, intestine, kidney, skeletal muscle, stomach and visceral fat did not differ significantly ($p<0.05$) between different sampling dates (Table 1.1). However, the percent body burden of [^{3}H]BaP-derived radioactivity in the skin, gill, liver and bile of coho salmon varied between sampling periods as illustrated in Figure 1.6. The percent body burden of [^{3}H]BaP-derived radioactivity in the skin decreased with time of sampling. Specifically, it was low on February 8, then increased ten-fold by the end of the month (February 22), and declined steadily from March 8 to June 21. Changes in the percent body burden in the gill did not appear to follow a clear pattern, however the highest accumulation of the chemical in the gill occurred on February 8 and April 19. The percent body burden in the liver increased in March when it reached 30 % and decreased in the following months.

The percent body burden of [^{3}H]BaP-derived radioactivity in the bile increased significantly with time. The transfer of [^{3}H]BaP between the liver and

Table 1.1. Percent body burden of total [^{3}H] radioactivity in tissues of coho salmon during the parr-smolt transformation. Values are the means (±SE) for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 µg (1 µCi)/L. There were no significant differences between the means of the percent body burden of these tissues between the sampling times at $p<0.05$.

Tissue	Date											
	Feb. 8	Feb. 22	Mar. 8	Mar. 22	Apr. 5	Apr. 19	May 10	May 17	May 25	May 31	Jun. 14	Jun. 21
Brain	0.14 (±0.01)	0.96 (±0.48)	0.32 (±0.18)	0.21 (±0.11)	0.21 (±0.21)	0.33 (±0.14)	0.45 (±0.28)	0.30 (±0.13)	0.27 (±0.03)	0.05 (±0.04)	0 (±0)	0.10 (±0.04)
Intestine	17.49 (±9.55)	6.51 (±4.29)	8.53 (±5.43)	16.69 (±5.37)	11.07 (±3.88)	17.15 (±7.01)	13.34 (±2.79)	16.27 (±2.84)	18.58 (±2.49)	20.48 (±3.33)	28.53 (±5.55)	19.37 (±2.73)
Kidney	2.22 (±0.80)	1.10 (±0.09)	1.26 (±0.10)	1.75 (±0.42)	1.80 (±0.45)	2.19 (±0.14)	3.03 (±0.58)	4.71 (±0.87)	1.05 (±0.14)	1.19 (±0.24)	1.13 (±0.22)	1.41 (±0.48)
Muscle	16.28 (±10.67)	11.39 (±10.67)	13.71 (±2.56)	4.97 (±2.70)	11.83 (±11.37)	6.17 (±4.74)	15.06 (±5.57)	4.43 (±2.18)	0 (±0)	0.62 (±0.62)	0.25 (±0.25)	7.32 (±4.71)
Stomach	0.98 (±0.67)	3.14 (±1.25)	0.83 (±0.20)	0.72 (±0.36)	1.85 (±0.89)	1.70 (±0.98)	1.25 (±0.80)	2.51 (±1.19)	0.44 (±0.25)	0.23 (±0.16)	0.20 (±0.12)	0.27 (±0.05)
Visceral fat	4.79 (±3.96)	1.52 (±1.26)	0.72 (±0.24)	1.86 (±1.34)	3.53 (±0.95)	6.36 (±3.25)	3.00 (±0.84)	1.95 (±1.27)	1.81 (±0.81)	0.66 (±0.45)	0.87 (±0.11)	0.63 (±0.14)

Figure 1.6. Percent body burden of BaP-derived radioactivity in coho skin, gill, liver, and bile during the parr-smolt transformation from February to June. Values are the means ±SE for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 μg (1 μCi)/L. Values with common symbols are not significantly different between the sampling times at p <0.05.


Skin
Gill
Liver
Bile
% Body burden
% Body burden
Date
Date
0
10
20
30
40
50
60
a
c
b
b
b
b
b
b
b
a
a
a
a
a
b
b
b
February 8
22
March 8
22
April 5
19
May 10
17
25
31
June 14
21

the bile described as the ratio of the concentration of radioactivity in the bile to that in the liver (Kennedy et al. 1989a), was lowest on March 22 (0.5) and increased to 4.3, 3.9 and 2.8 on May 25, June 14 and 21, respectively. This coincided with an increase in the percent body burden in the bile from 13.6 % on March 22 to 46.2 %, 47.4 %, and 43.8 % on May 25, June 14 and 21, respectively.

[^{3}H]BaP-derived radioactivity content (in μg BaP-equivalent /g of tissue) in several tissues of coho salmon was also determined during the parr-smolt transformation. The liver had the highest [^{3}H]BaP-derived radioactivity content (0.7 - 3.1 μg BaP-equivalent/g), followed by the intestine (0.4 - 1.4 μg BaP-equivalent/g). The brain and the skeletal muscle contained the lowest concentrations of [^{3}H]BaP-derived radioactivity, ranging between 0 - 0.1 μg BaP-equivalent/g in both tissues. The [^{3}H]BaP-derived radioactivity content of tissues such as the brain, gill, intestine, kidney, skeletal muscle, stomach, and visceral fat was not significantly different between the different sampling times (Table 1.2).

As illustrated in Figure 1.7, the [^{3}H]BaP-derived radioactivity content of the skin decreased with time. Specifically, it was high on February 22 (0.9 μg BaP-equivalent/g) and declined until it reached the lowest concentration (0.06 μg BaP-equivalent/g) on June 14. [^{3}H]BaP-derived radioactivity content of the liver increased until March and subsequently declined in the following months. The concentration in the liver was highest on March 8 (3.2 μg BaP-equivalent/g) and lowest on May 25 (0.8 μg BaP-equivalent/g) (Figure 1.7).

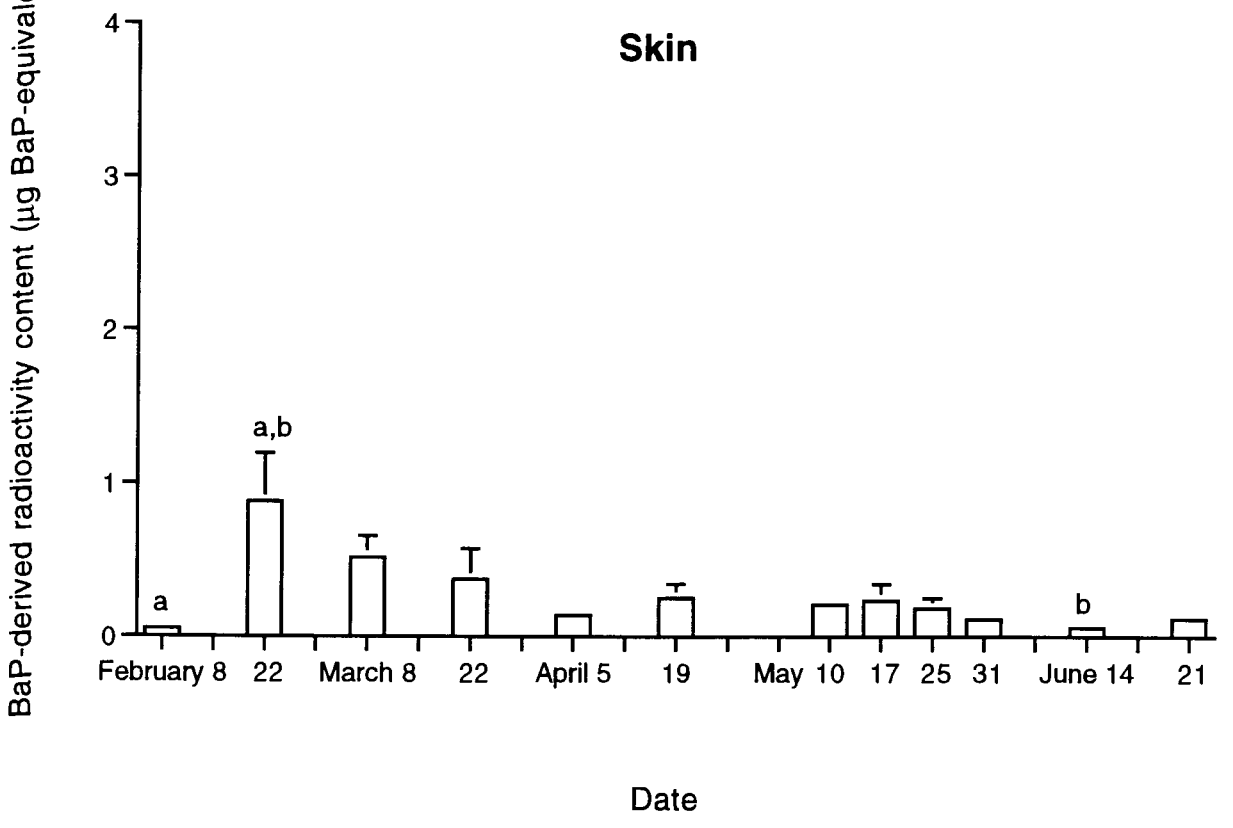
Table 1.2. [^{3}H]BaP-derived radioactivity content (µg BaP-equivalent/ g)* of several tissues and the total uptake of BaP-derived radioactivity by coho salmon during the parr-smolt transformation. Values are the means (±SE) for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 µg (1 µCi)/L. There were no significant differences between the means of tissue BaP-derived radioactivity content at different sampling days at $p<0.05$.

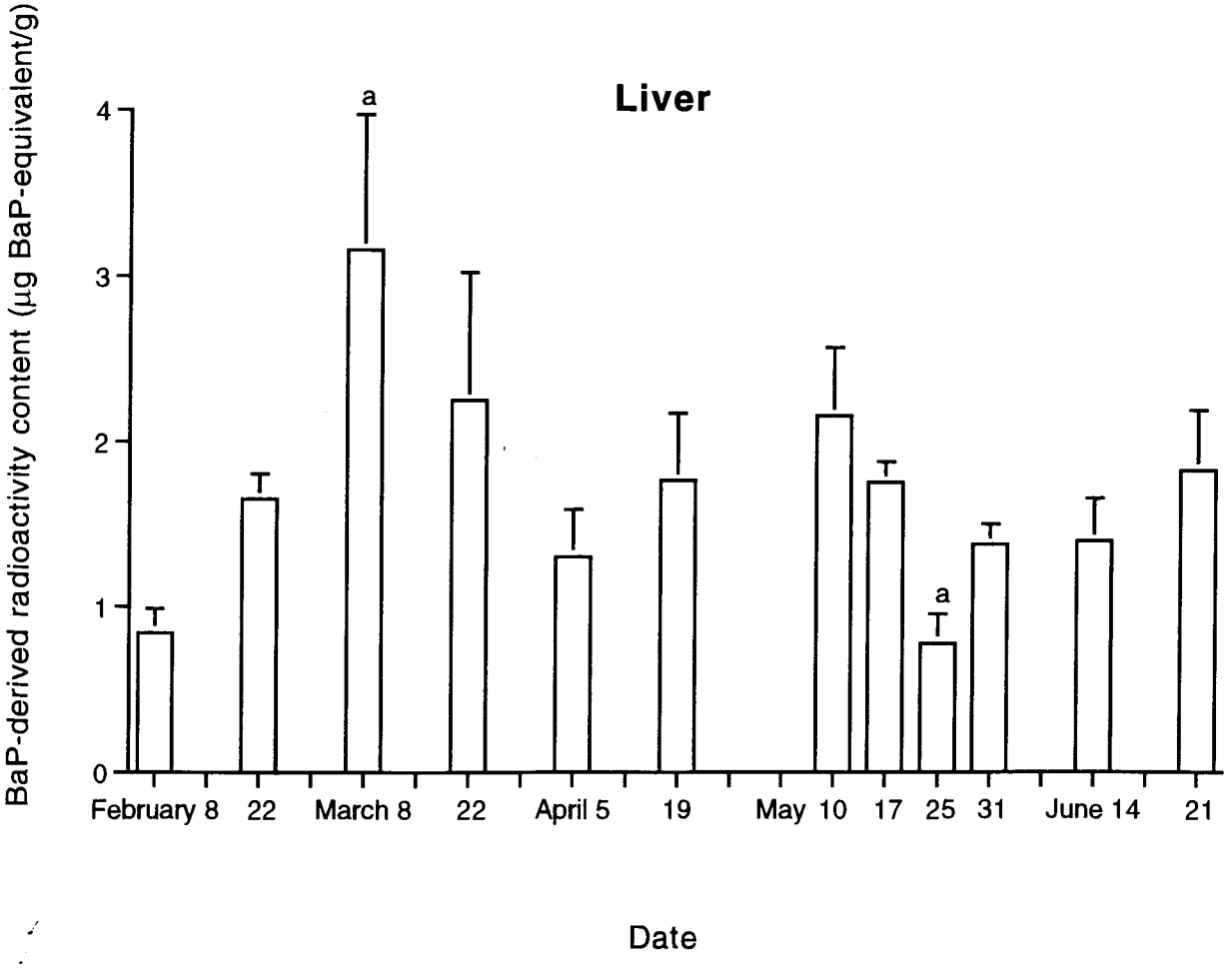
	Date											
Tissue	Feb. 8	Feb. 22	Mar. 8	Mar. 22	Apr. 5	Apr. 19	May 10	May 17	May 25	May 31	Jun. 14	Jun. 21
Gill	0.36 (±0.13)	0.30 (±0.06)	0.48 (±0.15)	0.45 (±0.17)	0.38 (±0.08)	0.63 (±0.01)	0.35 (±0.00)	0.35 (±0.10)	0.27 (±0.05)	0.32 (±0.04)	0.35 (±0.05)	0.40 (±0.03)
Brain	0.006 (±0.00)	0.124 (±0.05)	0.049 (±0.04)	0.015 (±0.01)	0.012 (±0.01)	0.030 (±0.01)	0.049 (0.03)	0.021 (±0.01)	0.022 (±0.01)	0.003 (±0.00)	0 (±0)	0.014 (±0.00)
Intestine	0.53 (±0.13)	0.46 (±0.11)	0.71 (±0.49)	0.55 (±0.12)	0.42 (±0.24)	0.74 (±0.24)	0.69 (±0.24)	0.57 (±0.12)	0.72 (±0.16)	0.73 (±0.14)	1.43 (±0.18)	1.39 (±0.25)
Kidney	0.15 (±0.03)	0.22 (±0.03)	0.20 (±0.04)	0.16 (±0.02)	0.17 (±0.01)	0.26 (±0.03)	0.38 (±0.08)	0.37 (±0.13)	0.11 (±0.03)	0.11 (±0.02)	0.18 (±0.03)	0.25 (±0.08)
Muscle	0.02 (±0.01)	0.04 (±0.02)	0.04 (±0.02)	0.01 (±0.01)	0.03 (±0.03)	0.02 (±0.01)	0.04 (±0.02)	0.01 (±0.00)	0 (±0)	0 (±0)	0.02 (±0.02)	0.03 (±0.02)
Stomach	0.10 (±0.04)	0.38 (±0.14)	0.14 (±0.06)	0.05 (±0.01)	0.16 (±0.10)	0.21 (±0.12)	0.17 (±0.12)	0.22 (±0.10)	0.04 (±0.02)	0.02 (±0.01)	0.04 (±0.01)	0.04 (±0.01)
Visceral fat	0.10 (±0.08)	0.07 (±0.04)	0.04 (±0.02)	0.04 (±0.02)	0.15 (±0.06)	0.32 (±0.11)	0.20 (±0.10)	0.21 (±0.10)	0.13 (±0.04)	0.18 (±0.14)	0.10 (±0.03)	0.34 (±0.10)
Total uptake (%)	41.6	47.7	43.9	47.1	53.8	54.8	46.6	55.2	60.9	57.7	60.6	56.6

* BaP-equivalent reflects BaP-derived radioactivity of both BaP and BaP metabolites.

Figure 1.7. BaP-derived radioactivity content (μg of BaP-equivalent/g)* in the skin and liver of coho salmon during the parr-smolt transformation. Values are the means ±SE for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 μg (1 μCi)/L. Values with common symbols are significantly different between the sampling times at p <0.05.

* BaP-equivalent reflects BaP-derived radioactivity of both BaP and BaP metabolites.


Skin
BaP-derived radioactivity content (µg BaP-equivalent/g)
4
3
2
1
0
a
a,b
b
February 8
22
March 8
22
April 5
19
May 10
17
25
31
June 14
21
Date


Liver
BaP-derived radioactivity content (µg BaP-equivalent/g)
4
3
2
1
0
a
a
February 8
22
March 8
22
April 5
19
May 10
17
25
31
June 14
21
Date

C. Metabolism of BaP

Metabolite patterns of BaP in coho salmon parr and smolts were investigated by analyzing the contents of the bile following an exposure to BaP. In particular, the extraction of the bile contents with an organic solvent and incubation with hydrolytic enzymes revealed the presence of both Phase I and Phase II metabolites (Table 1.3). The metabolite classes identified in the bile were: organic soluble metabolites which include Phase I metabolites, glucuronide conjugates, sulfate conjugates, other unidentified conjugates and unknown metabolites. The majority of [^{3}H] radioactive counts in the bile were as Phase I metabolites (30 -52 %) and unknown water-soluble metabolites (24 - 38 %) followed by glucuronide conjugates (9 - 17 %), other conjugates (5 -13 %), and sulfate conjugates (3 - 7 %). There were no significant differences in the proportions of the metabolite classes between each sampling time from February to June.

Based on the retention times of known standards, the reverse-phase HPLC separation of the organic soluble fraction of the bile from coho salmon during smoltification tentatively identified the presence of several metabolites and their abbreviations as listed in Table 1.4. A typical HPLC chromatogram is presented in Figure 1.8. Individual BaP quinones, 3-OH BaP, and 9-OH BaP were collected separately but listed collectively as quinones, and a 3-OH and 9-OH BaP fraction due to close separation times. There were no significant differences in the proportions of 7,8,9,10-tetrol, 9,10-dihydrodiol, quinones, and

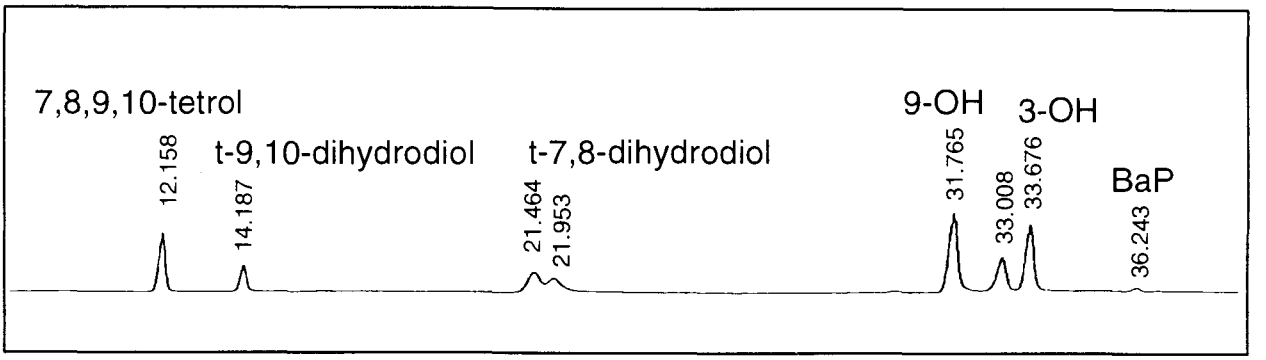
Table 1.3. Percent of total [^{3}H]BaP-derived radioactivity as several classes of metabolites in the bile of coho salmon during the parr-smolt transformation. Values are the means (±SE) for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 µg (1 µCi)/L. There were no significant differences between the means of the percent of these metabolite fractions for different sampling periods, at $p<0.05$.

	Date											
Metabolite fraction	Feb. 8	Feb. 22	Mar. 8	Mar. 22	Apr. 5	Apr. 19	May 10	May 17	May 25	May 31	Jun. 14	Jun. 21
Phase I	34.32 (±4.02)	51.89 (±1.43)	41.05 (±3.19)	40.80 (±8.08)	38.79 (±4.17)	31.02 (±1.86)	51.92 (±4.86)	39.50 (±2.49)	30.01 (±3.09)	33.39 (±2.33)	33.26 (±2.41)	38.56 (±3.57)
Glucuronide conjugates	12.24 (±2.96)	9.18 (±0.93)	13.58 (±3.04)	15.96 (±6.43)	14.88 (±2.96)	17.04 (±1.29)	12.50 (±2.82)	14.62 (±1.41)	14.60 (±2.48)	17.16 (±2.05)	12.94 (±0.04)	12.69 (±0.95)
Sulfate conjugates	4.32 (±0.92)	3.81 (±0.46)	6.64 (±1.70)	3.63 (±0.77)	3.01 (±0.87)	6.21 (±1.47)	5.08 (±1.06)	4.41 (±0.76)	7.56 (±0.93)	5.35 (±0.54)	7.23 (±1.24)	6.32 (±0.64)
Other conjugates	13.54 (±4.66)	5.38 (±2.02)	7.61 (±1.73)	11.06 (±2.67)	10.10 (±1.05)	9.69 (±1.08)	6.13 (±2.03)	10.53 (±1.48)	9.01 (±2.05)	6.72 (±1.10)	9.51 (±1.25)	5.01 (±0.99)
Unknown metabolites	35.58 (±4.52)	29.74 (±3.91)	31.12 (±2.25)	28.54 (±0.43)	33.23 (±1.81)	36.03 (±0.94)	24.37 (±1.45)	30.94 (±1.22)	38.82 (±2.62)	37.39 (±2.89)	37.06 (±0.15)	37.41 (±2.97)

Table 1.4. Tentatively identified BaP metabolites and the parent compound recovered from organic-soluble fraction of the bile of coho salmon by reverse phase HPLC.

Metabolite standard	Abbreviation
r-7,t-8,9,c-10-tetrahydroxytetrahydrobenzo[a]pyrene	7,8,9,10-tetrol
(-)-*trans*-9,10-dihydroxybenzo[a]pyrene	9,10-dihydrodiol
(-)-*trans*-7,8-dihydroxybenzo[a]pyrene	7,8-dihydrodiol
benzo[a]pyrene 1,6-quinone	1,6-quinone
benzo[a]pyrene 3,6-quinone	3,6-quinone
benzo[a]pyrene 6,12-quinone	6,12-quinone
9-hydroxybenzo[a]pyrene	9-OH BaP
3-hydroxybenzo[a]pyrene	3-OH BaP
benzo[a]pyrene	BaP

Figure 1.8. A typical HPLC chromatograph of a bile sample separated using the method of Gmur and Varanasi (1982).


7,8,9,10-tetrol
12.158
t-9,10-dihydrodiol
14.187
t-7,8-dihydrodiol
21.464
21.953
9-OH
31.765
3-OH
33.008
33.676
BaP
36.243

the parent compound between the sampling dates from February to June (Table 1.5). The proportion of 7,8-dihydrodiol of the total Phase I metabolite pool was highest in February and decreased significantly in the remaining sampling period as illustrated in Figure 1.8. As the proportion of 7,8-dihydrodiol of the total Phase I metabolite pool decreased with time, the proportion of 3-OH BaP and 9-OH BaP of the total Phase I metabolite pool, when grouped together, increased. Specifically, it was lowest on February 8, increased two-fold by February 22, and reached the highest levels by April 19 (Figure 1.9) and remained high until the end of sampling period.

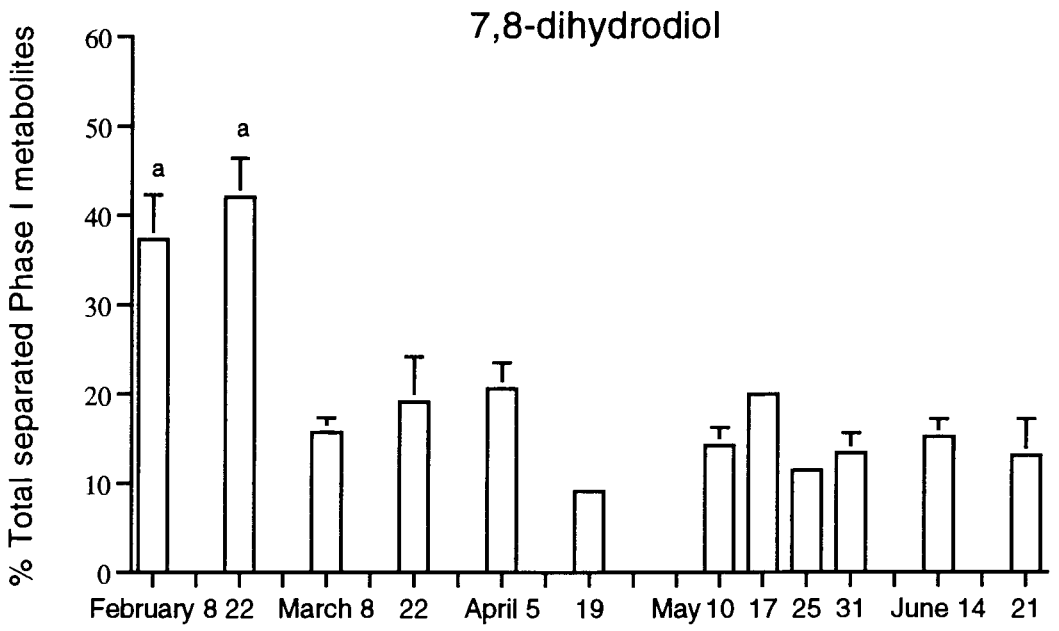
D. BaP-DNA adduct formation

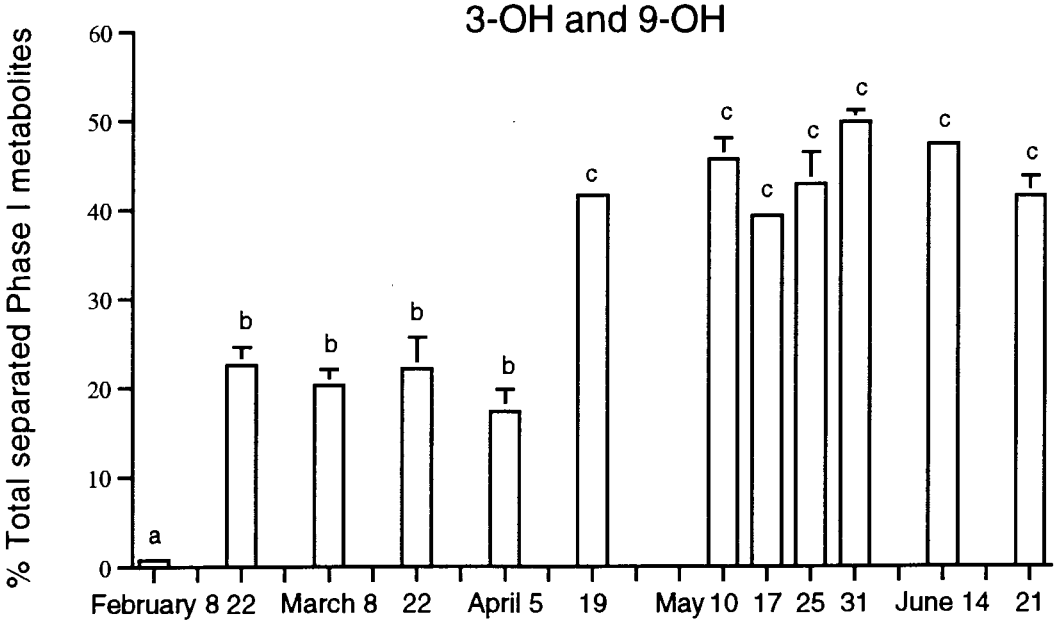
BaP-DNA adduct formation in coho salmon during smoltification was investigated by extracting hepatic DNA and analyzing it for bound [^{3}H] radioactivity following BaP exposure. The A_{260}/A_{280} ratio of absorbances of the DNA solutions extracted from the liver were always in the range of 1.7 to 2.0 indicating that the DNA was free of proteins and RNA. [^{3}H]BaP-derived radioactivity was found associated with the extracted DNA in the livers of juvenile coho salmon (Figure 1.10). The levels of BaP-DNA adducts showed a general trend of decline with time of sampling from February to June. Specifically, the levels of BaP-DNA adducts were significantly higher (10 - 15 pg BaP/μg DNA) on February 16, March 2, and March 30, than the levels on March 16, April 13, April 27, May 12, May 24, and June 6 (2 - 8 pg BaP/μg DNA).

Table 1.5. The percent of total organic soluble [^{3}H] radioactivity as individual metabolites or groups and BaP in the bile of coho salmon during the parr-smolt transformation. Values are the means (±SE) for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 μg (1 μCi)/L. There were no significant differences between means of each metabolite or group at different sampling periods, at $p<0.05$.

	Date											
Metabolite	Feb. 8	Feb. 22	Mar. 8	Mar. 22	Apr. 5	Apr. 19	May 10	May 17	May 25	May 31	Jun. 14	Jun. 21
tetrol	39.16 (±7.59)	9.61 (±2.02)	28.57 (±3.06)	29.88 (±8.59)	22.88 (±0.58)	9.17 (±0.92)	13.34 (±0.54)	9.96 (±0.97)	14.16 (±1.50)	10.15 (±5.51)	11.92 (±4.38)	12.44 (±2.23)
9,10 dihydrodiol	10.27 (±2.57)	10.02 (±4.40)	14.33 (±0.89)	12.68 (±3.41)	15.05 (±0.77)	13.76 (±1.36)	11.17 (±1.84)	15.59 (±1.65)	16.03 (±2.46)	3.87 (±2.47)	12.46 (±5.66)	15.93 (±3.59)
quinones	12.49 (±5.66)	9.20 (±0.27)	16.91 (±2.81)	13.72 (±5.82)	18.13 (±0.63)	18.29 (±1.90)	14.05 (±1.08)	12.29 (±1.30)	12.23 (±2.21)	16.36 (±3.66)	10.65 (±1.60)	14.64 (±4.26)
BaP	0 (±0)	6.43 (±4.26)	4.18 (±0.65)	2.41 (±1.37)	5.88 (±0.96)	8.02 (±0.76)	1.46 (±0.20)	2.87 (±0.30)	3.25 (±1.72)	6.40 (±5.25)	2.30 (±1.61)	2.26 (±0.39)

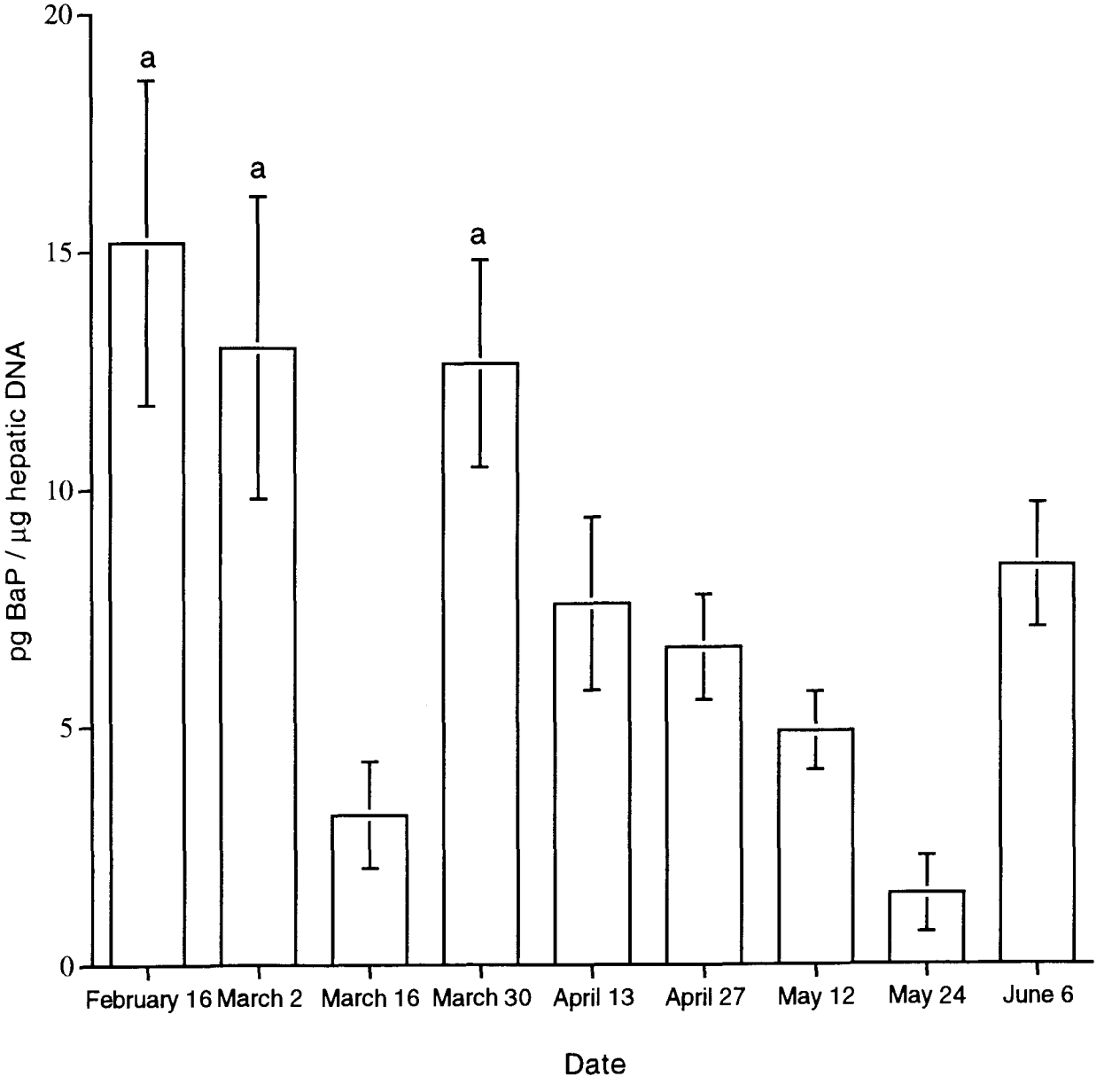
Figure 1.9. Percent of total organic-soluble radioactivity as BaP 7,8-dihydrodiol and 3-hydroxy and 9-hydroxy BaP (3-OH and 9-OH BaP) during the parr-smolt transformation from February to June. Values are the means ±SE for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 μg (1 μCi)/L. Values with common symbols are not significantly different between the sampling times at $p < 0.05$.


7,8-dihydrodiol
% Total separated Phase I metabolites
60
50
40
30
20
10
0
a
a
February 8 22 March 8 22 April 5 19 May 10 17 25 31 June 14 21


3-OH and 9-OH
% Total separated Phase I metabolites
60
50
40
30
20
10
0
a
b
b
b
b
c
c
c
c
c
c
February 8 22 March 8 22 April 5 19 May 10 17 25 31 June 14 21

Date

Figure 1.10. Levels of BaP-DNA adducts in the liver of coho salmon during the parr-smolt transformation from February to June. Values are means ±SE for eight fish injected with 10 mg/kg (1 μCi) [^{3}H]BaP . Values with common symbols are not significantly different between sampling times at p <0.05.


20
15
10
5
0
pg BaP / µg hepatic DNA
a
a
a
February 16
March 2
March 16
March 30
April 13
April 27
May 12
May 24
June 6
Date

D. The effects of smoltification on oxygen consumption rate

Oxygen consumption rates in coho salmon during smoltification were investigated using respirometry and involved the measurements of oxygen tension in a respirometry vessel containing 5 fish. Oxygen consumption rates on February 18, March 16, April 19, May 13, and June 15 were: 130. 6, 99.4, 188.2, 236.7, and 308.7 mg O_2 / g / h, respectively.

The concurrent exposure to BaP resulted in a BaP content in the whole fish body which was below the detection limit by HPLC (0.01 μg BaP/ml). Therefore, no correlation between oxygen consumption rates and the BaP content in the fish could be made.

DISCUSSION

During smoltification a variety of morphological, physiological and behavioral changes occur which may alter the sensitivity of juvenile salmonids to toxicants. In this study, the effects of smoltification on the uptake, distribution and metabolism of benzo[a]pyrene (BaP) in coho salmon were examined. Another objective was to determine if any alterations in BaP-DNA adduct formation take place during this transformation.

In order to assess the approximate status of hatchery-grown juvenile coho salmon with regards to the stage of smoltification, morphological observations of changes in body coloration such as the presence of characteristic parr marks and appearance and growth of teeth were conducted throughout the duration of all experiments. The fish retained their distinct parr marks until May and progressively increased their silvery appearance towards the end of sampling period. In addition, the changes in body coloration corresponded to the emergence of teeth. To confirm the transition of freshwater parr to seawater-migrating smolt, levels of Na^+,K^+-ATPase in the gills were measured by Seubert (1997) on the same stock of fish brought from the hatchery and indicated an increase in Na^+,K^+-ATPase activity in mid-March and May characteristic of juvenile salmon reared in hatchery conditions (Zaugg, 1982). As a consequence, it was concluded that the hatchery-grown coho salmon showed characteristic traits of parr in the beginning of the sampling

season (February) and exhibited traits typical to smolt by the end of the sampling period (June).

The uptake of [^{3}H]BaP from water from February to June was rapid: 45 - 60 % of the total radioactivity added to the flasks was removed from the water within 24 hours. A similar rapid uptake of [^{14}C]BaP, at the same initial concentration, was reported by Kennedy at al. (1989a) in the study on the uptake of BaP by the gulf toadfish. Lee et al. (1972) reported a fast uptake of [^{3}H]BaP and [^{14}C]naphthalene by marine fish and the presence of radiolabelled chemicals in fish tissues within a few minutes after the introduction of hydrocarbons. Other polycyclic aromatic hydrocarbons (PAHs) such as 2-methylnaphthalene, fluorene, and pyrene are quickly taken up by fish as observed by the presence of radiolabelled PAHs in the blood within minutes of chemical exposure (Kennedy and Law, 1990). This rapid uptake has been attributed to the highly lipophilic nature of these chemicals which cross biological membranes by passive diffusion (Heath, 1995; Kennedy, 1995).

The rates of [^{3}H]BaP uptake increased two-fold by the end of March and reached a four-fold increase in May and continued to be high through the release date of the fish in June as compared to rates in February. The increases in BaP uptake rates from water through smoltification may be related to changes in fish respiration rates and oxygen utilization by the fish. As suggested by McKim et al. (1985) and McKim and Erickson (1991), chemical uptake can be correlated to the rates of oxygen uptake in water-breathing organisms. An increase in routine oxygen consumption rates in smolts during

the months of April and May above those found in parr have been well documented (McCormick and Saunders, 1987; Boeuf, 1992). In a concurrent study outlined in this thesis, respirometer measurements of oxygen consumption rates of coho salmon during the parr-smolt transformation indicated a general trend of increased oxygen consumption rates in smolts as compared to parr. The levels of oxygen consumption were comparable to those found by Morgan and Iwama (1991) in juvenile fall chinook salmon. It is possible that, to meet increased oxygen demand during smoltification, fish may increase their respiration rates which may lead to an increase in the amount of water passing through the gill in a given time and may result in an increase in the exposure to water containing BaP.

There are a few factors affecting uptake of lipid-soluble chemicals in relation to oxygen uptake. Heath (1995) and Kennedy (1995) pointed to the importance of factors such as water flow over the gill (ventilation), diffusional properties of a membrane, and blood perfusion of the gill in relation to chemical uptake. Fish can alter the amount of water flowing via the gills by changing ventilation rate or volume (Heath, 1995) and it has been shown that the uptake of compounds with high log K_{ow} such as BaP is ventilation-limited (Schmieder and Weber, 1992). The diffusion rate across a membrane for hydrophobic chemicals may be dependent on the presence of stagnant water layers with different diffusion properties located next to the gill epithelium and on the composition of the epithelial membrane (Hayton and Barron, 1990). The fatty acid composition of epithelial membrane in the gill changes during

smoltification from the predominance of saturated fatty acids in parr to long-chain polyunsaturated fatty acids in smolts (Sheridan, 1989, Sweeting, 1989), however, it is unknown whether these changes in the structure of the membrane significantly affect the diffusion of hydrophobic chemicals. Under conditions of normal oxygen saturation, fish perfuse approximately 60 % of their gill lamellae (Booth, 1978) and can increase the blood perfusion of the gill when the demand for oxygen increases. In addition, the residence time of the blood in the gill filaments appears to be sufficient for hydrophobic chemicals to reach equilibrium with the transport proteins of blood such as albumin suggesting that the uptake of these compounds is not perfusion-limited (Kennedy, 1995). Therefore, the increase in BaP uptake during smoltification is most likely related to the changes in gill ventilation in response to an increase in oxygen demand during that time.

The levels of [^{3}H]BaP in the stomach throughout the experiment were low compared to levels found in the gills, possibly indicating that the oral route of exposure to the chemical was low. These findings agree with those of Lee et al. (1972) and Kennedy and Law (1990) which showed that PAHs administered orally to fish contributed little to the total body burden. It is also known that freshwater fish prevent osmotic water gain by low drinking rates and maintain a low rate of intestinal water absorption (Eddy, 1982; Loretz et al. 1982) which would limit oral route of exposure to chemicals.

The effects of smoltification on the distribution of BaP in juvenile coho salmon were determined by analyzing the radioactivity content in different

tissues after exposing the fish to [^{3}H]BaP in water. This study confirms that BaP can be widely distributed in fish tissues following a short exposure to the chemical in water which can be related to its high lipid-solubility. Similarly to the study of Lee et al. (1972), [^{3}H] radioactivity was detected in all major tissues within 24 hours of exposure. The highest levels were found in the skin (February) followed by the liver (March) and the bile (May and June).

The percent body burden of [^{3}H]BaP-derived radioactivity in the skin, gill, liver and bile was altered by the parr-smolt transformation. The levels of the percent body burden in the skin were much higher during the initial sampling days than reported for any species of fish (Kennedy et al. 1989a; Kennedy and Law, 1990). These high levels could indicate that a significant dermal absorption of BaP may have occurred at that time and it could be related to the small size of the fish as reported by Lien and McKim (1993). These researchers postulated that in smaller fish of 5 g or less, the importance of dermal absorption of hydrophobic chemicals is greater relative to the branchial route of uptake but decreases with the growth of the fish and the associated growth and development of gill tissue resulting in a large increase in respiratory surface area. Varanasi et al. (1978) have found that naphthalene accumulated in the skin of different species of salmonids due to a direct uptake via the skin. Satchell (1991) suggested that the microcirculation in the skin, which is a part of fish's secondary blood system, may facilitate the uptake of chemicals.

A small but significant increase in the percent body burden of [^{3}H]BaP-derived radioactivity of the gill observed from February to April may correspond

to an increase in number of gill epithelial cells such as chloride cells (Loretz et al., 1982) and change in morphology of gill filaments (McCormick and Saunders, 1987) associated with smoltification and seawater adaptation. The percent body burden of the gill decreased in May and June as compared to April even though BaP uptake rates increased four-fold during that time which may suggest that BaP may be preferentially distributed to other parts of the body such as the liver or the bile.

The effects of parr-smolt transformation on the percent body burden of [^{3}H]BaP-derived radioactivity in the liver were also evident. The higher percent body burden in parr (March) as compared to that in smolts (June) may correspond to the changes in the morphology of liver cells and a shift from anabolic to catabolic type of metabolism in the fish (McKeown, 1984). As mentioned previously, during the parr-smolt transformation, the liver cells shift from the storage role of large amounts of glycogen to glycogen utilization and increased enzymatic activity exemplified by an alteration in mitochondrial enzyme activities, an increase in volume of mitochondria, and an increase in volume and number of organelles such as smooth and rough endoplasmic reticulum, and Golgi complexes (Blake et al., 1984; Robertson and Bradley, 1991). This increase in metabolic activity later in the smoltification process correlates with the increased presence of radioactivity in the bile which may indicate an enhanced biotransformation of BaP and transfer of BaP metabolites produced in the liver to the gall bladder. This agrees with the findings of several other studies in which the bile appeared to be the main elimination route for

many PAHs in other teleost species (Lee et al. 1972; Gmur and Varanasi, 1982; Thomas and Rice, 1982; Kennedy et al. 1989a; Kennedy and Law, 1990). In contrast to high levels of [^{3}H] radioactivity in the bile, the percent body burden of [^{3}H]BaP-derived radioactivity in the kidney was very low indicating limited excretion of BaP metabolites by that route within a short exposure time which is in agreement with the findings of Kennedy et al. (1989a) who found a comparable levels of [^{14}C]BaP in the kidney of gulf toadfish after 24 hour exposure to the chemical in water.

At any time, the percent body burden in the visceral fat was low. Kennedy and Law (1990) found that the visceral fat of rainbow trout contained 5 to 7 times more PAHs than the liver after a single intravenous injection which is likely due to the hydrophobic nature of these chemicals. The low levels of BaP found in the fat of fish in this study may be attributed to the relatively short period between chemical exposure and sacrifice of the animal and to relatively poor perfusion of fat tissue (Heath, 1995). It is possible that a longer period of time is required for the redistribution of BaP or its metabolites to the fat from other tissues.

When expressing the [^{3}H]BaP-derived radioactivity content in the tissues as the actual concentration of BaP and/or its metabolites in that tissue, the findings of this study indicate that the liver had the highest concentration of [^{3}H]BaP-derived radioactivity throughout the sampling period and particularly in March. Only the concentration of [^{3}H]BaP-derived radioactivity in the liver and skin was altered by the smoltification process. A general increase in [^{3}H]BaP-

derived radioactivity concentration in the liver during March may correlate with an increase in activity of cytochrome P450 and enzymes associated with it such as ethoxyresorufin O-deethylase observed by Seubert (1997) in juvenile coho salmon. Even though the concentration of [^{3}H]BaP-derived radioactivity in the bile was not determined, high levels of [^{3}H]BaP-derived radioactivity in the liver indicate a high potential of that tissue to store and metabolize highly hydrophobic compounds such as BaP as suggested by Klaassen (1986).

[^{3}H]BaP-derived radioactivity content in the skin followed the same pattern as observed before: the levels were high in the initial sampling period (February) and declined as smoltification progressed (May and June). This finding could be attributed to the increase in the respiratory surface area of the gill relative to the surface area of the skin with the growth of fish, as suggested by Lien and McKim (1993), which could lead to a decrease in importance of branchial route of uptake of BaP as observed in smolts

In order to determine the effects of smoltification on the metabolism of BaP, the bile of juvenile coho salmon was analyzed after exposing the fish to [^{3}H]BaP in water. The analysis of bile revealed that juvenile coho salmon are capable of metabolizing BaP to both Phase I and Phase II metabolites during the parr-smolt transformation. Similar results have been observed in gulf toadfish (*Opsanus beta*) exposed to [^{14}C]BaP in water for 24 hours (Kennedy et al., 1989b) and in a variety of other fish species such as English sole (*Parophrys vetulus*) and starry flounder (*Platichthys stellatus*) injected with [^{3}H]BaP (Varanasi et al., 1989). Exposure of rats to [^{3}H]BaP resulted in the

presence of both Phase I and Phase II in the bile after 24 hours (Varanasi et al., 1989).

Metabolite classes determined in the bile were organic-soluble metabolites (30-52 %), glucuronide conjugates (9-17 %), sulfate conjugates (3-7 %), other conjugates (5-13 %), and unknown metabolites (24-38 %). Smoltification did not significantly affect the overall proportions of each metabolite class. The proportion of organic-soluble metabolites in the bile of coho salmon (*Oncorhynchus kisutch*) was slightly lower than reported by Seubert (1997) in his study on juvenile coho salmon injected i.p. with 10 mg/kg of BaP but were much higher than reported in other studies on the metabolism of PAHs in fish. Gmur and Varanasi (1982) reported that organic-soluble metabolites in the bile of English sole (*Parophrys vetulus*) constituted around 20 % of the total radioactivity found. Kennedy et al. (1989b) have found even lower levels of the organic-soluble fraction of the bile (<10 %) in gulf toadfish (*Opsanus beta*) exposed to the same initial BaP concentration as used in this study. The high level of organic-soluble metabolites in coho salmon parr and smolts may indicate a more effective transport of these metabolites from the bile or an inability to effectively biotransform BaP to water-soluble or conjugated metabolites.

In general, there was a higher proportion of glucuronide than sulfate conjugates observed in this study suggesting that conjugation to glucuronic acid may be more prevalent to conjugation to sulfate. This is in agreement with the findings of Varanasi et al. (1989) in English sole (*Parophrys vetulus*) and

starry flounder (*Platichthys stellatus*) and of Steward et al. (1991) in common carp (*Cyprinus carpio*). However, Kennedy et al. (1989b) have found equal proportions of glucuronide and sulfate conjugates in the bile of gulf toadfish. The proportions of glucuronide and sulfate conjugates found in that study were both below 5 % of the total radioactivity in the bile which is lower than observed in this study. The remaining unidentified conjugates found in the bile of coho salmon may be glutathione conjugates as suggested by several researchers (Stein et al. 1987; Varanasi et al. 1989; Kennedy et al. 1989b). Steward et al. (1991) have found that glutathione conjugates constituted about 16 % of extracted metabolite classes in common carp which is similar to the findings of this study. These low levels of glutathione conjugates may correspond to the low levels of glutathione S-transferase enzyme found in the livers of juvenile coho salmon during the same period of time (Seubert, 1997).

Following enzymatic hydrolyses, acid hydrolysis and extraction with organic solvent, the resulting aqueous fraction contained unknown water-soluble metabolites of BaP suggesting that BaP and its metabolites may be conjugated to other compounds such as amino acids, particularly taurine as observed in dogfish sharks (*Squalus acanthias*) (Bend and James, 1978). This water-soluble fraction constituted a high proportion of all extracted metabolite classes but was much below the levels found by Kennedy et al. (1989b) in gulf toadfish who found that the water-soluble fraction constituted as much as 85 % of the total bile radioactivity. However, the acid hydrolysis of the bile was not performed in that study which precluded the separation of possible glutathione

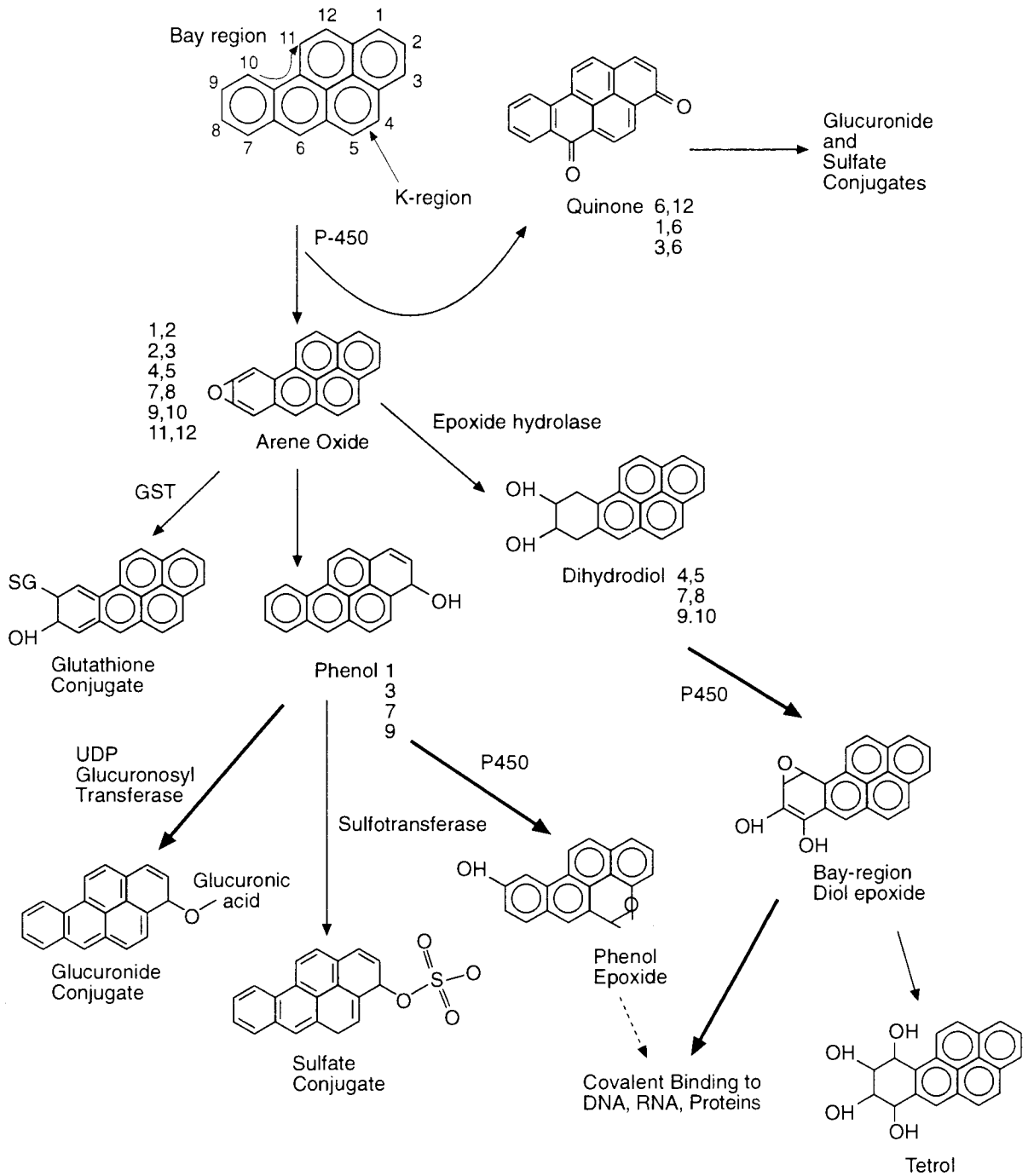
conjugates from amino acid conjugates and may have accounted for a high radioactivity content in the aqueous fraction of the bile.

Reverse phase HPLC analysis of organic-soluble fraction of the bile revealed a presence of variety of metabolites such as tetrols, diols, quinones and phenols as well as BaP in the bile of juvenile coho salmon. Smoltification affected the levels of 3-OH and 9-OH BaP which steadily increased with time and the levels of 7,8-dihydrodiol which decreased with time but the proportions of other metabolites and metabolite groups were not affected during that time. The levels of BaP quinones and 9,10-dihydrodiol found in the organic-soluble fraction of the bile were low. The levels of BaP quinones were similar to the levels reported by Stegeman et al. (1984) in microsomal preparations of scup (*Stenotomus chrysops*) and winter flounder (*Pseudopleuronectes americanus*) livers but lower than the levels found by Steward et al. (1991) in common carp (*Cyprinus carpio*) and Seubert (1997) in juvenile coho salmon. It is possible that further metabolism of quinones via conjugation to glucuronic acid and sulfate increased their water solubility and removal from the organic-soluble fraction of the bile. Contrary to the findings of Tan and Melius (1986) in other freshwater teleosts, juvenile coho salmon did not metabolize BaP to 9,10-dihydrodiol to great extent. These authors also suggested that fish liver monooxygenase system shows relative regioselectivity for the bay region of the BaP molecule (i.e., 7-,8-,9- and 10 position) rather than the K-region (i.e., 4- and 5 position) as seen in mammals. Physiological changes occurring during smoltification, including changes in the amount and proportion of saturated and

polyunsaturated fatty acids contained within biological membranes, may affect the characteristics of the medium in which some of the enzymatic systems, such as the cytochrome P450 system, are found and may lead to possible alterations in their specificity. In addition, the cytochrome P450 system is involved in chemical conversions and degradation of many endogenous compounds including steroid hormones (Klaassen, 1986) and the biotransformation potential of these enzymes may be altered during smoltification period due to the increased synthesis of cortisol in smolts as compared to parr (Barron, 1986).

There are several pathways by which BaP can be metabolized in the liver by the Phase I and Phase II enzymatic systems (Figure 1.11). The predominant pathway involves the formation of arene oxide by the action of the cytochrome P450. This oxidized unstable metabolite may be further metabolized to diols by epoxide hydrolase, spontaneously rearrange to form phenols which may be then conjugated to molecules such as glucuronic acid. BaP phenols and diols may be conjugated to a variety of endogenous compounds by the Phase II enzymes or be further oxidized by cytochrome P450 enzymes to multiple hydroxylated products such as triols and tetrols. The reactions described above can occur in either bay-region or K-region of the molecule resulting in different types of metabolites. Some of the intermediate and unstable metabolites, particularly epoxides, have an ability to covalently bind to macromolecules such as DNA or RNA (Gelboin, 1980; Varanasi et al., 1989).

Figure 1.11. Schematic representation of the biotransformation and metabolic activation of benzo[a]pyrene (Varanasi et al., 1989).


Bay region
12
1
11
2
10
3
9
4
8
7
6
5
K-region
Quinone 6,12
1,6
3,6
Glucuronide
and
Sulfate
Conjugates
P-450
1,2
2,3
4,5
7,8
9,10
11,12
Arene Oxide
Epoxide hydrolase
GST
OH
OH
Dihydrodiol 4,5
7,8
9.10
SG
OH
Glutathione
Conjugate
OH
Phenol 1
3
7
9
P450
UDP
Glucuronosyl
Transferase
P450
Sulfotransferase
OH
OH
Bay-region
Diol epoxide
Glucuronic
acid
Glucuronide
Conjugate
Phenol
Epoxide
Sulfate
Conjugate
Covalent Binding to
DNA, RNA, Proteins
OH
OH
OH
OH
Tetrol

As observed in this study, high levels of phenolic BaP metabolites indicate a preferential spontaneous conversion of arene oxide to BaP phenols over the enzymatic conversion of the same intermediate to diols and eventually 7,8,9,10-tetrol in smolts of juvenile coho salmon. Varanasi et al. (1986) have found that, although 3-OH and 9-OH BaP contributed a high proportion to the radioactivity of the organic phase in the bile taken from adult English sole and starry flounder, the predominant metabolite was 7,8-dihydrodiol. Similar results were reported by Gmur and Varanasi (1982) in juvenile flatfish and Kennedy et al. (1989b) in gulf toadfish. High levels of 7,8-dihydrodiol in February in February and March in coho salmon parr may indicate a relative regioselectivity of the monooxygenase system to metabolize BaP at the bay region rather than K-region as suggested by Stegeman et al. (1984), Tan and Melius (1986), and Varanasi et al. (1989). The presence of high levels of 7,8-dihydrodiol in parr may also indicate that possible conversion of 7,8-dihydrodiol to the reactive metabolite 7,8-diol-9,10 epoxide by liver monooxygenases can take place in juvenile coho salmon. This can also be confirmed by the presence of BaP triols and tetrols which are the products of further diol-epoxide conversion and have been found in the present study ranging from 10 to 40 % of the total radioactivity in the organic-soluble fraction of the bile. It has been shown that the reactive metabolite 7,8-diol-9,10 epoxide can react with DNA or RNA to form adducts in hepatic cells (Gelboin, 1980; Varanasi et al. 1986; Varanasi et al. 1989; Kennedy and Walsh, 1991). Based on the decrease in the levels of 7,8-dihydrodiol in the bile in February and the increase in levels of non-reactive

metabolites such as 3-OH and 9-OH BaP by May and June, it appears that coho salmon parr may have a higher risk of adduct formation as compared to smolts.

To determine if the parr-smolt transformation can alter levels of BaP-DNA adduct in juvenile coho salmon, the hepatic DNA was extracted and BaP-DNA adducts were quantified . Since the fish were injected i.p. with a known dose of [^{3}H]BaP, any changes in the BaP-DNA adducts formation could not be attributed to alterations in the uptake of the chemical due to smoltification as shown in the uptake experiment. The amounts of BaP-DNA adducts found in this study (2-15 pg BaP/μg hepatic DNA) were slightly higher than reported by Shugart et al. (1987) in bluegill sunfish (*Lepomis macrochirus*) (0.06-0.9 pg BaP/μg hepatic DNA) injected i.p. with 5 mg/kg of BaP and by Kennedy and Walsh (1991) in gulf toadfish (0.1-0.5 pg BaP/μg hepatic DNA) injected i.v. with a similar dose of BaP. However, Varanasi et al. (1985; 1986) reported higher levels of BaP-DNA adducts in English sole force-fed with BaP ranging from 1-10 μg BaP/μg hepatic DNA and 0.1-0.5 μg BaP/μg hepatic DNA depending on the study. It is possible that the differences in the levels of BaP-DNA adducts observed between the studies in flatfish and salmonids in the present study could be attributed to the different route of exposure to BaP and the activity levels of the fish result in higher BaP activation and BaP-DNA adduct formation when the fish absorbs BaP from the gastrointestinal tract.

The levels of BaP-DNA adducts generally declined with time from February to June. That finding correlates with the results of HPLC analysis of the bile where the levels of 7,8-dihydrodiol, one of the important precursors to

reactive metabolites, decrease from February to June as well. Since 7,8-dihydrodiol can be further converted to the ultimate carcinogen 7,8-diol-9,10 epoxide which binds covalently to DNA in liver. The binding of reactive metabolites to DNA (adduct formation) has been used as an indicator of carcinogenic potential of PAHs such as BaP in mammals and aquatic organisms (Varanasi et al. 1989). This binding is a first step in tumor initiation followed by fixation of the biochemical change in the macromolecule and uncontrolled cell proliferation (Harvey, 1985) leading to hepatic cytotoxicity, lesions and tumor formation in English sole and other marine fish (Conney, 1982; Baumann, 1989; Varanasi et al. 1989). The progression of carcinogenesis is dependent on the efficiency of variety of factors including the types of DNA adducts, levels of adducts, rate of DNA repair, or rates of mitotic division of tissues (Varanasi et al. 1989). For example, enzymatic excision repair of modified DNA, when fully operational, could eliminate the effects of adducts on the macromolecule. Since the changes in the efficiency of DNA repair mechanisms have not been investigated in this study, the trends in DNA adduct formation observed in the present study may serve only as an indicator of changes in risk of tumor formation in juvenile coho salmon during smoltification, particularly a higher risk of carcinogenesis in parr as compared to smolts.

PART II. The effects of salinity changes on the toxicokinetics of benzo[a]pyrene in smolts

INTRODUCTION

A prominent feature of the life history of Pacific salmon (genus *Oncorhynchus*) is their anadromous migration between freshwater and marine habitats. The transformation of juvenile salmon from freshwater parr to seawater smolt represents one of the most significant adaptations in the salmon life cycle. An increase in euryhalinity, an essential part of this transformation, allows smolts to live in salinities varying from freshwater to full-strength seawater (Clarke and Hirano, 1995). These different osmotic environments require a variety of different mechanisms which allow the fish to maintain the volume of water and electrolytes in their body fluids within a homeostatic range (Burton, 1986). For example, in freshwater, the blood and body fluids are highly concentrated compared to the medium and there is a tendency for water to enter the body by osmosis and salts are lost by passive diffusion (Eddy, 1982). In order to maintain water and electrolyte balance, salmon excrete excess water through the kidneys and actively take up ions through the gills (Clarke and Hirano, 1995). In seawater, the osmotic pressure of blood and body fluids is lower than the surrounding water and salmon must take up water by drinking and excrete excess salts, usually by an active process in the gills as the urine of seawater fish is greatly reduced and concentrated to reduce water loss. All of

these mechanisms take place in a number of tissues including the gills, intestine, kidney, urinary bladder, and, to some degree, in the skin (Clarke and Hirano, 1995).

It has been shown that juvenile salmonids exposed to changes in salinity exhibited a variety of physiological responses. These include changes in oxygen consumption rate and metabolic rate (Morgan and Iwama, 1991), variations in ionic content of blood plasma (Morgan and Iwama, 1991; Brauner et al., 1992), alterations in water status and morphology of gill epithelial cells such as chloride cells and associated changes in Na^+,K^+-ATPase activity (Sargent et al., 1975; Eddy, 1982), alterations in muscle moisture content and blood volume (Eddy, 1982; Brauner et al., 1992), and changes in swimming ability (Brauner et al., 1992). The general responses of Pacific salmon to the stress of change in salinity have been varied but in most studies resulted in decreased survival and growth of juvenile fish (Mahnken and Waknitz, 1979; Flagg et al., 1983).

Because the maintenance of hydromineral balance through osmoregulation involves changes in the routes of uptake and excretion of water and ions in euryhaline fish in freshwater and seawater, any alterations to the mechanisms of water and ion uptake may result in the differential uptake of waterborne contaminants as well as their elimination by the fish. For example, an early study on the effects of salinity change on the uptake of DDT, DDE, and DDD by mosquito fish (*Gambusia affinis*) indicated that the uptake of DDT decreased significantly with the increase in water salinity from 0.15 $^{o}/_{oo}$ to 15 $^{o}/_{oo}$

but the uptake of DDE and DDD was not affected (Murphy, 1970). Similarly, uptake of ^{14}C-2,2',4,5,5'-pentachlorobiphenyl by juvenile Atlantic salmon (*Salmo salar*) was more efficient from freshwater than from seawater resulting in higher tissue concentrations in the fish exposed to the chemical in freshwater (Tulp et al., 1979). The absorption rates of five halogenated phenols by rainbow trout (*Oncorhynchus mykiss*) in seawater were lower than in freshwater when the compounds were in nonionized form (pH 6.0) but were equal when the compounds were ionized (pH 9.0) (Pärt, 1989). Tachikawa and Sawamura (1994) reported that the accumulation of pentachlorophenol in killifish (*Oryzias latipes*) decreased with increase in salinity from 1.2 to 18.7 $^{o}/_{oo}$. In contrast, Canton et al. (1975; 1978) have found that the uptake and accumulation of alpha-hexachlorocyclohexane by the guppy (*Labistes reticulatus*) in seawater (32 $^{o}/_{oo}$) was higher than in freshwater.

As these numerous studies imply, salinity changes are of importance in modifying the absorption rates of organic chemicals. Changes in the permeability of gill epithelium and general dehydration associated with increase in salinity, as indicated by Pärt (1989), may affect the rates of chemical uptake. An increase in oxygen consumption rate and metabolic rate with increase in salinity (Morgan and Iwama, 1991) may also alter the rates of uptake for hydrophobic compounds. Increased rates of water ingestion and altered intestinal permeability in seawater may additionally affect uptake rates of chemicals (Eddy, 1982). A decrease in hematocrit and increase in blood plasma volume and resulting decrease in oxygen carrying capacity (Brauner et

al., 1992), observed in fish exposed to seawater, may alter the distribution patterns of absorbed chemicals. Energetic demands placed on fish coping with osmoregulatory stress at different salinity (Eddy, 1982) may alter distribution as well as metabolism of xenobiotics taken up by the fish. Reported decreases in urine production in fish in salt water (Eddy, 1982) may affect chemical elimination and half-life in the body.

The various changes in euryhaline juvenile salmonids undergoing smoltification have an important effect on fish physiology as well as uptake, disposition, metabolism, and elimination of chemicals in fish as discussed previously. Osmoregulatory adjustments required by fish exposed at the same time to different salinity levels may results in additional alterations in the toxicokinetics of xenobiotics. Therefore, the first objective of this study was to determine the effects of salinity change on the uptake, distribution, and metabolism of benzo[a]pyrene (BaP) in juvenile coho salmon undergoing smoltification. The second objective was to examine any changes in oxygen consumption rate in fish exposed to different salinity levels and to determine a possible correlation between oxygen consumption rate at varied salinity and the uptake of BaP in fish during the parr-smolt transformation. In both cases, the experiments were similar to those described in Part I of this thesis except that the fish were additionally exposed to water of 5, 10, and 20 $^{o}/_{oo}$ salinity containing BaP during the duration of the experiments from February to June.

MATERIALS AND METHODS

General

Fish

Juvenile coho salmon (*Oncorhynchus kisutch,* 0+) were obtained on 12 separate dates from the Capilano Hatchery, North Vancouver, British Columbia during the period from February to June, 1994. Fish were maintained in flow-through, filtrated, dechlorinated municipal water of pH 6.7, O_2 saturation >95%, and hardness 5.2 to 6.0 mg/L $CaCO_3$. Fish were kept under a natural photoperiod and natural temperature regime (5-12°C) for 1 week prior to an experiment. During that time, fish were fed commercial salmon pellets (Biodiet, Warrenton, OR, USA) until 2 days before an experiment.

Chemicals

[1,3,6-^{3}H] Benzo[*a*]pyrene (BaP) (specific activity of 52 Ci/mmol) was purchased from NEN Research Products (Boston, MA, USA). Unlabelled BaP (>99% purity) was purchased from Sigma Chemical Company (St. Louis, MO, USA). BaP metabolite standards were obtained from the National Cancer Institute Chemical Repository, Midwest Research Institute (Kansas City, MO, USA). ß-glucuronidase (with no sulfatase activity) and sulfatase were purchased from Sigma Chemical Company (St. Louis, MO, USA). Sodium citrate, HPLC-grade methanol and ethyl acetate were purchased from BDH Inc. (Toronto, ON, Canada). Sulfuric acid (1 N H_2SO_4) and sodium hydroxide (1 N

NaOH) were obtained from Fisher Scientific (Napean, ON, Canada). Tricaine methane sulfonate (MS-222) was purchased from Syndel Laboratories Ltd. (Vancouver, B.C., Canada). Sodium bicarbonate ($NaHCO_3$) and sodium chloride (NaCl) were purchased from Sigma Chemical Company (St. Louis, MO, USA).

A. Uptake of BaP

This experiment was performed to determine the effects of environmental salinity changes on benzo[a]pyrene (BaP) uptake in coho salmon as the fish undergo the parr-smolt transformation. Fish were collected on 12 different days from February to June and exposed to radiolabelled BaP.

Salinity regime and exposure to BaP

Individual fish at each sampling time (February to June) were placed in 1-liter aerated Erlenmeyer flasks which were submerged in a waterbath to maintain a constant water temperature. The water temperature was adjusted for seasonal changes in the ambient water temperature registered at the hatchery. Flasks were grouped into 4 different salinity levels: 0 ‰, 5 ‰, 10 ‰ and 20 ‰. [^{3}H]BaP, dissolved in 1-2 drops of Mulgosen EL-719 (GAS Ltd., Manchester, United Kingdom) and 20 ml of water, was added to the water in the flasks by a glass syringe after 2 hours following the addition of the fish. The initial concentration of BaP in each flask was 5 µg BaP/L (1 µCi [^{3}H]BaP/L) as in

previous experiment on the uptake of BaP described in Part I of this thesis. Control flasks contained the chemical but no fish. All flasks, including the control flasks, were placed behind a dark screen to reduce disturbance of the fish.

Sampling and analysis

BaP uptake rates were estimated as described by Kennedy et al. (1989a) and previously in Part I of this thesis. Uptake rates were calculated as the inverse rate of disappearance of the radioactivity from the water. As described previously in the section on the uptake of BaP in Part I, water samples (1.0 ml) were collected at 0, 0.5, 1, 2, 4, 5, 6, 8, and 24 hours following the addition of the chemical. The samples were mixed with 10 ml of BCS, dark adapted for 24 h, and counted for total [^{3}H] radioactivity by liquid scintillation counting (LSC). An additional water sample (15 ml) was collected at the end of each experiment and hydrolyzed by the addition of 1 N H_2SO_4 to pH 2.0, incubated for 24 h in 80°C water bath, and extracted 3 times with 15 ml of ethyl acetate. The combined extracts were evaporated under nitrogen and resuspended in 1 ml of methanol and separated by reverse phase HPLC using the method of Elnenaey and Schoor (1981) as outlined earlier. The identity of BaP or its metabolites was confirmed by comparing the retention time of recorded peaks with the retention times of known standards shown in Figure 1.1.

Calculations and statistical analysis

Uptake rates of BaP (µg BaP/g/h) by juvenile coho salmon at each salinity level were calculated, as described previously in Part I of this thesis in the section on the uptake of BaP, as the inverse of the disappearance of radioactivity from the water. The first derivative of the fitted exponential curve at each sampling time was calculated with respect to the decreasing chemical concentration, using the volume of the flask, specific activity of BaP and the weight of the fish. Uptake rates at the initial chemical concentration of 5 µg/L were calculated and compared statistically.

A two-way analysis of variance with repetition (ANOVA) and Student-Newman-Kuels multiple range comparison test (Zar, 1984) were used to test for significant effects on BaP uptake in different salinity treatments and/or between the sampling dates. Results were considered significant at $p < 0.05$. All analyses were performed using the SYSTAT statistical program (Wilkinson, 1988).

In all figures, standard error values smaller than 5 % of the mean could not be distinguished graphically.

B. Distribution of BaP

The objective of this experiment was to investigate possible changes in the distribution patterns of benzo[a]pyrene (BaP) in tissues due to different environmental salinities in fish undergoing the parr-smolt transformation. Fish

were collected on 12 different days from February to June and exposed to radiolabelled BaP as described in the previous section on the uptake of BaP.

Sampling and analysis

Tissue samples

Following the 24 h exposure to [^{3}H]BaP, as described previously for the uptake of BaP, fish were anaesthetized in 0.2 g/L MS-222 buffered $NaHCO_3$ (1:1, w/w) and sacrificed by cephalic blow, weighed and dissected. Similarly to the procedure described in the Part I of this thesis on the distribution of BaP, whole organs including the liver, stomach, intestine, kidney, gills, brain and visceral fat and a subsample of skin and skeletal muscle were dissected and weighed. All tissues were homogenized in 1 ml of 0.9% saline and aliquots of the tissue homogenates were oxidized followed by the addition of OX-162 Tritium Cocktail and counted for [^{3}H] radioactivity after dark-adapting for 24 h.

Calculations and statistical analysis

A two-way analysis of variance with repetition (ANOVA) and Student-Newman-Kuels multiple range comparison test (Zar, 1984) were used to test for significant effects of different salinity levels and /or time of sampling on the percent body burden of radioactivity in the tissues and on the [^{3}H]BaP-derived radioactivity content of examined tissues. Since the amount of radioactivity in each tissue could be attributed to both BaP and BaP metabolites, [^{3}H]BaP-derived radioactivity content was represented as µg BaP-equivalent / g of tissue using the specific activity of [^{3}H]BaP, and the amount of [^{3}H]BaP and BaP added.

Percent data were arcsin transformed before statistical analysis was performed. Results were considered significant at $p < 0.05$. All analyses were performed using the SYSTAT statistical program (Wilkinson, 1988).

In all figures, standard error values smaller than 5 % of the mean could not be distinguished graphically.

D. Metabolism of BaP

The objective of this study was to investigate possible alterations of hepatic metabolism of benzo[a]pyrene (BaP) due to different salinity levels in smolting coho salmon. Fish were collected on 12 dates from February to June and exposed to radiolabelled BaP as described in the previous section on the uptake of BaP.

Sampling and analysis

Bile analysis

Following the 24 h exposure to [^{3}H]BaP, fish were anaesthetized and sacrificed as described in the section on the distribution of BaP. Fish were weighed, the gall bladder was removed and the collected bile was mixed with 1 ml of 0.01 M sodium citrate buffer (pH 7.0). An aliquot of this bile solution was mixed with 10 ml of BCS, dark adapted for 24 h, and counted by LSC. As described in the Part I of this thesis in the section on metabolism of BaP, organic soluble metabolites and several of Phase II conjugates were separated from the

remaining bile solution by extraction with ethyl acetate. The extracted organic layers were combined, evaporated under nitrogen, and resuspended in methanol for further analysis of BaP and Phase I metabolites by reverse-phase HPLC. A small aliquot was removed, mixed with 10 ml of BCS, dark adapted for 24 h, and counted by LSC. The remaining aqueous layer was hydrolyzed with ß-glucuronidase and extracted again with ethyl acetate. The organic extracts, containing Phase I metabolites previously conjugated to glucuronic acid, were combined, evaporated under nitrogen, resuspended in methanol and an aliquot was mixed with 10 ml of BCS, dark adapted for 24 h, and counted by LSC. The remaining aqueous layer was hydrolyzed with sulfatase and extracted with ethyl acetate. The organic extracts, containing Phase I metabolites previously conjugated to sulfate, were combined, evaporated under nitrogen, resuspended in methanol and an aliquot was mixed with 10 ml of BCS, dark adapted for 24 h, and counted by LSC. The remaining aqueous layer was hydrolyzed with 1 N H_2SO_4 and extracted with ethyl acetate. The resulting organic extracts were combined, evaporated under nitrogen, resuspended in methanol and an aliquot was mixed with 10 ml of BCS, dark adapted for 24 h, and counted by LSC. The aqueous layer, containing unknown residues of BaP, was neutralized to pH 7.0 with 1 N NaOH, mixed with 10 ml of BCS, dark adapted for 24 h, and counted by LSC.

HPLC analysis of Phase I metabolites

Organic extracts collected after the first extraction of the bile and resuspended in 1 ml of methanol were separated by reverse-phase HPLC as described in Part I of this thesis in the section on metabolism of BaP and using the modified linear gradient method of Gmur and Varanasi (1982). The identity of BaP or its metabolites was confirmed by comparing the retention times of recorded peaks with the retention times of known standards as shown in Figure 1.2. To determine the presence and retention times of BaP quinones which absorbance is maximized in the visible range of light spectrum, the standards were run using a fluorescence detector set at 430 nm for excitation and 480 nm for emission and running the same solvent gradient. The amount of BaP or its metabolites was estimated by collecting 1 min interval fractions and counting [^{3}H] radioactivity in the fractions by adding 5 ml of BCS and dark adapting for 24 h.

Calculations and statistical analysis

A two-way analysis of variance with repetition (ANOVA) and Student-Newman-Kuels multiple range comparison test (Zar, 1984) were used to test for significant effects of different salinity levels and /or time of sampling on the proportions of metabolite classes in the total bile and in the proportions of several organic soluble metabolites and BaP in the Phase I fraction. Percent data were arcsin transformed before statistical analysis was performed. Results

were considered significant at $p < 0.05$. All analyses were performed using the SYSTAT statistical program (Wilkinson, 1988).

In all figures, standard error values smaller than 5 % of the mean could not be distinguished graphically.

D. The effects of salinity on oxygen consumption rate

The objective of this experiment was to investigate the effects of salinity on oxygen consumption rate in coho salmon as the fish undergo smoltification. In addition, this study intended to determine if any changes in oxygen uptake could in turn affect the uptake of benzo[a]pyrene (BaP) by the fish during the same period of time.

Fish

Juvenile coho salmon (*Oncorhynchus kisutch,* 0+) were obtained on a monthly basis from the Capilano Hatchery, North Vancouver, British Columbia during the period from February to June, 1995. Fish were maintained in flow-through, filtrated, dechlorinated municipal water of pH 6.7, O_2 saturation >95%, and hardness 5.3 to 6.1 mg/L $CaCO_3$. Fish were kept under a natural photoperiod and natural temperature regime (4-11°C) for 1 week prior to an experiment. During that time, fish were fed commercial salmon pellets (Biodiet, Warrenton, OR, USA) until 2 days before an experiment.

Chemicals

Benzo[a]pyrene (BaP) (>99% purity) and 1,2 benz[a]anthracene (>99% purity) were purchased from Sigma Chemical Company (St. Louis, MO, USA). [1,3,6-^{3}H] Benzo[*a*]pyrene (specific activity of 52 Ci/mmol) was purchased from NEN Research Products (Boston, MA, USA). HPLC-grade methanol, ethyl acetate and acetonitrile were purchased from BDH Inc. (Toronto, ON, Canada). HPLC-grade pentane and dichloromethane were obtained from Anachemia (Montreal, PQ, Canada). Potassium hydroxide (KOH) pellets and sodium chloride (NaCl) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Hydrochloric acid (12 N HCl) was purchased from BDH Inc. (Toronto, ON, Canada). Sulfuric acid (1 N H_2SO_4) was obtained from Fisher Scientific (Napean, ON, Canada).

Oxygen consumption measurements and exposure to BaP

At each sampling time (February 16, March 2, April 4, May 2, and June 9, 1995), 5 fish were placed in a 9-liter glass respirometer (Figure 1.3) submerged in a waterbath and placed behind a dark screen to reduce disturbance of the fish. The water temperature in the water bath was adjusted to reflect seasonal temperatures registered at the hatchery. The fish were kept in the respirometer for two days in flow-through freshwater system prior to change in salinity and the addition of the chemical. Shortly before the addition of BaP, water of either 0 ‰, 5 ‰, 10 ‰, or 20 ‰ salinity was passed for 5 min through the vessel from a controlled reservoir with the use of a Little Giant Submersible Circulating pump

(Fisher Scientific, Ottawa, ON, Canada). The desired final salinity level was confirmed using a hand refractometer (ATAGO S/Mill, Fisher Scientific, Ottawa, ON, Canada). The water flow was stopped and BaP, dissolved 1-2 drops of in Mulgosen EL-719 and water, was added to the chamber. The initial concentration of the chemical in the chamber was 5 μg BaP/L. No chemical was added to the control run. As an additional control, bacterial oxygen consumption was estimated by measuring oxygen partial pressure in the chamber containing freshwater without fish in it. After BaP was added and the water flow was stopped, the partial pressure of oxygen was measured continuously for 1 h as described previously in the Part I of this thesis on the oxygen consumption rates. Following the exposure, fish were removed for analysis of BaP content.

Sampling and analysis

Tissue BaP determination

To determine the tissue content of BaP, the fish were sacrificed by cephalic blow, weighed and homogenized as described earlier in Part I of this thesis in the section on the oxygen consumption rates. To increase the sensitivity for BaP quantitation by HPLC, the homogenates, enriched with 1,2 benz[a]anthracene as an internal standard, were saponified with concentrated KOH (50% w/w) and methanol following the modified method of Lebo et al. (1991) as described previously. Following the saponification, the subsamples were acidified to pH 5.9-6.1 with concentrated HCl and extracted with pentane

to separate BaP from the formed soaps. The extracts with small amount of isooctane were evaporated under nitrogen and run through a silica gel column to remove the coextracted residuals. The samples were eluted with pentane and dichloromethane, and each eluted fraction was collected and evaporated under nitrogen. [^{3}H]BaP, used to determine which eluted fractions contained most of BaP, was eluted in the same way by collecting fractions, evaporating them under nitrogen, resuspending in methanol, mixing with BCS, dark adapting for 24 h and counting for [^{3}H] radioactivity. The last two dichloromethane fractions contained majority of [^{3}H] BaP (83% efficiency) and were thus collected from the samples and resuspended in methanol and separated by HPLC using the 90/10 acetonitrile/water mixture and an internal standard of 1,2 benz[a]anthracene.

Calculations

Recorded partial pressures of oxygen were converted into oxygen concentrations (mg O_2/L) as described previously in Part I of this thesis using the formulas listed by Cech (1990), oxygen solubility tables and water vapour pressure tables (Green and Carritt, 1967), measured partial pressures of oxygen in water, and total barometric pressures.

Oxygen consumption rates were estimated over the linear portion of the measurements (40-50 min) for the reasons outlined in the section on the effects of smoltification on oxygen consumption rates in Part I of this thesis. The recorded oxygen partial pressures were fitted into a regression line and the

regression slope was used as an estimate of oxygen consumption rate and was expressed in terms of wet weight over a 1 h period (mg O_2/kg/h) and subtracting the rates of bacterial oxygen consumption. The oxygen consumption rates represented total oxygen consumption by the 5 fish in a chamber. Due to a small size of replicates, statistical analysis of the data was not performed.

RESULTS

A. Uptake of BaP

In investigating the effects of salinity on the uptake of BaP by juvenile coho salmon parr and smolts, the experimental procedure followed that of Kennedy et al. (1989a), which was used in determination of the effects of temperature on the uptake, distribution, and metabolism of BaP in the gulf toadfish (*Opsanus beta*).

By following the disappearance of the BaP-derived radioactivity from the water, it was confirmed that less than 5 % of the [^{3}H]BaP added to the control flasks was lost due to adsorption onto the glass walls or by volatilization (Figure 1.4). The analysis of 15 ml water samples by HPLC, taken at the end of each experiment, revealed no BaP metabolites in the water indicating that no excretion of BaP metabolites occurred by the fish, thus eliminating possible re-exposure of the fish to radioactivity. Therefore, the loss of the chemical from the water was indicative of the uptake into fish which remained in the fish for the duration of the experiment.

The disappearance of [^{3}H]BaP from the water displayed an exponential decline in all salinity treatments as shown previously in Figure 1.4. The results of the two-way ANOVA indicate that, at the initial chemical concentration of 5 μg BaP/L, the uptake rates of the chemical at 0 $^{o}/_{oo}$ salinity level (0 ppt) were significantly higher (p <0.05) than the uptake rates of BaP at 20 $^{o}/_{oo}$ (20 ppt) independent of the time of sampling (Table 2.1).

Table 2.1. Differences between salinity treatments as determined by two-way ANOVA with repetition in the uptake rates of BaP by coho salmon independent of time of sampling.

Salinity	0 ‰	5 ‰	10 ‰	20 ‰
0 ‰	x	ns	ns	s
5 ‰	ns	x	ns	ns
10 ‰	ns	ns	x	ns
20 ‰	s	ns	ns	x

ns - not statistically significant at $p<0.05$
s - statistically significant at $p<0.05$
x - not compared

In general, at the initial chemical concentration of 5 μg BaP/L at most salinities except 10 $^{o}/_{oo}$ (10 ppt), uptake rates of BaP were lower at the start of the study and increased towards the end of the sampling period (Figure 2.1). The rates of BaP uptake in April and May were three to four times higher than the rates of BaP uptake in February and beginning of March. At the same initial BaP concentration, the uptake rates of BaP at 10 $^{o}/_{oo}$ salinity remained unchanged at all sampling dates.

B. Distribution of BaP

The analysis for radioactivity in several tissues of juvenile coho salmon exposed to BaP at different salinities during the parr-smolt transformation revealed a rapid distribution of [^{3}H]BaP after the initial uptake of the chemical.

The percent body burden of BaP-derived radioactivity in the tissues such as the gills, intestine, and the bile was significantly different at different salinities independent of time of sampling. However, the percent body burden of BaP in the tissues such as the brain, kidney, liver, skeletal muscle, stomach, visceral fat and skin was not affected by the exposure to different salinity levels, independent of time of sampling. The highest levels were found in the bile followed by the liver, intestine, gill, skin, muscle, kidney, stomach, visceral fat, and the brain.

The percent body burden of BaP-derived radioactivity in the gills of coho salmon parr and smolts at 10 $^{o}/_{oo}$ was significantly higher than at 20 $^{o}/_{oo}$ (Table 2.2). The percent body burden of BaP-derived radioactivity in the intestine

Figure 2.1. Uptake rates of BaP by coho salmon at different salinities during the parr-smolt transformation from February to June at an initial BaP concentration of 5 μg/L. Values are means ±SE for three fish. Values with common symbols are not significantly different between sampling periods at $p < 0.05$.

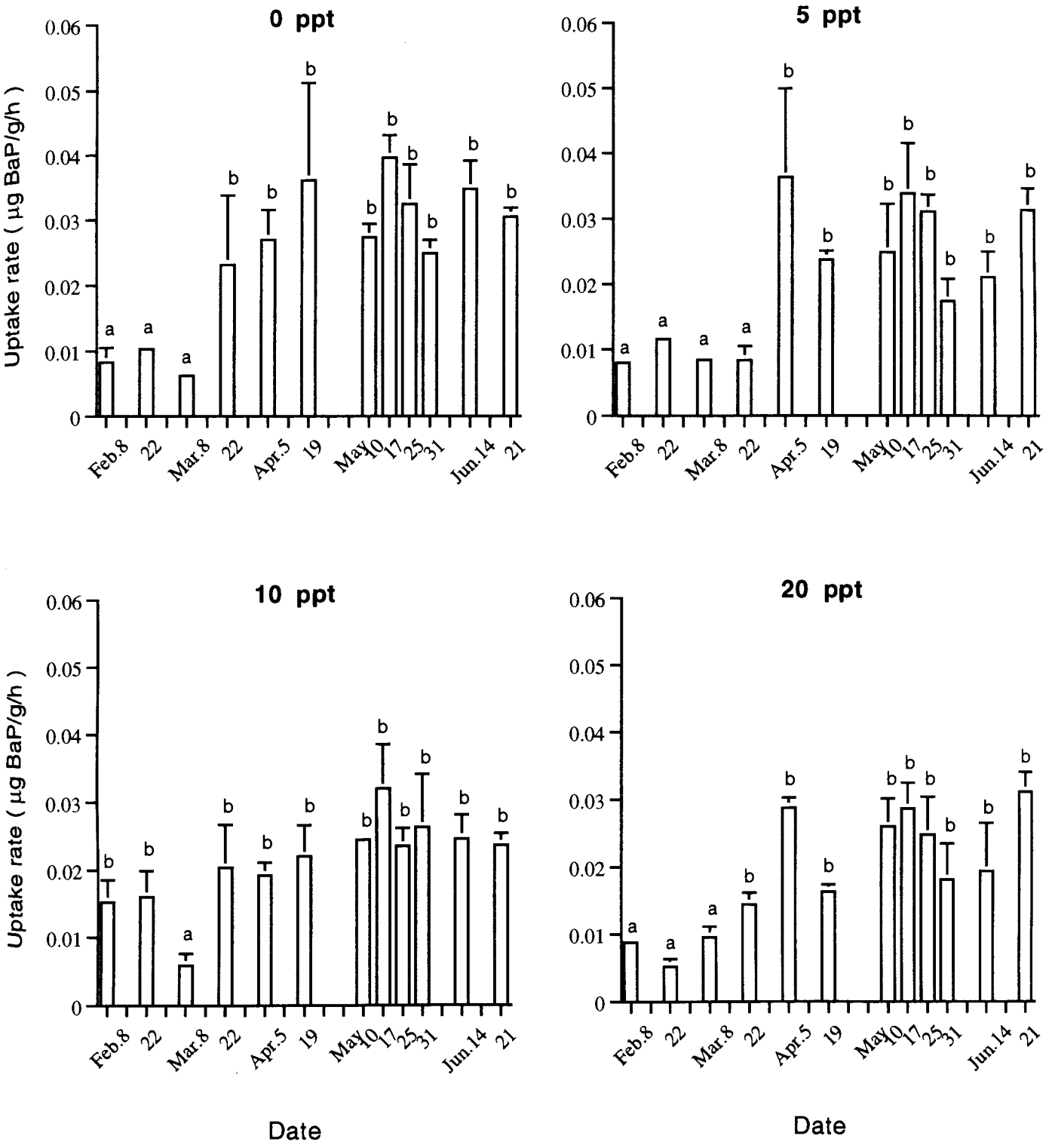

0 ppt
5 ppt
10 ppt
20 ppt
Uptake rate (µg BaP/g/h)
0.06
0.05
0.04
0.03
0.02
0.01
0
Feb.8
22
Mar.8
22
Apr.5
19
May 10
17
25
31
Jun.14
21
Date

Table 2.2. Differences between salinity treatments as determined by two-way ANOVA with repetition in the percent body burden of BaP-derived radioactivity in the gill of coho salmon independent of time of sampling.

Salinity	0 ‰	5 ‰	10 ‰	20 ‰
0 ‰	x	ns	ns	ns
5 ‰	ns	x	ns	ns
10 ‰	ns	ns	x	s
20 ‰	ns	ns	s	x

ns - not statistically significant at $p<0.05$
s - statistically significant at $p<0.05$
x - not compared

decreased with an increase in salinity (Table 2.3) where the values at 0 $^{o}/_{oo}$ were higher than the values at 5 $^{o}/_{oo}$, 10 $^{o}/_{oo}$, and 20 $^{o}/_{oo}$ and the values at 5 $^{o}/_{oo}$ were higher than the values at 20 $^{o}/_{oo}$. The percent body burden of BaP-derived radioactivity in the bile decreased with an increase in salinity (Table 2.4), values at 0 $^{o}/_{oo}$ were significantly lower than at 5 $^{o}/_{oo}$, 10 $^{o}/_{oo}$, and 20 $^{o}/_{oo}$.

Table 2.5 presents the results of the analysis of percent body burden of BaP-derived radioactivity of several tissues of coho salmon at different salinity levels during the parr-smolt transformation. The levels of percent body burden of BaP-derived radioactivity in the brain, kidney, liver, skeletal muscle, stomach, and visceral fat were similar to the levels found in the study on the distribution of BaP during smoltification outlined in Part I of this thesis.

As observed in the previous study during smoltification, there was a significant trend in the percent body burden of BaP-derived radioactivity in the skin which decreased with time from February to June (Figure 2.2). The percent body burden of BaP-derived radioactivity in the gill was low in the beginning of the study, increased during April sampling times (over 15 %) and declined afterwards (Figure 2.3). It was found that the percent body burden of BaP-derived radioactivity in the intestine was increasing with time and was highest in May (20 %) or June (28 %) (Figure 2.4). The percent body burden of BaP-derived radioactivity in the bile increased significantly from February to June when it reached over 60 % of the total body burden (Figure 2.5).

The content of BaP-derived radioactivity (μg BaP-equivalent/g of tissue) in the several tissues of juvenile coho salmon at different salinities during the

Table 2.3. Differences between salinity treatments as determined by two-way ANOVA with repetition in the percent body burden of BaP-derived radioactivity in the intestine of coho salmon independent of time of sampling.

Salinity	0 ‰	5 ‰	10 ‰	20 ‰
0 ‰	x	s	s	s
5 ‰	s	x	ns	s
10 ‰	s	ns	x	ns
20 ‰	s	s	ns	x

ns - not statistically significant at $p<0.05$
s - statistically significant at $p<0.05$
x - not compared

Table 2.4. Differences between salinity treatments as determined by two-way ANOVA with repetition in the percent body burden of BaP-derived radioactivity in the bile of coho salmon independent of time of sampling.

Salinity	0 ‰	5 ‰	10 ‰	20 ‰
0 ‰	x	s	s	s
5 ‰	s	x	ns	ns
10 ‰	s	ns	x	ns
20 ‰	s	ns	ns	x

ns - not statistically significant at $p<0.05$
s - statistically significant at $p<0.05$
x - not compared

Table 2.5. Percent body burden of total [^{3}H] radioactivity in several tissues of coho salmon at different salinity levels during the parr-smolt transformation. Values are means ± SE for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 μg (1 μCi)/L. Values with common symbols are significantly different between sampling periods at $p<0.05$.

Tissue	Date											
	Feb. 8	Feb. 22	Mar. 8	Mar. 22	Apr. 5	Apr. 19	May 10	May 17	May 25	May 31	Jun. 14	Jun. 21
Brain												
0 ‰	0.11^{a} ±0.03	1.08^{a-j} ±0.30	0.32^{b} ±0.18	0.21^{c} ±0.11	0.21^{d} ±0.21	0.33^{e} ±0.14	0.45 ±0.28	0.30^{f} ±0.13	0.27^{g} ±0.03	0.05^{h} ±0.04	0^{i} ±0	0.10^{j} ±0.04
5 ‰	$0.11^{a,h}$ ±0.01	1.31^{a-g} ±0.73	1.31^{h-o} ±0.46	0.26 ±0.26	0.35 ±0.32	0.32^{j} ±0.04	$0.23^{b,j}$ ±0.12	$0.18^{c,k}$ ±0.16	$0.19^{d,l}$ ±0.11	$0^{e,m}$ ±0	$0^{f,n}$ ±0	$0.01^{g,o}$ ±0.01
10 ‰	$0.13^{a,f}$ ±0.00	0.72^{a-e} ±0.40	0.69^{f-k} ±0.11	0.18^{g} ±0.12	$0.06^{b,h,l}$ ±0.06	0.45 ±0.18	0.41 ±0.16	0.56^{i-o} ±0.26	0.24 ±0.18	$0^{c,i,m}$ ±0	$0.01^{d,j,n}$ ±0.01	$0.03^{e,k,o}$ ±0.03
20 ‰	$0.15^{a,i}$ ±0.01	0.70^{a-h} ±0.07	0.68^{i-o} ±0.25	0.47 ±0.13	$0^{b,j,p}$ ±0	0.22^{c} ±0.07	$0.15^{d,k}$ ±0.08	$0^{e,l,q}$ ±0	0.81^{p-r} ±0.64	$0.11^{f,m}$ ±0.08	$0^{g,n,r}$ ±0	$0.14^{h,o}$ ±0.08
Kidney												
0 ‰	2.37 ±0.49	1.73 ±0.62	1.26 ±0.10	1.75 ±0.42	1.80 ±0.45	2.19 ±0.14	$3.03^{a,b}$ ±0.58	$3.72^{c,d}$ ±1.11	$1.05^{a,c}$ ±0.14	$1.19^{b,d}$ ±0.24	1.45 ±0.34	1.41 ±0.48
5 ‰	1.32 ±0.15	2.28 ±0.12	2.30 ±0.27	2.05 ±0.37	1.55 ±0.41	$2.87^{a,b}$ ±0.44	2.51 ±0.34	$2.83^{c,d}$ ±0.23	$0.94^{a,c}$ ±0.16	1.30 ±0.18	$1.17^{b,d}$ ±0.15	1.63 ±0.15
10 ‰	1.53^{c} ±0.09	1.74^{d} ±0.34	1.66^{e} ±0.13	2.00^{f} ±0.11	1.93^{g} ±0.30	2.40^{h} ±0.47	$3.32^{a,b}$ ±0.55	5.21^{c-l} ±1.19	2.24^{i} ±0.78	$1.15^{a,j}$ ±0.23	1.90^{k} ±0.44	$1.22^{b,l}$ ±0.15
20 ‰	2.16^{j} ±0.16	2.86^{a-c} ±0.75	1.67^{k} ±0.43	1.61^{l} ±0.41	1.80^{m} ±0.10	3.04^{d-f} ±0.46	3.09^{g-i} ±0.54	3.51^{j-p} ±0.42	$1.08^{a,d,g,n}$ ±0.32	2.70 ±0.81	$1.08^{b,e,h,o}$ ±0.35	$1.44^{c,f,i,p}$ ±0.22

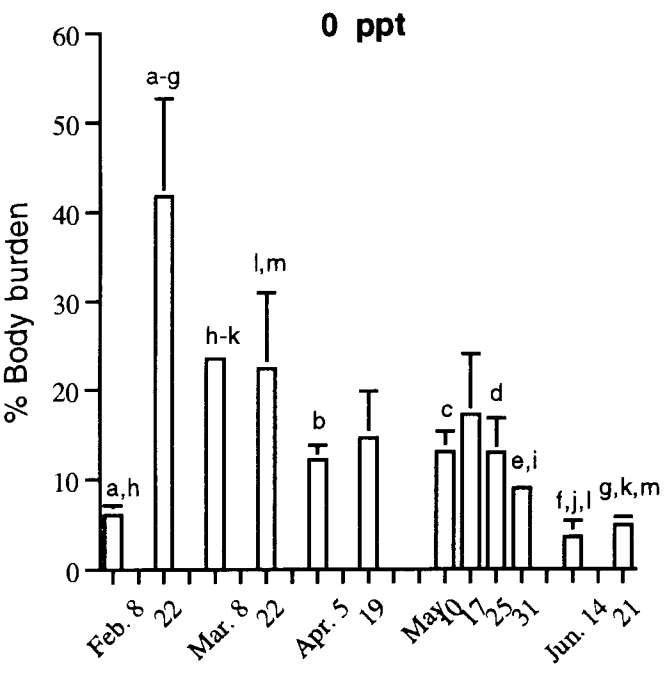
Table 2.5. cont.

Tissue	Date											
	Feb. 8	Feb. 22	Mar. 8	Mar. 22	Apr. 5	Apr. 19	May 10	May 17	May 25	May 31	Jun. 14	Jun. 21
Liver												
0 ‰	19.38 ±9.92	14.40 ±3.92	22.40 ±2.17	25.41[a] ±4.29	19.03 ±6.12	15.90 ±1.60	18.47 ±2.05	23.95 ±6.25	10.75[a] ±1.46	18.64 ±1.25	14.86 ±3.80	15.59 ±1.55
5 ‰	23.86 ±4.74	15.46 ±4.08	34.59[a,c] ±11.89	24.01 ±1.89	24.15 ±4.34	10.98[a] ±1.29	21.79 ±6.83	20.49 ±1.14	11.32[b] ±1.79	22.40 ±3.10	13.14[c] ±1.27	18.03 ±1.45
10 ‰	21.79 ±4.54	19.30 ±3.02	29.62[a,b] ±1.31	28.52[c,d] ±2.67	21.43 ±1.82	18.65 ±3.42	14.04[a,c] ±0.84	20.12 ±6.36	19.32 ±5.58	22.93 ±5.03	18.29 ±4.41	15.19[b,d] ±0.35
20 ‰	24.04 ±7.80	25.06 ±7.34	29.57[a,b] ±1.21	22.76 ±1.58	14.49[a] ±0.22	18.76 ±5.40	15.13[b] ±0.87	27.79 ±3.55	20.57 ±3.68	25.04 ±5.65	20.02 ±4.23	16.17 ±3.58
Muscle												
0 ‰	15.98 ±6.17	18.36 ±9.30	13.71 ±2.56	4.97 ±2.70	11.83 ±11.37	6.17 ±4.74	15.06 ±5.57	4.43 ±2.18	0 ±0	0.62 ±0.62	10.01 ±9.76	7.32 ±4.71
5 ‰	17.05[a-d] ±5.14	3.72 ±1.90	15.92[c-h] ±5.08	6.06 ±4.40	0.21[a,e] ±0.21	8.01 ±5.37	4.83 ±0.96	6.40 ±3.12	1.67[b,f] ±1.67	4.36 ±0.92	1.86[c,g] ±0.75	1.42[d,h] ±0.71
10 ‰	12.88 ±0.75	12.34 ±2.09	17.76[a,f] ±8.89	13.55 ±5.05	0.14[a,g] ±0.14	4.57[b] ±0.51	5.16[c] ±1.22	3.50[d] ±1.83	10.67 ±4.79	0.34[e,h] ±0.34	17.95[g,h] ±14.50	1.29[f] ±0.70
20 ‰	31.45[a-d] ±10.50	9.81 ±8.24	9.20 ±4.71	5.61[a,e] ±5.09	25.01[e-h] ±9.73	1.86[b,f] ±0.55	20.13 ±11.59	13.19 ±6.49	8.48 ±8.19	3.17[c,g] ±3.04	11.28 ±9.98	4.09[d,h] ±1.77

Table 2.5. cont.

Tissue	Date											
	Feb. 8	Feb. 22	Mar. 8	Mar. 22	Apr. 5	Apr. 19	May 10	May 17	May 25	May 31	Jun. 14	Jun. 21
Stomach												
0 ‰	1.90 ±1.00	2.88 [a-f] ±0.77	0.83 [a] ±0.20	0.72 [b] ±0.36	1.85 ±0.89	1.70 ±0.98	1.25 ±0.80	2.51 [g-j] ±1.19	0.44 [c,g] ±0.25	0.23 [d,h] ±0.16	0.32 [e,i] ±0.14	0.27 [f,j] ±0.05
5 ‰	0.49 [a,f,k] ±0.01	2.32 [a-e] ±0.32	2.38 [f-j] ±0.14	1.51 ±1.02	2.94 [k-n] ±1.97	1.70 ±1.17	0.80 ±0.22	1.21 ±0.30	0.45 [b,g] ±0.36	0.31 [c,h,l] ±0.06	0.18 [d,i,m] ±0.04	0.20 [e,j,n] ±0.08
10 ‰	0.27 [a,c] ±0.05	1.29 ±0.39	1.71 [a-d] ±0.57	1.02 ±0.48	1.89 ±1.58	0.68 ±0.17	0.84 ±0.43	1.89 [e-h] ±0.92	1.21 ±0.86	0.28 [b,f] ±0.09	0.52 [c-g] ±0.15	0.31 [d,h] ±0.06
20 ‰	0.83 ±0.33	2.01 [a-c] ±0.51	1.33 ±0.36	0.38 [a,d] ±0.08	0.63 [e] ±0.37	1.43 ±0.24	1.52 ±0.72	2.31 [d-h] ±0.73	0.75 ±0.46	0.22 [b,f] ±0.09	0.54 [g] ±0.33	0.25 [c,h] ±0.08
Visceral fat												
0 ‰	3.92 ±2.45	1.52 ±0.73	0.72 [a] ±0.24	1.86 ±1.34	3.53 ±0.95	6.36 [a-d] ±3.25	3.00 ±0.84	1.95 ±1.27	1.81 ±0.81	0.66 [b] ±0.45	0.93 [c] ±0.09	0.63 [d] ±0.14
5 ‰	0.56 [a,c] ±0.06	0.52 [b,f] ±0.08	3.00 ±0.42	1.69 ±1.41	4.08 [a-d] ±2.38	6.78 [e-j] ±2.87	2.11 ±0.18	2.44 ±1.07	0.49 [c,g] ±0.20	0.92 [h] ±0.26	0.62 [d,i] ±0.28	0.74 [j] ±0.37
10 ‰	0.29 [a,f] ±0.16	0.27 [b,g] ±0.11	1.75 ±0.82	1.94 ±0.79	4.78 [a-e] ±2.07	5.21 [f-j] ±2.88	2.09 ±0.32	2.08 ±0.79	0.74 [c,h] ±0.28	1.46 ±0.36	0.29 [d,i] ±0.13	0.31 [e,j] ±0.09
20 ‰	1.13 ±0.38	0.26 [a,c] ±0.22	1.40 ±0.88	0.39 [b,f] ±0.05	3.39 [a-d] ±1.90	4.31 [e-k] ±1.36	1.04 ±0.69	0.98 [g] ±0.26	0.42 [c,h] ±0.09	0.74 [i] ±0.41	0.39 [d,j] ±0.17	0.70 [k] ±0.40

Figure 2.2. Percent body burden of BaP-derived radioactivity in coho skin at different salinities during the parr-smolt transformation from February to June. Values are the means ±SE for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 µg (1 µCi)/L. Values with common symbols are significantly different between sampling periods at p <0.05.


0 ppt
% Body burden
60
50
40
30
20
10
0
a,h
a-g
h-k
l,m
b
c
d
e,i
f,j,l
g,k,m
Feb. 8
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Mar. 8
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Apr. 5
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May 10
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Jun. 14
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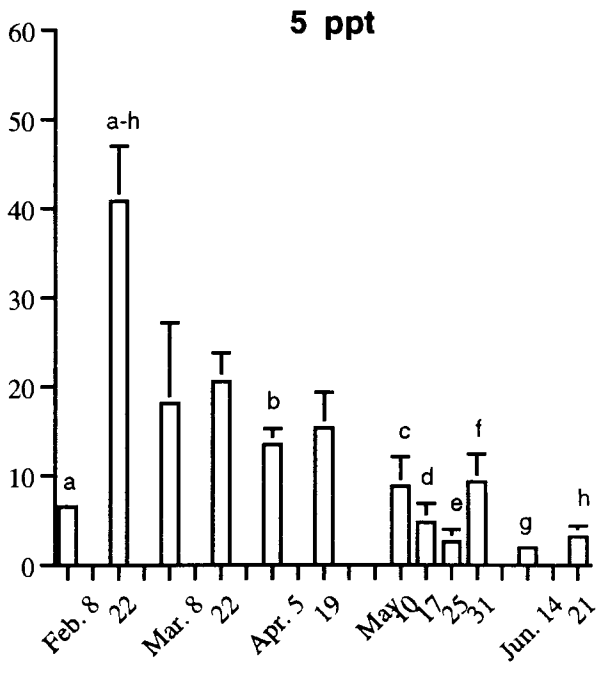
5 ppt
60
50
40
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Feb. 8
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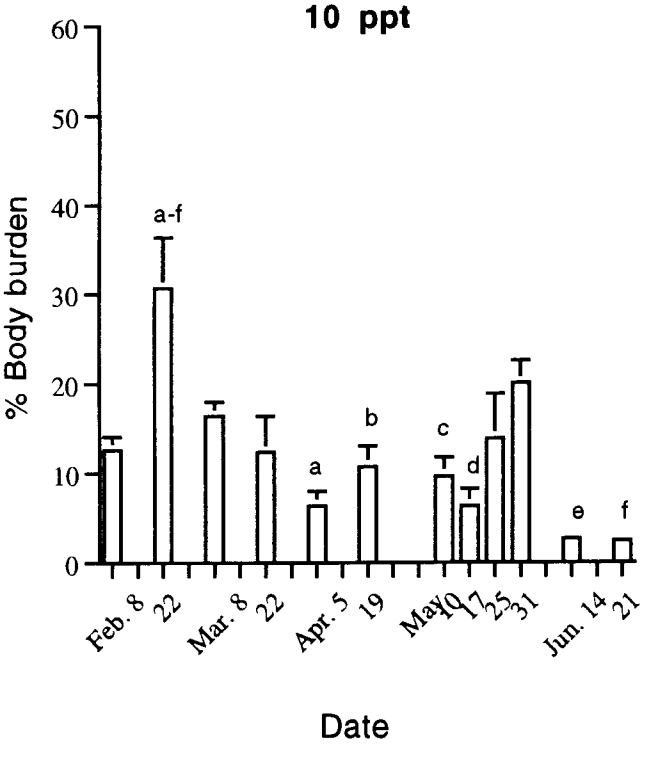
Mar. 8
22
Apr. 5
19
May 10
17
25
31
Jun. 14
21


10 ppt
% Body burden
60
50
40
30
20
10
0
a-f
a
b
c
d
e
f
Feb. 8
22
Mar. 8
22
Apr. 5
19
May 10
17
25
31
Jun. 14
21
Date

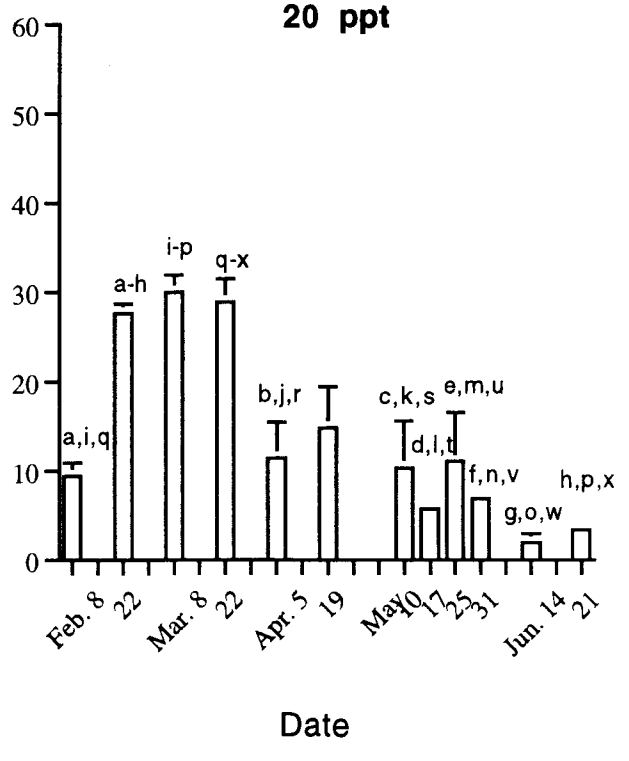

20 ppt
60
50
40
30
20
10
0
a,i,q
a-h
i-p
q-x
b,j,r
c,k,s
d,l,t
e,m,u
f,n,v
g,o,w
h,p,x
Feb. 8
22
Mar. 8
22
Apr. 5
19
May 10
17
25
31
Jun. 14
21
Date

Figure 2.3. Percent body burden of BaP-derived radioactivity in coho gill at different salinities during the parr-smolt transformation from February to June. Values are the means ±SE for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 μg (1 μCi)/L. Values with common symbols are significantly different between sampling periods at p <0.05.

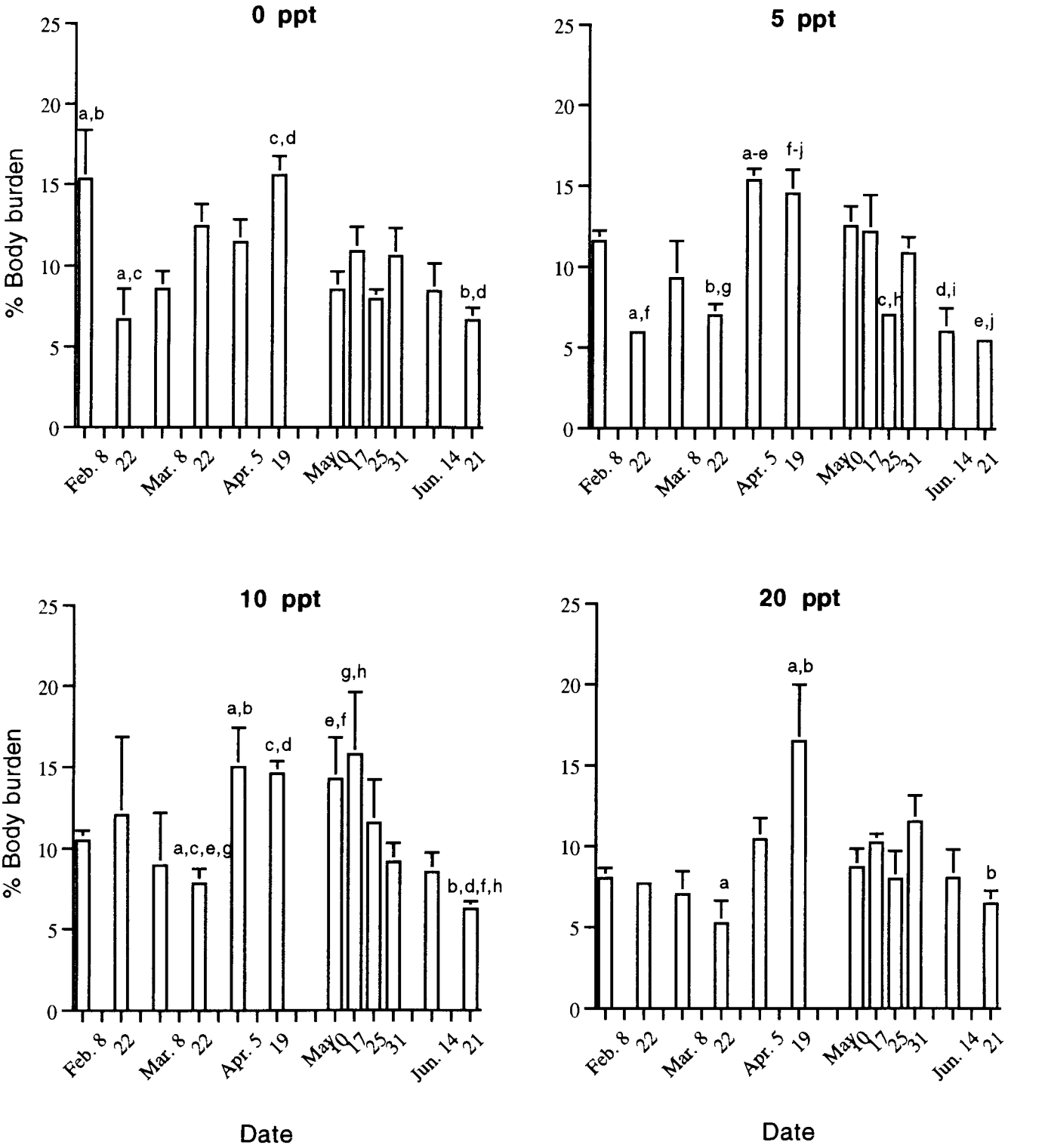

0 ppt
5 ppt
10 ppt
20 ppt
% Body burden
Date
Feb. 8
22
Mar. 8
22
Apr. 5
19
May 10
17
25
31
Jun. 14
21
a,b
a,c
c,d
b,d
a,f
b,g
a-e
f-j
c,h
d,i
e,j
a,c,e,g
a,b
c,d
e,f
g,h
b,d,f,h
a
a,b
b

Figure 2.4. Percent body burden of BaP-derived radioactivity in coho intestine at different salinities during the parr-smolt transformation from February to June. Values are the means ±SE for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 µg (1 µCi)/L. Values with common symbols are significantly different between sampling periods at p <0.05.

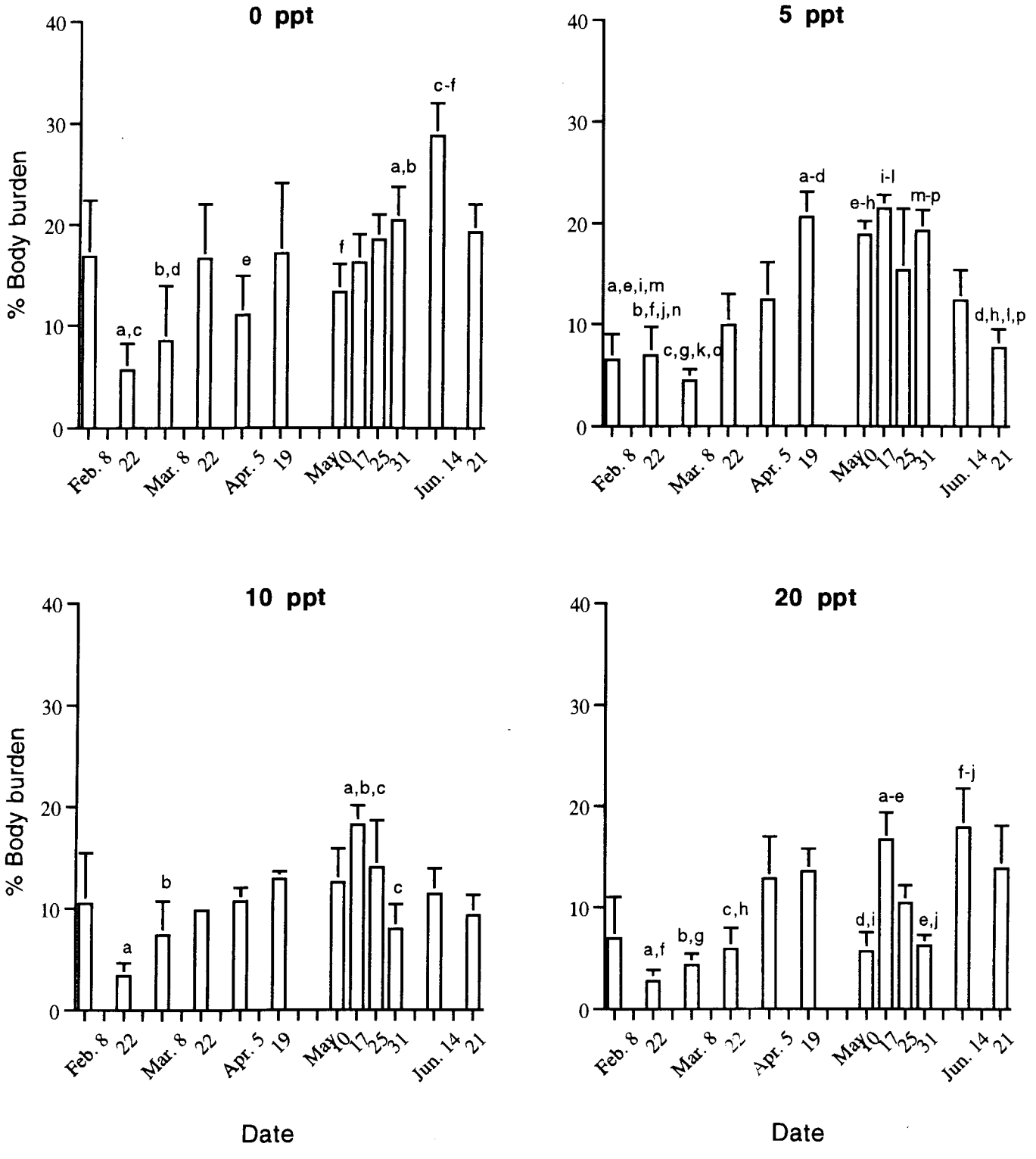
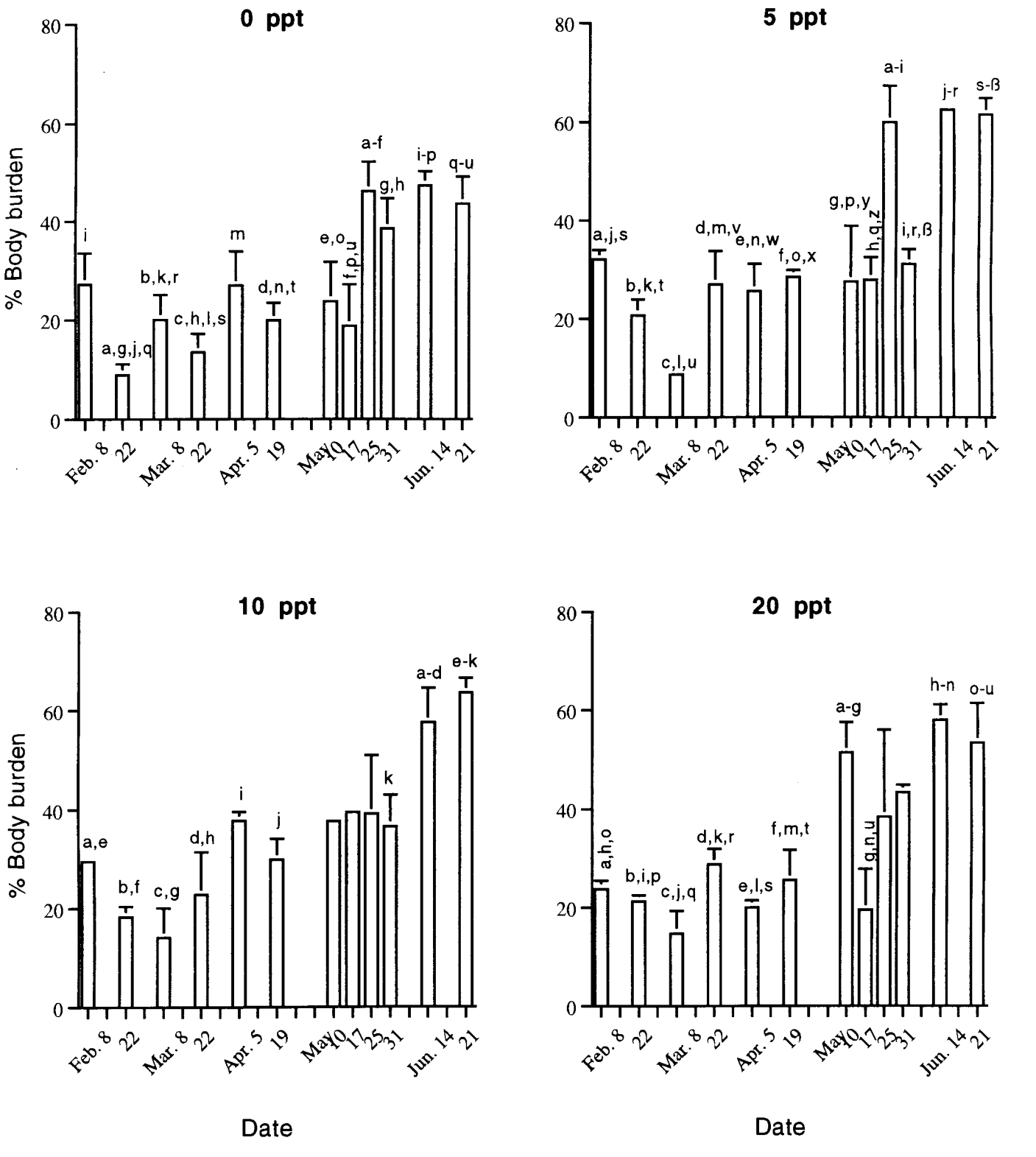

0 ppt
5 ppt
10 ppt
20 ppt
% Body burden
Date
40
30
20
10
0
Feb. 8
22
Mar. 8
22
Apr. 5
19
May 10
17
25
31
Jun. 14
21
a,c
b,d
e
f
a,b
c-f
a,e,i,m
b,f,j,n
c,g,k,o
a-d
e-h
i-l
m-p
d,h,l,p
a
b
a,b,c
c
a,f
b,g
c,h
d,i
a-e
e,j
f-j

Figure 2.5. Percent body burden of BaP-derived radioactivity in coho bile at different salinities during the parr-smolt transformation from February to June. Values are the means ±SE for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 µg (1 µCi)/L. Values with common symbols are significantly different between sampling periods at p <0.05.


0 ppt
5 ppt
10 ppt
20 ppt
% Body burden
Date
Feb. 8
22
Mar. 8
22
Apr. 5
19
May 10
17
25
31
Jun. 14
21
i
a,g,j,q
b,k,r
c,h,l,s
m
d,n,t
e,o
f,p,u
a-f
g,h
i-p
q-u
a,j,s
b,k,t
c,l,u
d,m,v
e,n,w
f,o,x
g,p,y
h,q,z
a-i
i,r,ß
j-r
s-ß
a,e
b,f
c,g
d,h
i
j
k
a-d
e-k
a,h,o
b,i,p
c,j,q
d,k,r
e,l,s
f,m,t
g,n,u
a-g
h-n
o-u

parr-smolt transformation revealed no salinity treatment effect in the tissues such as the brain, kidney, liver, skeletal muscle, stomach, and visceral fat independent of time of sampling. However, tissues such as the skin, gill and intestine have shown significant differences in the BaP-derived radioactivity content at different salinites independent of time of sampling. The highest concentrations of BaP-derived radioactivity were found in the liver followed by the intestine, skin, gill, kidney, stomach, visceral fat, brain and skeletal muscle.

BaP-derived radioactivity content of the skin decreased with an increase in salinity and was higher at 0 $^{o}/_{oo}$ salinity than at 5, 10 and 20 $^{o}/_{oo}$ (Table 2.6). The percent body burden of BaP-derived radioactivity in the gills decreased with salinity as well (Table 2.7) where the values at 0 $^{o}/_{oo}$ were significantly higher than the values at 20 $^{o}/_{oo}$. Similarly, BaP-derived radioactivity content in the intestine decreased with salinity (Table 2.8) where the values at 0 $^{o}/_{oo}$ were higher than at 5 $^{o}/_{oo}$, 10 $^{o}/_{oo}$, and 20 $^{o}/_{oo}$.

Table 2.9 summarizes the results of the analysis of BaP-derived radioactivity content of several tissues of coho salmon at different salinity levels during the parr-smolt transformation. BaP-derived radioactivity content in the brain, kidney, liver, skeletal muscle, stomach, and visceral fat was similar to the findings of the study on the distribution of BaP during smoltification.

BaP-derived radioactivity in the skin showed a significant trend in the decline of levels with time (Figure 2.6). BaP-derived radioactivity in the gill increased until April and May and decreased afterwards (Figure 2.7). At isotonic salinity (10 $^{o}/_{oo}$) the values were not significantly different between

Table 2.6. Differences between salinity treatments as determined by two-way ANOVA with repetition in the BaP-derived radioactivity content in the skin of coho salmon independent of time of sampling.

Salinity	0 ‰	5 ‰	10 ‰	20 ‰
0 ‰	x	s	s	ns
5 ‰	s	x	ns	ns
10 ‰	s	ns	x	ns
20 ‰	ns	ns	ns	x

ns - not statistically significant at $p<0.05$
s - statistically significant at $p<0.05$
x - not compared

Table 2.7. Differences between salinity treatments as determined by two-way ANOVA with repetition in BaP-derived radioactivity content in the gill of coho salmon independent of time of sampling.

Salinity	0 ‰	5 ‰	10 ‰	20 ‰
0 ‰	x	ns	ns	s
5 ‰	ns	x	ns	ns
10 ‰	ns	ns	x	ns
20 ‰	s	ns	ns	x

ns - not statistically significant at $p<0.05$
s - statistically significant at $p<0.05$
x - not compared

Table 2.8. Differences between salinity treatments as determined by two-way ANOVA with repetition in BaP-derived radioactivity content in the intestine of coho salmon independent of time of sampling.

Salinity	0 ‰	5 ‰	10 ‰	20 ‰
0 ‰	x	s	s	s
5 ‰	s	x	ns	ns
10 ‰	s	ns	x	ns
20 ‰	s	ns	ns	x

ns - not statistically significant at $p<0.05$
s - statistically significant at $p<0.05$
x - not compared

Table 2.9. BaP-derived radioactivity content (µg BaP-equivalent/g)* of several tissues of coho salmon at different salinity levels during the parr-smolt transformation. Values are means ± SE for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 µg (1 µCi)/L. Values with common symbols are significantly different between the sampling periods at $p<0.05$.

Tissue	Date											
	Feb. 8	Feb. 22	Mar. 8	Mar. 22	Apr. 5	Apr. 19	May 10	May 17	May 25	May 31	Jun. 14	Jun. 21
Brain												
0 ‰	0.01 ^{a} ±0.00	0.12 $^{a-j}$ ±0.04	0.04 ±0.03	0.01 ^{b} ±0.01	0.01 ^{c} ±0.01	0.02 ^{d} ±0.01	0.04 ±0.02	0.02 ^{e} ±0.01	0.02 ^{f} ±0.00	0 ^{g} ±0	0 ^{h} ±0	0.01 ^{i} ±0.00
5 ‰	0.01 ^{a,j} ±0.00	0.08 $^{a-i}$ ±0.02	0.11 $^{j-r}$ ±0.04	0.02 ^{b,k} ±0.02	0.03 ±0.03	0.02 ^{c,l} ±0.00	0.02 ^{d,m} ±0.01	0.01 ^{e,n} ±0.01	0.01 ^{f,o} ±0.01	0 ^{g,p} ±0	0 ^{h,q} ±0	0 ^{i,r} ±0
10 ‰	0.01 ^{a,f} ±0.00	0.05 $^{a-c}$ ±0.02	0.08 $^{f-k}$ ±0.02	0.01 ^{g} ±0.01	0 ^{b,h} ±0	0.03 ±0.01	0.02 ±0.01	0.02 ±0.01	0.02 ±0.02	0 ^{c,j} ±0	0 ^{d,j} ±0	0 ^{e,k} ±0
20 ‰	0.01 ^{a,j} ±0.00	0.05 $^{a-h}$ ±0.00	0.05 $^{j-p}$ ±0.01	0.03 ±0.00	0 ^{b,j,q} ±0	0.01 ^{c,k} ±0.00	0.02 ^{d,l} ±0.01	0 ^{e,m,r} ±0	0.06 $^{q-t}$ ±0.04	0 ^{f,n,s} ±0	0 ^{g,o,t} ±0	0.01 ^{h,p} ±0.01
Kidney												
0 ‰	0.15 ^{a} ±0.02	0.22 ±0.03	0.19 ±0.04	0.15 ^{b} ±0.01	0.16 ^{c} ±0.01	0.26 ±0.03	0.38 $^{a-f}$ ±0.07	0.36 ^{g,h} ±0.12	0.11 ^{d,g} ±0.02	0.11 ^{e,h} ±0.02	0.18 ^{f} ±0.03	0.25 ±0.07
5 ‰	0.12 ±0.02	0.25 ±0.08	0.28 ±0.09	0.15 ±0.02	0.17 ±0.05	0.23 ±0.03	0.30 ^{a,b} ±0.05	0.20 ±0.02	0.09 ^{a} ±0.02	0.10 ^{b} ±0.01	0.15 ±0.02	0.22 ±0.05
10 ‰	0.15 ±0.01	0.14 ±0.03	0.23 ±0.03	0.20 ±0.01	0.09 ^{a,c} ±0.01	0.22 ±0.05	0.32 ^{a,b} ±0.04	0.33 ^{c,d} ±0.11	0.15 ±0.01	0.11 ^{b,d} ±0.03	0.24 ±0.05	0.22 ±0.03
20 ‰	0.16 ±0.01	0.26 ±0.04	0.20 ±0.04	0.13 ^{a} ±0.03	0.13 ^{b} ±0.03	0.26 ±0.06	0.40 $^{a-d}$ ±0.08	0.28 ±0.07	0.13 ^{c} ±0.04	0.15 ±0.05	0.12 ^{d} ±0.01	0.22 ±0.05

Table 2.9. cont.

Tissue	Date											
	Feb. 8	Feb. 22	Mar. 8	Mar. 22	Apr. 5	Apr. 19	May 10	May 17	May 25	May 31	Jun. 14	Jun. 21
Liver												
0 ‰	0.84[a] ±0.14	1.65 ±0.14	3.16[a,b] ±0.81	2.24 ±0.77	1.30 ±0.28	1.76 ±0.40	2.15 ±0.41	1.75 ±0.12	0.78[b] ±0.17	1.38 ±0.12	1.40 ±0.26	1.82 ±0.36
5 ‰	1.34[a] ±0.36	1.78 ±0.87	2.93[a-c] ±0.11	1.59 ±0.19	2.05 ±0.91	1.47 ±0.84	2.37 ±0.91	1.15[b] ±0.21	0.95[c] ±0.19	1.33[d] ±0.38	1.36[c] ±0.25	1.72 ±0.17
10 ‰	1.44[a] ±0.17	1.31[b] ±0.05	3.75[a-g] ±0.90	2.59 ±0.50	1.06[c] ±0.21	1.42[d] ±0.29	1.06[e] ±0.08	0.83[f] ±0.16	1.07[g] ±0.13	1.52 ±0.56	1.53 ±0.29	1.97 ±0.09
20 ‰	1.19[a] ±0.29	1.97 ±0.45	3.43[a-c] ±0.98	1.73 ±0.21	1.13[b] ±0.18	1.71 ±0.38	1.97 ±0.53	1.59 ±0.29	1.90 ±0.28	0.92[c] ±0.09	1.70 ±0.16	1.90 ±0.51
Muscle												
0 ‰	0.02 ±0.01	0.04[a,b] ±0.02	0.04[c,d] ±0.01	0.01 ±0.01	0.03 ±0.03	0.01 ±0.01	0.04[e,f] ±0.01	0.01 ±0.00	0[a,c,e] ±0	0[b,d,f] ±0	0.02 ±0.02	0.02 ±0.01
5 ‰	0.02 ±0.00	0.01 ±0.01	0.04[a-d] ±0.02	0.01 ±0.01	0[a] ±0	0.01 ±0.01	0.01 ±0.00	0.01 ±0.00	0[b] ±0	0.01 ±0.00	0[c] ±0	0[d] ±0
10 ‰	0.02 ±0.00	0.01 ±0.00	0.05[a-c] ±0.02	0.02 ±0.01	0[a] ±0	0.01 ±0.00	0.01 ±0.00	0.01 ±0.00	0.01 ±0.01	0[b] ±0	0.04 ±0.03	0[c] ±0
20 ‰	0.04[a,b] ±0.02	0.02 ±0.02	0.02 ±0.02	0.01 ±0.01	0.04[c,d] ±0.02	0[a,c,e] ±0	0.06[e,f] ±0.05	0.01 ±0.01	0.01 ±0.01	0[b,d,f] ±0	0.01 ±0.01	0.01 ±0.01

Table 2.9. cont.

Tissue	Date											
	Feb. 8	Feb. 22	Mar. 8	Mar. 22	Apr. 5	Apr. 19	May 10	May 17	May 25	May 31	Jun. 14	Jun. 21
Stomach												
0 ‰	0.10 ±0.04	0.38 [a-c] ±0.13	0.13 ±0.05	0.04 [a] ±0.01	0.15 ±0.09	0.20 ±0.12	0.16 ±0.11	0.22 ±0.10	0.04 [b] ±0.02	0.01 [c] ±0.01	0.03 [d] ±0.01	0.04 [c] ±0.01
5 ‰	0.03 [a,c] ±0.00	0.24 [a-d] ±0.11	0.25 [c-i] ±0.07	0.09 ±0.05	0.15 ±0.10	0.11 ±0.06	0.07 ±0.01	0.07 ±0.02	0.03 [f] ±0.03	0.02 [b,g] ±0.01	0.02 [e,h] ±0.01	0.02 [d,i] ±0.01
10 ‰	0.02 [a] ±0.01	0.09 ±0.03	0.21 [a,b] ±0.07	0.09 ±0.05	0.07 ±0.05	0.05 ±0.01	0.07 ±0.03	0.12 ±0.08	0.06 ±0.03	0.02 [b] ±0.01	0.05 ±0.01	0.05 ±0.01
20 ‰	0.06 ±0.02	0.19 [a-c] ±0.07	0.16 ±0.07	0.03 [a] ±0.01	0.04 ±0.02	0.11 ±0.03	0.23 [d] ±0.16	0.14 ±0.03	0.06 ±0.03	0.01 [b,d] ±0.00	0.04 ±0.02	0.03 [c] ±0.01
Visceral fat												
0 ‰	0.10 ±0.07	0.07 [a,d] ±0.04	0.03 [b,e] ±0.01	0.03 [e,f] ±0.01	0.14 ±0.06	0.31 [a-c] ±0.11	0.20 ±0.09	0.20 ±0.09	0.12 ±0.04	0.18 ±0.13	0.09 ±0.02	0.33 [d-f] ±0.10
5 ‰	0.01 ±0.00	0.02 ±0.01	0.13 ±0.06	0.03 ±0.03	0.07 ±0.04	0.18 ±0.06	0.11 ±0.02	0.13 ±0.04	0.05 ±0.03	0.15 ±0.11	0.05 ±0.02	0.08 ±0.04
10 ‰	0.01 [a] ±0.01	0.01 [b] ±0.00	0.07 ±0.03	0.06 ±0.03	0.09 ±0.01	0.17 ±0.10	0.11 ±0.03	0.04 ±0.01	0.04 ±0.01	0.13 ±0.07	0.08 ±0.02	0.25 [a,b] ±0.14
20 ‰	0.02 ±0.01	0.01 [a] ±0.01	0.04 ±0.02	0.01 [b] ±0.00	0.15 ±0.11	0.13 ±0.03	0.13 ±0.09	0.05 ±0.01	0.04 ±0.01	0.07 ±0.02	0.04 ±0.02	0.47 [a,b] ±0.39

* BaP-equivalent reflects BaP-derived radioactivity of both BaP and BaP metabolites.

Figure 2.6. BaP-derived radioactivity content (μg of BaP-equivalent/g)* in the skin of coho salmon at different salinities during the parr-smolt transformation from February to June. Values are means ±SE for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 μg (1 μCi)/L. Values with common symbols are significantly different between sampling periods at p <0.05.

* BaP-equivalent reflects BaP-derived radioactivity of both BaP and BaP metabolites.

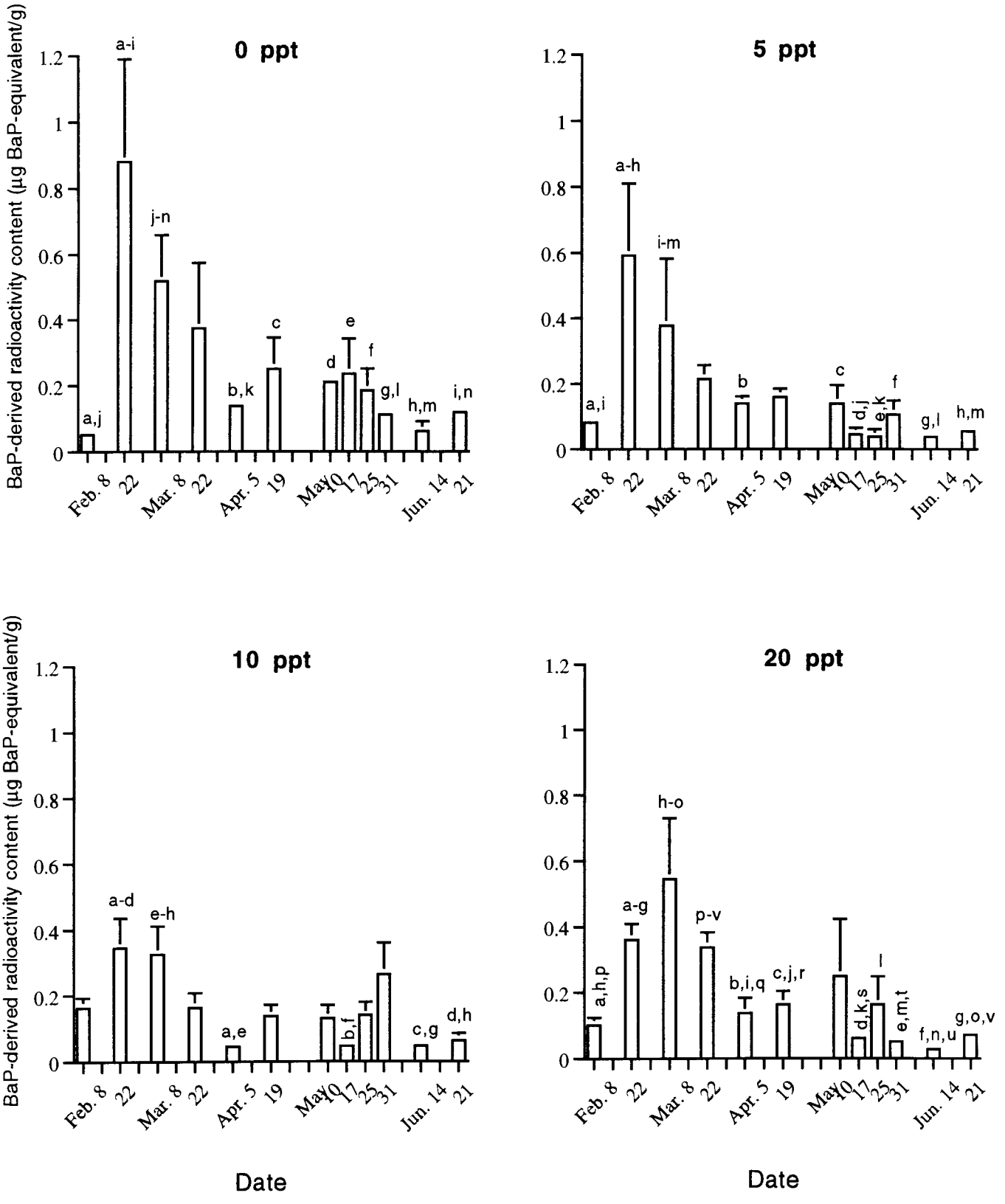
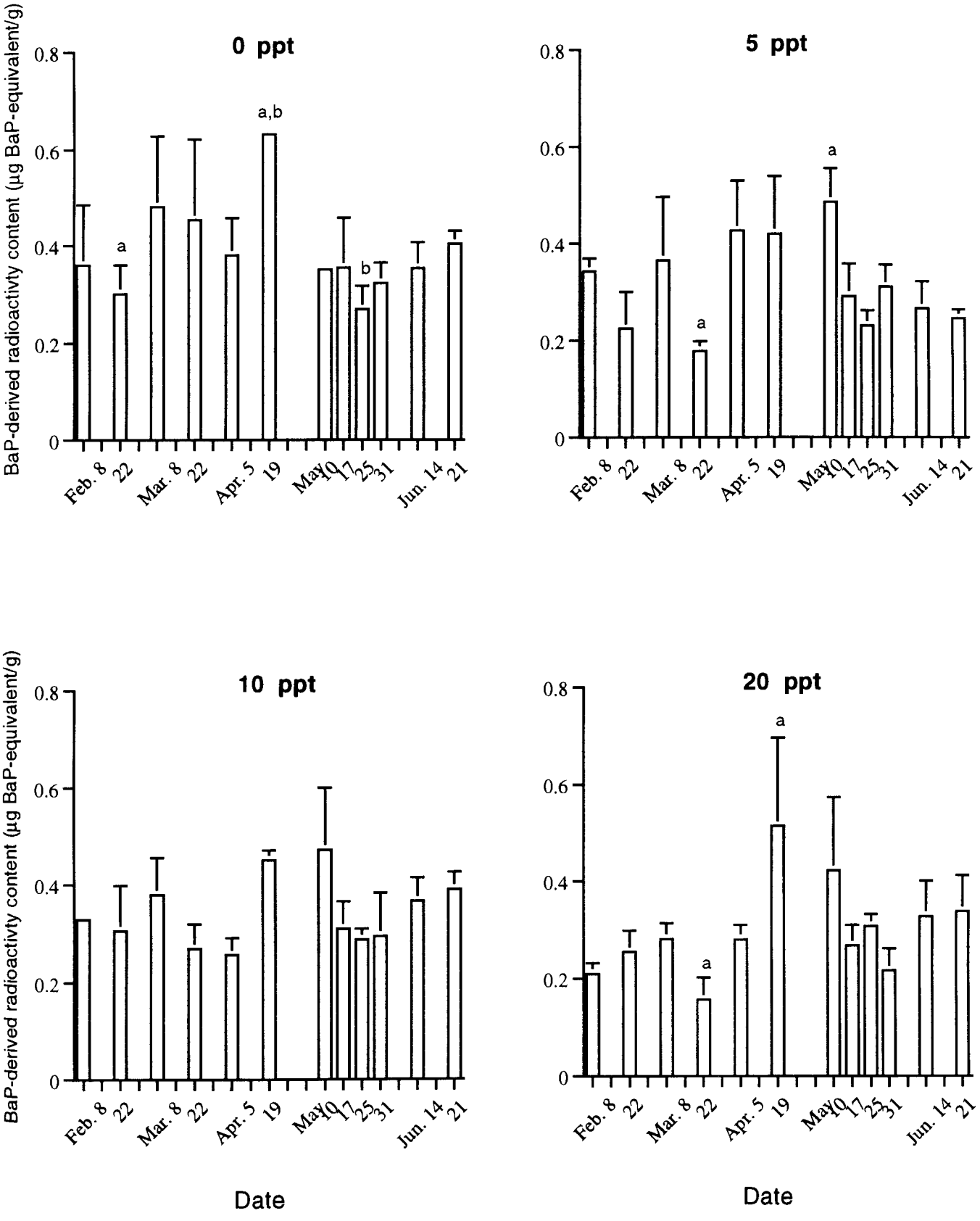

0 ppt
5 ppt
10 ppt
20 ppt
BaP-derived radioactivity content (µg BaP-equivalent/g)
1.2
1
0.8
0.6
0.4
0.2
0
a,j
a-i
j-n
b,k
c
d
e
f
g,l
h,m
i,n
a,i
a-h
i-m
b
c
d,j
e,k
f
g,l
h,m
a,h,p
a-d
e-h
a,e
b,f
c,g
d,h
a-g
h-o
p-v
b,i,q
c,j,r
d,k,s
l
e,m,t
f,n,u
g,o,v
Feb. 8
22
Mar. 8
22
Apr. 5
19
May 10
17
25
31
Jun. 14
21
Date

Figure 2.7. BaP-derived radioactivity content (μg of BaP-equivalent/g)* in the gills of coho salmon at different salinities during the parr-smolt transformation from February to June. Values are means ±SE for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 μg (1 μCi)/L. Values with common symbols are significantly different between sampling periods at p <0.05.

* BaP-equivalent reflects BaP-derived radioactivity of both BaP and BaP metabolites.


0 ppt
5 ppt
10 ppt
20 ppt
BaP-derived radioactivty content (µg BaP-equivalent/g)
0.8
0.6
0.4
0.2
0
a
a,b
b
Feb. 8
22
Mar. 8
22
Apr. 5
19
May 10
17
25
31
Jun. 14
21
Date

sampling times. It was found that BaP-derived radioactivity content in the intestine increased with time (Figure 2.8).

C. Metabolism of BaP

Metabolic patterns of BaP in coho salmon at different salinity levels were investigated by analyzing the contents of the bile during the parr-smolt transformation. The extraction of the bile contents with an organic solvent and incubation with hydrolytic enzymes revealed the presence of both Phase I and Phase II metabolites (Table 2.10). The metabolite classes extracted from the bile were: organic-soluble fraction, glucuronide conjugates, sulfate conjugates, other unidentified conjugates and unknown residues. There were no significant differences in the proportions of metabolite classes between different salinity treatments independent of time of sampling.

The percent of total [^{3}H]BaP-derived radioactivity in the bile representing Phase I metabolites varied between 22 - 52 % (Table 2.10). There was no apparent trend in the levels of radioactivity in Phase I at all salinity treatments between sampling times. Glucuronide conjugates represented 8 - 21 % of the total [^{3}H]BaP-derived radioactivity in the bile and sulfate conjugates ranged between 3 - 10 % of the total bile content. There were no apparent trends in the levels of glucuronide and sulfate conjugates during the parr-smolt transformation. The percent of other conjugates in the total bile was 13 - 17 % as in the previous study. Unknown residues and compounds constituted as

Figure 2.8. BaP-derived radioactivity content (μg of BaP-equivalent/g)* in the intestine of coho salmon at different salinities during the parr-smolt transformation from February to June. Values are means ±SE for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 μg (1 μCi)/L. Values with common symbols are significantly different between sampling periods at p <0.05.

* BaP-equivalent reflects BaP-derived radioactivity of both BaP and BaP metabolites.

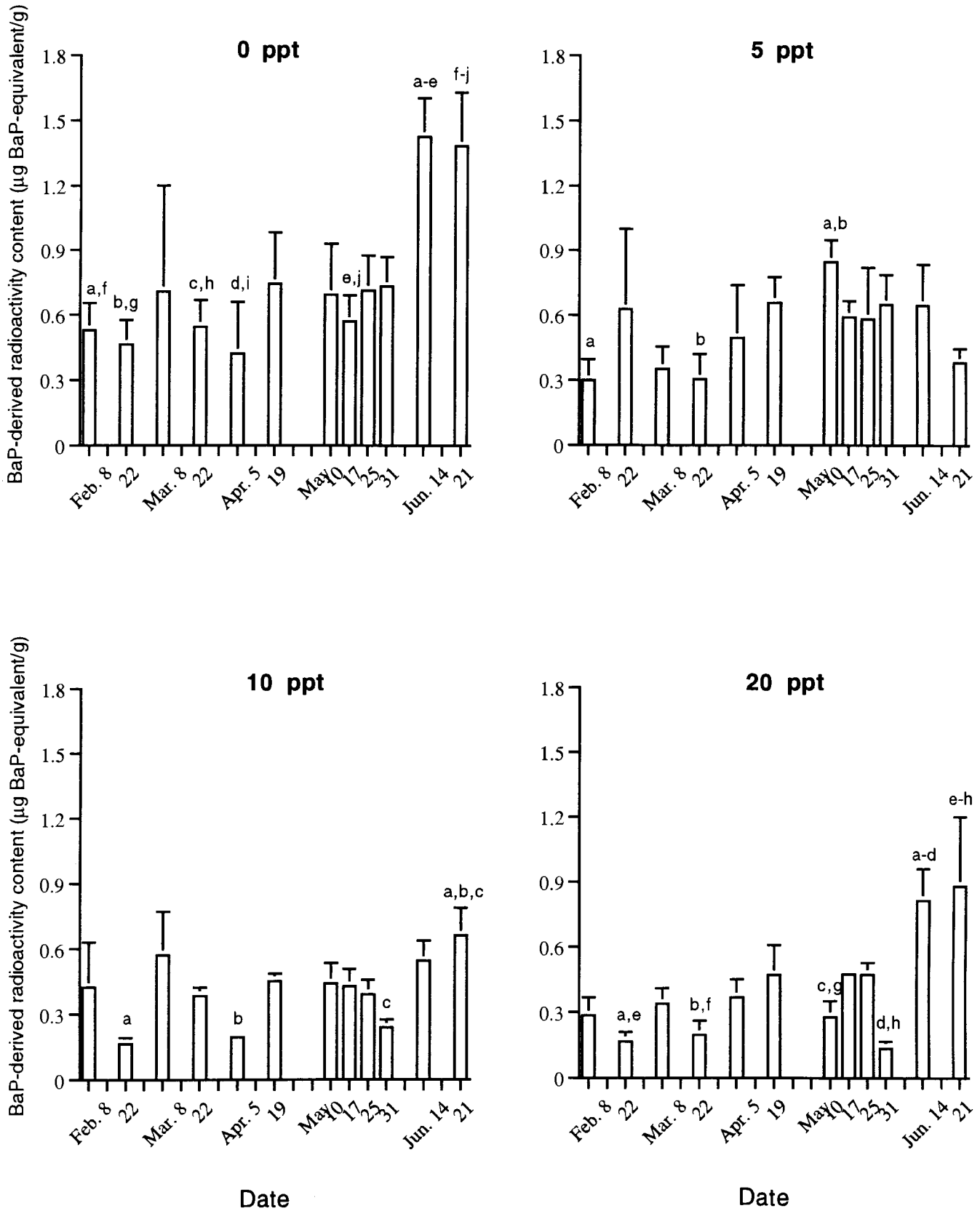

0 ppt
5 ppt
10 ppt
20 ppt
BaP-derived radioactivity content (µg BaP-equivalent/g)
1.8
1.5
1.2
0.9
0.6
0.3
0
a,f
b,g
c,h
d,i
e,j
a-e
f-j
a
b
a,b
a
b
a,b,c
a,e
b,f
c,g
d,h
a-d
e-h
Feb. 8
22
Mar. 8
22
Apr. 5
19
May 10
17
25
31
Jun. 14
21
Date

Table 2.10. Percent of total [^{3}H]BaP-derived radioactivity in several classes of metabolites in the bile of coho salmon at different salinity levels during the parr-smolt transformation. Values are means ± SE for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 µg (1 µCi)/L. Values with common symbols are significantly different between sampling periods at $p<0.05$.

Metabolite fraction	Date											
	Feb. 8	Feb. 22	Mar. 8	Mar. 22	Apr. 5	Apr. 19	May 10	May 17	May 25	May 31	Jun. 14	Jun. 21
Phase I												
0 ‰	34.32 ±4.02	51.89 ±1.43	41.05 ±3.19	40.80 ±8.08	38.79 ±4.17	31.02 ±1.86	51.92 ±4.86	39.50 ±2.49	30.01 ±3.09	33.39 ±2.33	33.26 ±2.41	38.56 ±3.57
5 ‰	22.18^{a,e} ±0.81	45.40^{a-d} ±8.90	45.27^{e-h} ±7.70	39.07 ±3.56	37.34 ±3.09	36.12 ±0.12	33.67 ±4.59	35.76 ±4.87	34.88 ±6.73	26.53^{b,f} ±3.47	27.51^{c,g} ±3.44	29.84^{d,h} ±2.35
10 ‰	25.40^{a,e} ±4.04	34.64 ±3.39	41.53 ±11.01	38.41 ±4.45	31.23 ±1.82	33.66 ±6.81	48.19^{a-d} ±5.45	33.63 ±2.62	44.97^{e-h} ±2.85	28.49^{b,f} ±2.10	29.91^{c,g} ±0.04	26.30^{d,h} ±2.91
20 ‰	31.67 ±2.75	45.18^{a,b} ±9.77	44.12^{c,d} ±9.56	28.63^{e} ±1.30	35.75 ±6.71	32.89 ±4.59	32.26 ±4.44	44.11^{e-g} ±5.87	37.52 ±0.81	25.33^{a,c,f} ±2.82	28.47^{b,d,g} ±0.24	35.09 ±4.00
Glucuronide conjugates												
0 ‰	12.24 ±2.96	9.18^{a,b} ±0.92	13.58 ±3.04	15.96 ±6.43	14.88 ±2.96	17.04^{a} ±1.29	12.50 ±2.82	14.62 ±1.41	14.60 ±2.48	17.16^{b} ±2.05	12.94 ±0.06	12.69 ±0.95
5 ‰	12.89 ±0.25	11.59 ±1.56	9.51^{a,b,c,d} ±2.15	12.56 ±1.64	14.13 ±1.62	12.89 ±0.85	16.88^{a} ±1.76	15.06 ±0.91	10.80 ±0.62	16.49^{b} ±1.94	17.87^{c} ±1.89	18.86^{d} ±4.02
10 ‰	16.08 ±3.74	16.93^{a} ±1.84	8.24^{a,b,c,d,e} ±1.46	13.17 ±1.53	15.67 ±0.76	16.62 ±3.75	10.00 ±1.99	17.80^{b} ±0.15	16.15^{c} ±0.87	14.69 ±1.62	21.51^{d} ±0.14	16.32^{e} ±1.93
20 ‰	13.91 ±2.29	12.00 ±2.87	10.30^{a,b} ±2.50	13.22 ±1.26	14.78 ±2.53	15.00 ±0.78	15.37 ±1.80	11.84 ±3.03	12.49 ±1.12	15.79 ±1.03	17.88^{a} ±0.20	20.29^{b} ±0.63

Table 2.10. cont.

Metabolite fraction	Date: Feb. 8	Feb. 22	Mar. 8	Mar. 22	Apr. 5	Apr. 19	May 10	May 17	May 25	May 31	Jun. 14	Jun. 21
Sulfate conjugates												
0 ‰	4.32 ±0.92	3.81 ±0.46	6.64 ±1.70	3.63 ±0.77	3.01[a] ±0.87	6.21 ±1.47	5.08 ±1.06	4.41 ±0.76	7.56[a] ±0.93	5.35 ±0.54	7.23 ±1.24	6.32 ±0.64
5 ‰	5.58 ±0.45	5.60 ±0.94	6.52 ±1.01	5.76 ±1.63	4.33[a] ±0.55	4.54[b] ±0.48	5.38 ±0.85	6.47 ±0.75	7.20 ±2.42	6.80 ±1.17	9.05[a,b] ±0.79	7.99 ±1.13
10 ‰	5.49 ±0.68	6.85 ±0.22	4.96 ±1.47	7.07 ±1.08	5.19 ±0.32	4.66[a] ±0.51	3.90[b] ±0.58	10.01[a,b] ±0.32	5.91 ±0.38	5.54 ±0.90	6.92 ±0.75	6.43 ±0.89
20 ‰	3.47[a,d,g] ±0.03	5.11 ±0.26	5.76 ±1.85	3.65[b,e,h] ±0.96	3.82[c,f,i] ±0.90	5.37 ±0.35	4.85 ±0.46	3.92 ±0.53	7.68[a,c] ±0.44	10.89[d,f] ±0.18	7.64[g,i] ±0.34	5.72 ±0.28
Other conjugates												
0 ‰	13.53[a,b] ±4.65	5.37[a] ±2.02	7.60 ±1.72	11.06 ±2.67	10.09 ±1.05	9.69 ±1.08	6.77 ±1.92	10.52 ±1.47	9.01 ±2.05	6.71 ±1.10	9.50 ±1.25	5.01[b] ±0.98
5 ‰	17.56[a,b] ±2.71	6.09[a] ±1.07	10.05 ±1.41	8.68 ±1.95	9.98 ±0.86	9.57 ±1.38	13.41 ±1.76	7.28[b] ±1.31	11.34 ±3.27	9.16 ±4.05	11.76 ±4.33	7.94 ±2.19
10 ‰	13.62[a,c] ±2.13	8.11 ±0.60	11.11 ±2.85	6.11 ±2.03	9.26 ±0.94	9.13 ±1.97	7.14 ±0.88	4.06[a] ±0.02	5.37[b] ±0.88	9.89 ±2.49	4.07[c] ±0.27	8.10 ±0.58
20 ‰	15.10[a,b] ±1.21	10.55 ±3.38	7.99 ±2.99	13.27 ±0.45	10.65 ±4.20	6.59[a] ±1.34	15.11 ±2.38	5.74[b] ±2.67	10.53 ±1.00	9.93 ±3.75	11.47 ±0.81	7.35 ±0.60

Table 2.10 cont.

Metabolite fraction	Date											
	Feb. 8	Feb. 22	Mar. 8	Mar. 22	Apr. 5	Apr. 19	May 10	May 17	May 25	May 31	Jun. 14	Jun. 21
Unknown												
0 ‰	35.58 ±4.52	29.73 ±3.91	31.11 ±2.25	28.53 ±0.42	33.23 ±1.81	36.03 ±0.94	24.37 ±1.44	30.94 ±1.22	38.81 ±2.61	37.39 ±2.89	37.06 ±0.14	37.41 ±2.97
5 ‰	41.77 ±2.11	31.31 ±6.11	28.64 ±4.35	33.92 ±2.62	34.20 ±2.42	36.88 ±0.16	30.64 ±0.36	35.43 ±2.45	35.77 ±2.62	40.99 ±4.28	33.80 ±1.68	35.35 ±3.17
10 ‰	39.41 ±1.16	33.46 ±1.56	34.15 ±7.50	35.24 ±4.52	38.64 ±2.63	35.92 ±2.03	30.77 ±2.66	34.49 ±2.81	27.58 ±0.72	41.47 ±3.25	37.58 ±1.21	42.84 ±1.75
20 ‰	35.84 ±3.85	27.15 ±3.42	31.82 ±2.90	41.21 ±0.98	34.98 ±0.99	40.14 ±4.08	32.40 ±8.16	34.37 ±2.36	31.77 ±0.41	38.04 ±0.61	34.52 ±0.72	31.53 ±4.12

high as 40 % of the total [^{3}H]BaP-derived radioactivity in the bile. There were no significant differences in the values of this class between the sampling dates.

Based on the retention times of available standards, reverse-phase HPLC analysis of organic-soluble fraction of the bile from fish exposed to BaP at different salinity levels during the parr-smolt transformation, identified the presence of several metabolites listed in Table 1.4. There were no significant differences in the proportions of 7,8,9,10 tetrol, 7,8-dihydrodiol, quinones, 3-OH and 9-OH BaP, and the parent compound between different salinities independent of time of sampling. However, the proportion of 9,10-dihydrodiol at 10 $^{o}/_{oo}$ was significantly higher than the proportion at 0, 5, and 20 $^{o}/_{oo}$ (Table 2.11).

Table 2.12 summarizes the results of HPLC analysis of organic-soluble fraction of the bile. The proportion of 7,8,9,10 tetrol varied considerably between the time of sampling (30 - 60 %). The proportion of 7,8-dihydrodiol in the Phase I was highest from February (40 %) to early April and decreased significantly in the remaining sampling months (below 10 %). The levels of 1,6 quinone, 3,6 quinone and 6,12 quinone, when grouped together, varied from 5 - 28 %. The levels of the parent compound were less than 10 %. There were no significant differences between the levels of 9,10-dihydrodiol in the Phase I (5 - 30 %) between the sampling times. The proportion of 3-OH and 9-OH BaP in the Phase I increased gradually from February to June and reached over 40 % of the total Phase I radioactivity (Figure 2.9).

Table 2.11. Differences between salinity treatments as determined by two-way ANOVA with repetition in the pecent of total Phase I radioactivity as 9,10 dihydrodiol in coho salmon independent of time of sampling.

Salinity	0 ‰	5 ‰	10 ‰	20 ‰
0 ‰	x	ns	s	ns
5 ‰	ns	x	s	ns
10 ‰	s	s	x	s
20 ‰	ns	ns	s	x

ns - not statistically significant at $p<0.05$
s - statistically significant at $p<0.05$
x - not compared

Table 2.12. Percent of total Phase I [^{3}H] radioactivity as several metabolites and BaP separated from the bile of coho salmon at different salinity levels during the parr-smolt transformation. Values are means ± SE for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 µg (1 µCi)/L. Values with common symbols are significantly different between sampling periods at $p<0.05$.

	Date											
Metabolite	Feb. 8	Feb. 22	Mar. 8	Mar. 22	Apr. 5	Apr. 19	May 10	May 17	May 25	May 31	Jun. 14	Jun. 21
7,8,9,10 tetrol												
0 ‰	39.16 [a-h] ±7.59	9.61 [a,i,q] ±2.02	28.57 [i-p] ±3.06	29.88 [q-x] ±8.59	22.88 ±0.58	9.17 [b,j,r] ±1.86	13.34 [c,k,s] ±0.54	9.96 [d,l,t] ±0.69	14.16 [e,m,u] ±1.50	10.15 [f,n,v] ±5.51	11.92 [g,o,w] ±4.38	12.44 [h,p,x] ±2.23
5 ‰	61.94 [a-k] ±2.45	3.08 [a] ±2.17	22.32 [b] ±3.06	20.07 [c] ±8.39	18.62 [d] ±1.76	10.54 [e] ±0.08	15.79 [f] ±3.43	15.26 [g] ±1.30	17.21 [h] ±1.18	10.02 [i] ±3.99	16.75 [j] ±1.04	10.00 [k] ±0.70
10 ‰	40.15 [a-h] ±2.06	4.98 [a,i] ±2.72	25.40 ±5.43	32.99 [i-p] ±6.10	20.53 ±2.35	14.36 [b,j] ±0.21	16.08 [c,k] ±0.51	17.88 [d,l] ±0.64	14.88 [e,m] ±0.96	14.96 [f,n] ±3.41	16.75 [g,o] ±1.27	13.52 [h,p] ±0.65
20 ‰	31.17 [a,b] ±0.28	8.07 [a,c] ±1.39	20.11 ±7.59	25.59 [c,d] ±1.31	23.30 ±2.40	10.02 [b,d] ±2.57	15.66 ±0.60	17.59 ±1.47	16.14 ±0.63	14.62 ±1.61	17.19 ±0.52	18.14 ±4.74
7,8 diol												
0 ‰	37.31 [a,b] ±15.03	42.13 [c-i] ±4.38	15.70 [c] ±1.61	19.15 ±5.11	20.67 ±2.92	9.14 [a,d] ±0.93	14.24 [e] ±2.05	19.96 ±2.00	11.40 [b,f] ±0.15	13.35 [g] ±2.28	15.17 [h] ±2.05	13.07 [i] ±4.13
5 ‰	17.99 ±4.04	40.52 [a-c] ±10.39	16.21 ±3.87	34.35 [f-j] ±15.51	36.44 [k-o] ±14.54	2.64 [a,f,k] ±1.51	9.44 [b,g,l] ±1.53	8.30 [c,h,m] ±1.75	11.56 [d,i,n] ±1.67	7.05 [e,j,o] ±1.64	16.61 ±0.96	18.36 ±1.31
10 ‰	24.02 [a-c] ±1.69	23.10 [d-f] ±3.20	21.40 [g-j] ±2.84	11.70 ±4.42	26.01 [j-l] ±0.00	15.59 ±1.58	10.96 [a,d,g,j] ±0.13	11.86 [b,e, ±0.22 h,k]	9.47 [c,f,i,l] ±2.65	16.18 ±2.07	16.94 ±2.88	14.45 ±2.55
20 ‰	26.34 [a,b] ±3.46	20.93 [c,d] ±2.23	21.26 [e,f] ±2.96	15.98 ±1.55	22.14 [g,h] ±2.12	12.75 ±2.58	10.09 [a,c,e,g] ±1.12	15.42 ±3.95	12.08 [b,d,f,h] ±0.90	12.78 ±2.04	13.72 ±1.39	16.18 ±5.58

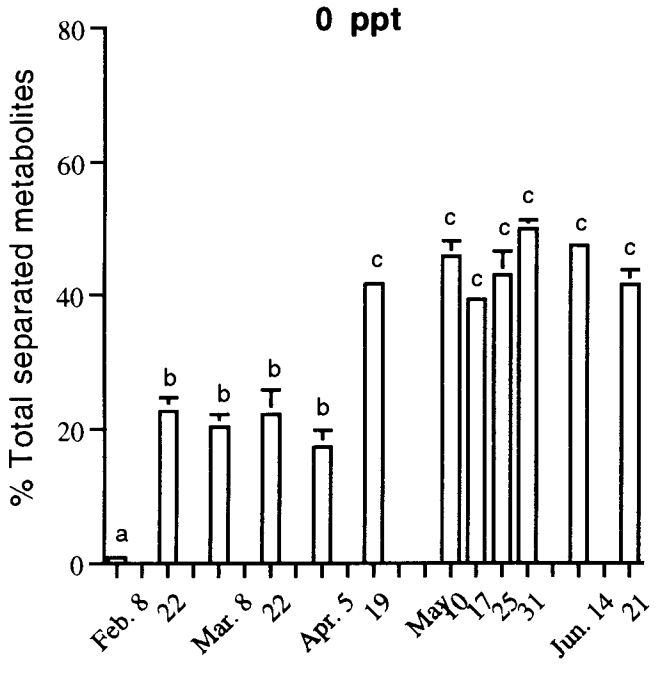
Table 2.12. cont.

Metabolite	Date											
	Feb. 8	Feb. 22	Mar. 8	Mar. 22	Apr. 5	Apr. 19	May 10	May 17	May 25	May 31	Jun. 14	Jun. 21
9,10 diol												
0 ‰	10.26 ±2.56	10.01 ±4.40	14.33 ±0.89	12.67 ±3.41	15.05 ±0.76	13.76 ±1.36	11.16 ±1.83	15.58 ±1.85	16.03 ±2.46	3.87 ±2.47	12.46 ±5.66	15.93 ±3.58
5 ‰	14.79 ±4.63	5.15 ±1.32	13.82 ±1.22	9.29 ±2.17	13.70 ±2.19	9.19 ±0.34	9.15 ±1.05	18.20 ±5.53	14.59 ±3.80	12.06 ±1.21	11.39 ±1.65	12.67 ±0.69
10 ‰	11.46 ±0.75	29.42 ±3.37	14.01 ±0.97	19.49 ±5.06	16.29 ±1.99	11.81 ±0.61	7.24 ±0.70	15.74 ±0.20	10.40 ±0.80	20.77 ±3.80	12.59 ±1.40	17.96 ±1.84
20 ‰	11.06 ±1.81	15.17 ±3.76	18.84 ±4.12	10.83 ±1.12	10.44 ±1.94	10.40 ±2.04	17.23 ±3.91	11.12 ±6.08	12.39 ±0.45	9.64 ±0.02	15.31 ±1.72	18.98 ±7.40
Quinones												
0 ‰	12.49 ±5.66	9.20[a,c] ±0.27	16.91 ±2.81	13.72 ±5.82	18.13[a,b] ±0.63	18.29[c,d] ±1.28	14.05 ±1.08	12.29 ±1.23	12.23 ±2.21	16.36 ±3.66	10.65[b,d] ±1.60	14.64 ±4.26
5 ‰	5.09[a,d] ±2.94	5.18[b,e] ±0.14	19.55[a,c] ±3.17	15.50 ±5.08	21.93[d,f] ±4.24	12.64 ±0.48	10.95 ±1.55	13.44 ±1.01	13.90 ±0.77	7.56[e,f] ±3.00	14.94 ±0.33	11.53 ±1.81
10 ‰	10.62 ±0.45	8.27[a,c,e,g] ±1.49	17.07[a,b] ±4.10	14.50 ±6.16	20.15[c,d] ±0.21	13.26 ±0.04	15.57[e,f] ±0.42	11.63 ±1.35	16.46[g,h] ±0.35	12.80 ±0.49	7.53[b,d,f,h] ±1.16	12.81 ±0.88
20 ‰	28.09[a,b] ±0.08	10.31[a,c,e,g] ±2.72	21.80[c,d] ±2.60	18.51[e,f] ±0.52	18.88[g,h] ±1.90	12.91 ±7.17	15.69 ±0.20	13.70 ±1.26	13.87 ±0.46	13.10 ±1.47	13.32 ±1.81	5.07[b,d,f,h] ±2.04

Table 2.12. cont.

Metabolite	Date											
	Feb. 8	Feb. 22	Mar. 8	Mar. 22	Apr. 5	Apr. 19	May 10	May 17	May 25	May 31	Jun. 14	Jun. 21
BaP												
0 ‰	0[a,c,e,j] ±0	6.43[a,b] ±4.26	4.18[c,d] ±0.65	2.41[j] ±1.37	5.88[e-h] ±0.96	8.02[i-o] ±0.81	1.46[b,d,f,k] ±0.20	2.87[g,l] ±0.30	3.25[m] ±1.72	6.40 ±5.25	2.30[n] ±1.61	2.26[h,o] ±0.39
5 ‰	0.17[a,b,i,q,r, w] ±0.10	4.11[a,j] ±0.12	8.97[b-h] ±1.66	1.72[c,k,s] ±1.07	1.85[d,l,t] ±0.86	12.78[i-p] ±4.96	5.58[m,q] ±0.49	2.60[f,n] ±0.70	1.58[g,o,u] ±0.58	6.61[r-v] ±2.16	1.54[h,p,v] ±0.64	6.08[w] ±4.03
10 ‰	2.41[a,f] ±1.39	6.55[a-c] ±0.89	4.76 ±1.64	1.24[b,g] ±0.29	2.15[c,h] ±0.00	2.94[j] ±0.84	0.92[d,j] ±0.20	3.64 ±0.98	11.02[f,k] ±4.46	4.45 ±1.64	4.15 ±0.39	2.48[e,k] ±0.35
20 ‰	0.20[a,d,g] ±0.10	3.49[a-c] ±0.05	4.14[d-f] ±1.36	2.56 ±0.77	3.38 ±0.39	6.94[g-j] ±2.86	0.90[b,e,h] ±0.11	3.07 ±0.31	1.36[e,f,i] ±0.15	2.80 ±1.04	1.83[j] ±0.39	2.66 ±0.95

Figure 2.9. Percent of total Phase I radioactivity as 3-hydroxy BaP and 9-hydroxy BaP (3-OH and 9-OH BaP) at different salinities during the parr-smolt transformation from February to June. Values are means ±SE for three fish exposed for 24 h to an initial [^{3}H]BaP concentration of 5 µg (1 µCi)/L. Values with common symbols are not significantly different between sampling periods at $p < 0.05$.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60
40
20
0
a
b
b
b
b
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c
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22
Apr. 5
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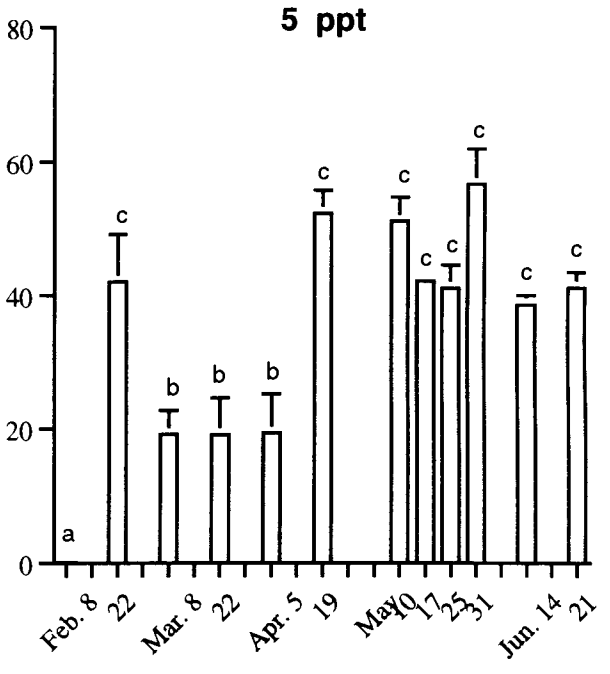
May 10
17
25
31
Jun. 14
21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80
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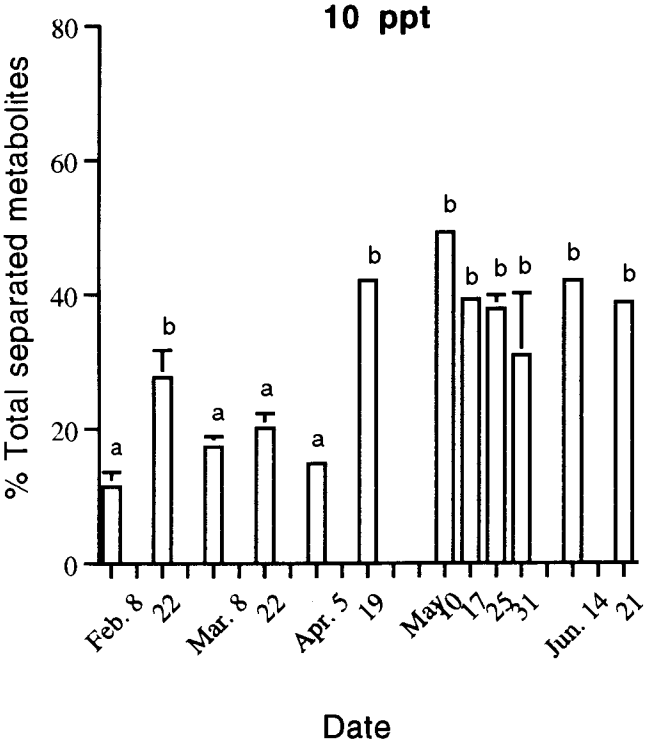
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% Total separated metabolites
80
60
40
20
0
a
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Jun. 14
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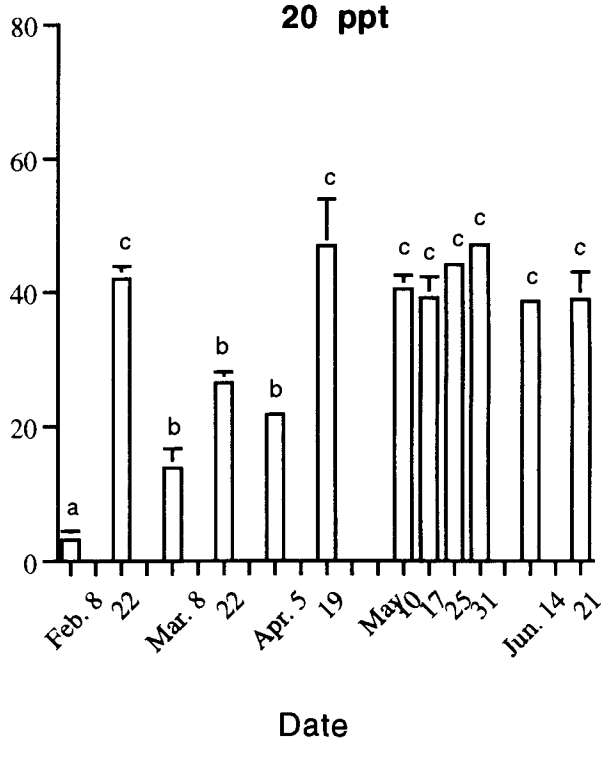
Date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80
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0
a
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Date

D. The effects of salinity on the oxygen consumption rate in parr and smolts

The changes in oxygen consumption rates of coho salmon at four levels of salinity (0, 5, 10, and 20 $^{o}/_{oo}$) during the parr-smolt transformation were investigated using respirometry and involved the measurements of oxygen tension in the vessel containing 5 fish. Oxygen consumption rates of coho salmon (mg O_2/g/h) on February 18, March 16, April 19, May 13, and June 15 are summarized in Table 2.13. At all salinities, the levels of oxygen consumption rate appear to increase with time of sampling. In addition, exposure of fish to higher salinity (10 and 20 $^{o}/_{oo}$) appear to result in higher oxygen consumption rates in February and March. This trend appears to be reversed in June when the oxygen consumption rates are lower at the highest salinities.

The concurrent exposure to BaP resulted in BaP content in the fish body which was below the detection limit by HPLC (0.01 μg BaP/ml).

Table 2.13. Oxygen consumption rates (mg O_2/g/h) of juvenile coho salmon at different salinity levels during the parr-smolt transformation. The values represent the rates of the group of 5 fish exposed concurrently for 1 hour to 5 µg BaP/L in the water.

	Date				
Treatment	February 18	March 16	April 19	May 13	June 15
0 ‰	130.6	99.4	188.2	236.7	308.7
5 ‰	80.2	61.3	132.3	234.3	214.2
10 ‰	75.7	138.6	171.9	290.7	189.9
20 ‰	161.1	188.1	221.4	207.2	195.3

DISCUSSION

Many water quality changes require alterations in the physiology and biochemistry of fish in order to successfully cope with the stressor (Fagerlund et al., 1995). One of these stressors is a change in environmental salinity which can have a profound effect on a variety of physiological processes including changes in oxygen consumption rate as well as on the toxicokinetics of xenobiotics in fish as was shown in this study. In particular, smolts of juvenile salmonids are known to be more sensitive to environmental pollution than parr as reported by Lorz and McPherson (1976) and Beckman and Zaugg (1988). The additional effects of salinity change on the sensitivity of juvenile coho salmon to contaminants during smoltification were the focus of this section of the thesis. The first objective of the present study was to examine the effects of an acute salinity change on the uptake, distribution, and metabolism of benzo[a]pyrene (BaP) in juvenile coho salmon during the parr-smolt transformation.

The approximate smolt status of the hatchery-grown juvenile coho salmon used in this study was determined by morphological observations of body coloration and appearance of teeth and by measuring changes in gill Na^+,K^+-ATPase levels conducted and reported by Seubert (1997). Similar to the results described in Part I of this thesis, juvenile coho salmon in this study exhibited characteristic parr traits in the beginning of the sampling season

(February) and showed typical smolts traits by the end of the sampling period (June).

In investigating the effects of salinity on the uptake, distribution, and metabolism of BaP in coho salmon during smoltification, fish were exposed to [^{3}H]BaP in water for 24 hours. As in the study of Kennedy et al. (1989a) and Part I of this thesis, it was assumed and later shown that the excretion of BaP-derived radioactivity into the water by the fish within the exposure period was insignificant. This was confirmed by HPLC analysis of water samples at the end of the sampling period which revealed that no BaP metabolites were present in the water. In addition, it was determined that chemical loss due to volatilization and adsorption onto walls was minimal.

The uptake of [^{3}H]BaP from water at all salinity levels during the entire smoltification period was rapid and resulted in the removal of 45 to 55% of the radioactivity from the water within 24 hours. Similar results were obtained in the investigation of the effects of smoltification on the uptake of BaP in coho salmon described in Part I of this thesis as well as in the study of Kennedy et al. (1989a) using gulf toadfish (*Opsanus beta*). This rapid uptake has been observed for a variety of polycyclic aromatic hydrocarbons (PAHs) in other freshwater fish such as rainbow trout (*Oncorhynchus mykiss*) (Kennedy and Law, 1990) and in marine species such as mudsucker (*Gillichthys mirabilis*), sculpin (*Oligocottus maculosus*), and sand dab (*Citharichthys stigmaeus*) (Lee et al., 1972). The observed rapid uptake of compounds such as BaP into fish has been linked to

their rapid diffusion through the membranes driven by their high lipid-solubility (McKim and Erickson, 1991; Heath, 1995; Kennedy, 1995).

Exposure to BaP at higher salinity had a significant effect on BaP uptake rates. The uptake rates of BaP decreased at the highest salinity (20 $^{o}/_{oo}$). This finding agrees with the results of the study of Murphy (1970) on the uptake of DDT, DDE and DDD in mosquito fish in which an acute transfer of fish into different salinities (0.15 $^{o}/_{oo}$, 10 $^{o}/_{oo}$, and 15 $^{o}/_{oo}$) resulted in the decreased uptake of DDT at 15 $^{o}/_{oo}$ as compared to freshwater-exposed fish. Acclimation of fish to higher salinities seems to have a similar effect on the uptake of chemicals. Acclimation of juvenile Atlantic salmon to seawater resulted in a decreased uptake of ^{14}C-2,2',4,5,5'-pentachlorobiphenyl (Tulp et al., 1979) and killifish acclimated to 18.7 $^{o}/_{oo}$ salinity took up less pentachlorophenol than freshwater-acclimated fish (Tachikawa and Sawamura, 1994). In the study of Pärt (1989), seawater-adapted rainbow trout had lower absorption rates of five nonionized halogenated phenols than freshwater-adapted fish. It was suggested that a characteristic reduction in water permeability of the gill epithelium during the adaptation from freshwater to seawater may result in the reduction of the efficiency of transportation of lipophilic compounds across the gill epithelium. Since the juvenile coho salmon experienced acute exposure to salinity change in this study, the osmoregulatory adaptations necessary to cope with higher salinity would not be fully developed yet. A different explanation was proposed by Brauner et al. (1992) who studied the effects of short-duration seawater exposure on plasma ion concentrations and swimming performance of coho

salmon parr. They noted a decrease in delivery of oxygen to active tissues attributed to a decrease in hematocrit following seawater transfer and an increase in plasma volume related to an increase in drinking of seawater and a decrease in urination rate. It was postulated that the overall effects of osmoregulatory stress, which the fish experienced by rapid seawater transfer, may be correlated with a reduction in cardiac output as observed in other species following exposure to high NaCl concentrations. If the salmonid cardiovascular system responds similarly to a rapid increase in salinity, cardiac output and thus perfusion of the gill arches may be compromised as compared to freshwater fish. Based on several studies on the absorption efficiency of hydrophobic chemicals such as BaP, Kennedy (1995) indicated that the uptake rate of these chemicals in freshwater fish is normally not likely to be perfusion-limited. However, the decreased blood delivery to the gill and an increase in water content of the blood plasma, associated with rapid exposure to high salinity, may affect the time and binding efficiency of blood transport proteins such as albumin for hydrophobic chemicals making it perfusion-limited. As a consequence, the uptake rates of chemicals such as BaP would decrease with an increase in salinity as observed in this study.

A decrease in BaP uptake rates at higher salinity as compared to freshwater may be also related to the changes in BaP solubility in relation to ionic strength of water. According to the data summarized by McElroy et al. (1989), solubility of BaP decreases two fold with the increase in salinity from 0 $^{o}/_{oo}$ to 33 $^{o}/_{oo}$. This inverse relationship between the solubility and salinity could

result in lower availability of BaP for uptake at higher salinities and account for the results obtained in the present study.

At all salinities, except at an isotonic salinity (10 $^{o}/_{oo}$), uptake rates of BaP increased during smoltification. A similar pattern was previously observed in the study on the effects of smoltification on the uptake of BaP during the parr-smolt transformation described in Part I of this thesis. This apparent increase in BaP uptake rates may be attributed to an increase in oxygen consumption rates and metabolic rates as observed by McCormick and Saunders (1987) and Boeuf (1992) in smolts as compared to parr. Since chemical uptake is correlated with oxygen uptake, as postulated by McKim et al. (1985) and McKim and Erickson (1991), an increase in oxygen consumption rate via an increase in ventilation rate or ventilation volume during smoltification would lead to an enhanced uptake of BaP by the fish. Under normoxic conditions, uptake of hydrophobic compounds has shown to be ventilation-limited and changes in oxygen demand resulted in an increase in ventilation leading to an increase in the amount of water containing a chemical which passed via the gills per unit time thus facilitating the uptake of the chemical (Kennedy, 1995). Levels of [^{3}H]BaP in the stomach were low as compared to the gill throughout the experiment possibly indicating that the oral absorption of [^{3}H]BaP was low which is in agreement with the findings of Lee et al. (1972) and Kennedy and Law (1990) that orally administered PAHs do not contribute significantly to the total body burden of the fish.

In a concurrent study on the effects of salinity on the oxygen consumption rate during smoltification outlined in this thesis, oxygen consumption rates in all salinity treatments indicated a general increase from February to June which correlates with a reported increase in energy demand of the fish along smoltification period. In addition, it appears that parr had higher oxygen consumption rates at higher salinities (20 $^{o}/_{oo}$) when compared to smolts. Since seawater adaptation progresses with time from parr stage to smolt stage (Eddy, 1982; McCormick et al., 1989), the initial high oxygen consumption rates at higher salinity may be attributed to the lack of seawater tolerance in parr resulting in general ionic and osmotic stress leading to general energy mobilization and increased oxygen demand regulated by catecholamine and corticosteroid hormones (Fagerlund et al., 1995). When the fish acquire osmotic tolerance to seawater later in the smoltification process, the trend may be reversed, as observed in this study.

Uptake rates of BaP were not affected by the parr-smolt transformation at the isotonic salinity (10 $^{o}/_{oo}$) at which body fluids of the fish and the external environment are in osmotic and ionic equilibrium (Evans, 1993). Several researchers reported that the oxygen consumption rate and metabolic rate is minimum in fish acclimated to isotonic salinity but increases at lower and higher salinities (Rao, 1968; Farmer and Beamish, 1969; DeSilva et al., 1986) and could be attributed to lower energetic cost of ion regulation in an isotonic environment. However, Morgan and Iwama (1991) reported in their study that metabolic rate in juvenile rainbow and steelhead trout and chinook salmon

increased with salinity. There is, however, a lack of agreement on the effects of salinity on the oxygen consumption rates during the parr-smolt transformation. The results of the respirometer experiment summarized in this thesis indicate that at salinity of 10 $^{o}/_{oo}$ oxygen consumption rates increased with smoltification. Since the increase in oxygen consumption rates did not coincide with an increase in BaP uptake rate during the same period of time, it is possible that some changes in the gill membrane permeability impeded the absorption of the chemical form the water. These changes may be related to the changes in the composition of lipids in the epithelial membrane of the gill from the predominance of saturated fatty acids in parr to long-chain polyunsaturated fatty acids in smolts (Sheridan, 1989). However, the effects of these structural changes on gill membrane permeability to hydrophobic chemicals have not yet been studied.

The effects of salinity on the distribution of BaP in juvenile coho salmon during smoltification were determined by analyzing the radioactivity content in different tissues following exposure to [^{3}H]BaP. Similar to the results of the Part I of this thesis, it was confirmed that BaP is widely distributed in fish tissues following a short exposure time. The highest levels were found in the bile followed by the liver and the intestine. The remaining tissues contained radioactivity in the following order: gill > skin > muscle > kidney = stomach > visceral fat > brain which corresponded with the findings of the study on the effects of parr-smolt transformation on the distribution of BaP outlined in Part I of this thesis and with the results of Kennedy et al. (1989a) in gulf toadfish. The

distribution of BaP and other lipid-soluble compounds in different tissues may be related to the blood flow to the tissue as well as its fat content (Klaassen, 1986).

The percent body burden of BaP in the bile increased with an increase in salinity. It appears that marine fish excrete higher levels of chemicals through the bile than freshwater fish (Lee et al., 1972; Kennedy et al. 1989a; Kennedy and Law, 1990). It is possible that even a short term exposure to higher salinity stimulated a more efficient excretion of BaP or its metabolites to the bile. As in the previous study, the transfer of BaP or its metabolites to the bile was enhanced at all salinities as the smoltification progressed possibly indicating a more efficient biotransformation of BaP in smolts than in parr.

Salinity had an effect on the percent body burden of the gill which decreased at highest salinity (20 $^{o}/_{oo}$). It is possible that at high salinity some change to perfusion occurs in the gills limiting the chemical entry. An increase in the levels of the gill in April at all salinities may correspond to an increase in gill epithelial cells such as chloride cells at that time (Sargent and Thomson, 1974; Eddy, 1982).

In addition, an increase in salinity resulted in a decrease in the percent body burden of the intestine which also appeared to be higher in smolts as compared to parr. When coping with osmotic stress at higher salinities, the blood flow to the tissues such as the intestine may decline due to changes in cardiovascular efficiency as suggested by Brauner et al. (1992).

The percent body burden of the skin and the liver showed similar patterns to the results obtained in the Part I of this thesis in accumulation of BaP-derived radioactivity with time. These patterns were possibly related to the decrease in the importance of dermal route of uptake of BaP with the growth of the fish and the associated relative increase in the surface area of the gills with time (Lien and McKim, 1993) and to an increase in metabolic rate during smoltification (Boeuf, 1992). The levels of percent body burden of remaining tissues were comparable to the levels found in Part I and were not affected by the salinity treatment.

When expressing the [^{3}H]BaP-derived radioactivity content (μg BaP-equivalent/g of tissue) in several tissues of coho salmon as actual concentration values of BaP and/or its metabolites, the highest concentration of BaP-derived radioactivity was found in the liver followed by the intestine. The remaining tissues contained radioactivity in the following order: skin > gill >> kidney > stomach > visceral fat >> brain = muscle which corresponded to the findings of the study on the effects of parr-smolt transformation on the distribution of BaP outlined previously and by Kennedy and Law (1990) with the exemption of visceral fat which contained much higher amounts of 2-methylnaphthalene, fluorene, and pyrene after 6 days of exposure than observed in the present study. However, a longer exposure time may have accounted for the preferential distribution of these lipid-soluble compounds to the fat.

BaP-derived radioactivity content in skin decreased with salinity. It is possible that, due to osmotic stress, there were changes in the perfusion of the

skin resulting from a change in cardiac output at high salinity which may have led to a lower content of BaP or its metabolites in that tissue (Brauner et al., 1992). In addition, solubility of BaP decreases with salinity and may result in a lower availability and absorption of that chemical across the membranes (McElroy et al., 1989). Again, the dermal absorption of the chemical decreased at all salinities as the fish increased in size during smoltification and the gill respiratory surface area increased in size and dominance (Lien and McKim, 1993; Heath, 1995).

As seen previously, higher salinity had an effect on the percent body burden of the gill. BaP-derived radioactivity content in the gill decreased with an increase in salinity which was explained by some limitation to perfusion in the gills limiting the chemical entry (Brauner et al., 1992) or a decrease in solubility of BaP at higher salinities (McElroy et al., 1989). An increase in BaP-derived radioactivity content of the gill in April at all salinities may be related to an increase in the gill epithelial cells such as chloride cells (Sargent and Thomson, 1974; Eddy, 1982).

Similarly, an increase in salinity resulted in a decrease in the BaP-derived radioactivity content of the intestine with an accompanying increase in the levels with time as seen previously. This could be explained by changes in blood flow to the intestine due to changes in cardiovascular efficiency as suggested earlier. The levels of the remaining tissues remained unchanged by the salinity treatment.

In order to determine the effects of salinity on the metabolism of BaP during the parr-smolt transformation, the bile of juvenile coho salmon was analyzed after exposing the fish to [^{3}H]BaP at different salinity levels. The analysis of bile revealed that juvenile coho salmon are capable of metabolizing BaP to both Phase I and Phase II metabolites, however, salinity did not affect the proportion of metabolites classes in the bile. The levels of each class in the bile were comparable to the levels found in Part I of this thesis and indicated that organic-soluble metabolites as well as unknown water-soluble conjugates are the predominant BaP metabolites. The proportion of organic-soluble metabolites in the bile of coho salmon was again lower than reported by Seubert (1997) in his study on juvenile coho salmon at different salinities and injected i.p. with 10 mg/kg of BaP but were much higher than reported by Gmur and Varanasi (1982) where organic-soluble metabolites in the bile of English sole constituted around 20 % of the total radioactivity found. The high level of organic-soluble metabolites points towards a more effective transport of these metabolites from the bile or an inability to effectively biotransform BaP to water-soluble or conjugated metabolites. The levels of glucuronide and sulfate as well as other unidentified conjugates in the bile were similar to the levels found in the previous study and indicated a predominant glucuronation over sulfonation in juvenile coho salmon.

Reverse phase HPLC analysis of organic-soluble fraction of the bile revealed that salinity had no effect on formation of most metabolites such as tetrols, diols, quinones and phenols as well as BaP in the bile of juvenile coho

salmon. Only at isotonic salinity, the levels of 9,10-dihydrodiol were higher than at other salinity treatments. 9,10-dihydrodiol has been reported as predominant metabolite of Phase I in freshwater species of teleosts (Tan and Melius, 1986). It is possible that in salmonids, the regioselectivity of BaP biotransformation in the liver may be altered by osmoregulatory demands of either hypotonic or hypertonic environment resulting in the formation of different metabolites then observed in other freshwater species.

Similarly to the findings of the study on the metabolism of BaP during smoltification, the major Phase I metabolite at all salinities were 3-OH and 9-OH BaP which increased in smolts as compared to parr and 7,8-dihydrodiol which declined in smolts but salinity had no effect on the proportions of these metabolites. The proportions of other metabolites and metabolite groups in organic-soluble fraction of the bile were similar to the proportions found in the study on the effects of smoltification on the metabolism of BaP. The results of HPLC analysis indicate that coho salmon smolts biotransform BaP to phenols rather than to diols such as 7,8-dihydrodiol or 9,10-dihydrodiol. Due to the presence of higher levels of 7,8-dihydrodiol, one of the precursors of reactive BaP metabolites, in February and March as compared to the following months, parr may be possibly at greater risk of tumor formation than smolts. The reactive epoxides such as 7,8-diol-9,10 epoxide may react with DNA or RNA to form adducts in hepatic cells and result in the initiation of liver lesions and tumors as shown for a variety of marine and freshwater fish (Gelboin, 1980; Varanasi et al. 1986; Varanasi et al. 1989).

SUMMARY AND CONCLUSIONS

Each year, myriad juvenile Pacific salmon (genus *Oncorhynchus*) leave streams and rivers of North Pacific rim countries in their migration to feeding grounds in the North Pacific Ocean and adjacent seas. After periods ranging from a few months to several years, adult salmon enter rivers along the coast of Asia and North America to spawn and complete their life cycle. Within that time, the fish exploits numerous freshwater, estuarine, and marine habitats and encounters may hazards including water pollution caused by humans, such as the introduction of effluent from pulp mills and sewage treatment systems, runoff from agricultural activity, physical barriers to migration such as hydroelectric dams as well as the use of chemical contaminants along a waterway. These environmental stressors and constraints on salmon elicit diverse physiological and behavioural responses in fish which may vary depending on the stage of development of the fish or other environmental factors involved. Information gathered from studying the effects of developmental modulators and other environmental variables on the physiological and toxicological parameters in organisms such as fish can help in determination of the fate of chemicals in the environment and aid in predictive risk assessment (Suter, 1993).

The objective of the studies presented in this thesis was to investigate the effects of one particular developmental modulator, the parr-smolt transformation, and alterations in environmental salinity on the toxicokinetics of a model compound benzo[a]pyrene (BaP) in juvenile coho salmon.

BaP uptake rates increased throughout smoltification resulting in higher BaP uptake in smolts than in parr. This finding may imply that juvenile coho salmon may be more vulnerable to exposure to chemicals during smoltification and seaward migration. The distribution of BaP to various tissues was also altered by the parr-smolt transformation indicating that half life and the concentrations at the target site and toxicity of xenobiotics in fish may be altered by physiological changes during different stages in organism's development. It was found that the fish increased their capacity to metabolize BaP in the liver and to efficiently eliminate it to the bile as smoltification progressed. Changes in metabolism of xenobiotics, as observed in this study, may have a differential influence on the fate of chemicals, their persistence in the biota and their toxic effects in organisms such as fish. Despite efficient metabolism and elimination of the compound via hepatobiliary system, presence of significant levels of BaP-DNA adducts in both parr and smolts points to the ability of hepatic detoxification systems in fish to bioactivate and increase the susceptibility of juvenile salmonids to xenobiotics with a carcinogenic potential including, several PAHs, which may be detrimental to individual fish and whole populations.

Exposure to different environmental salinities resulted in further modulation of the toxicokinetics of BaP in juvenile coho salmon undergoing smoltification. In particular, the uptake rates of BaP decreased with salinity indicating that changes in the chemical availability in seawater and coping with osmoregulatory stress may decrease chemical absorption by fish. Xenobiotic

distribution patterns, particularly to tissues in contact with the external environment, were also affected by the changes in salinity. This finding suggests that adaptations required for a rapid change in the osmotic environment may lead to changes in chemical disposition and fate during physiological adaptations for marine existence. The biliary elimination of BaP and its metabolites increased with salinity indicating that acute changes in water ion concentrations may affect chemical half life and persistence in organisms during critical stages of development and may result in alterations of the fate of pollutants in aquatic organisms and in changes in sensitivity of fish to xenobiotics. No differences were seen in the metabolic patterns of biotransformation of BaP between different salinities.

Changes in the quality of the aquatic environment, including changes in salinity and the chemical exposure received by each susceptible life-stage may affect life support systems in fish (Fagerlund et al., 1995). The results of this study show that the physiological and biochemical changes associated with a developmental stage of adaptation to a marine residence can affect the toxicokinetics of xenobiotics. In addition, it was demonstrated that chemical disposition may be further modified by osmoregulatory mechanisms associated with acute changes in the ionic content of water. This study shows that understanding the physiology of an organism in relation to its environment and its developmental stage may help in toxicological evaluations and in the prediction of toxic effects. Specifically, the integration of the knowledge of relevant physiological processes with toxicological parameters and effects is

needed to conduct environmental impact and risk assessment. The knowledge of modulatory effects of factors such as salinity and development on the differential uptake of chemicals and their disposition in organisms may aid in conducting and evaluating chemical toxicity testing as well as in predictive modeling of chemical fate. In particular, measurements of the levels of DNA adducts may be used as a good molecular indicator of stress caused by contaminant exposure and as an estimate of a potential risk of carcinogenesis in salmonids and other fish. Together with an analysis of the efficiency of DNA repair mechanisms, adduct formation may provide an aid in assessment of the likely impact of an environmental carcinogen or chemical mixture on an individual and whole fish populations.

Thus, the information gathered in this study may assist in several areas of aquatic toxicology by providing information on variety of toxicokinetic parameters and their modulation in aquatic organisms.

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