

Altered growth and related physiological responses in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) from dietary exposure to polycyclic aromatic hydrocarbons (PAHs)

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Abstract: A dietary feeding study with polycyclic aromatic hydrocarbons (PAHs) was conducted with juvenile Chinook salmon (*Oncorhynchus tshawytscha*) to mimic exposure from urban estuaries during their transition from freshwater to seawater. A significant reduction in mean fish dry weight was observed only for the highest doses; however, analysis of variance (ANOVA) using standard deviations and examination of the cumulative frequency plots revealed high variability among all treatments. The skewed fish weight distribution revealed a large number of small fish in several treatments compared with control fish. Analyses of whole-body lipids and several parameters in blood plasma related to growth and metabolism indicated alterations for most treatments. These results and trends in growth, plasma chemistry, and lipids as a consequence of PAH exposure were similar to those in fish exhibiting starvation, which we have termed "toxicant-induced starvation". Based on these results, we conclude that PAHs are toxic to salmonids at this life stage and the reduction in biomass and lipid stores observed here would have the potential to cause increased mortality for individuals during their first winter.

Résumé : Nous avons mené une étude d'alimentation diététique avec des hydrocarbures aromatiques polycycliques (PAHs) chez de jeunes saumons chinook (*Oncorhynchus tshawytscha*) afin de mimer leur exposition aux estuaires urbains durant leur transition de l'eau douce vers la mer. Une réduction significative de la masse sèche des poissons s'observe seulement avec les doses les plus élevées; cependant, une analyse de variance (ANOVA), qui utilise les écarts types et qui examine les graphiques de fréquences cumulées, montre qu'il existe une forte variabilité dans tous les traitements expérimentaux. La distribution asymétrique des masses des poissons montre qu'il y a un grand nombre de petits poissons dans plusieurs groupes expérimentaux par comparaison aux groupes témoins. Les analyses des lipides du corps entier et de plusieurs variables du plasma sanguin reliées à la croissance et au métabolisme indiquent qu'il y a des modifications dans la plupart des groupes expérimentaux. Ces résultats et les tendances dans la croissance, la chimie du plasma et les lipides qui se développent à la suite de l'exposition aux PAH font penser aux conditions de poissons qui manquent de nourriture; nous qualifions cet état de « disette provoquée par les produits toxiques ». Sur la foi de ces résultats, nous concluons que les PAHs sont toxiques pour les salmonidés à cette étape de leur vie et que la réduction de biomasse et de réserves lipidiques observée peut potentiellement causer une augmentation de la mortalité individuelle durant leur premier hiver.

[Traduit par la Rédaction]

Introduction

Several studies and reviews have shown that polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in aquatic systems, especially estuaries, and can often occur at high

concentrations in sediment, water, and tissue (Meador et al. 1995; Eisler 2000; van Metre et al. 2000). These contaminants come from multiple sources, such as stormwater runoff, atmospheric transport, petroleum operations (including drilling, refining, transport, combustion, spills, and natural seeps),

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municipal and industrial wastewater, and combustion of most organic material.

During smoltification, juvenile salmonids, especially wild fish, lose significant amount of lipids, usually falling to 1%–3% of wet weight (Brett 1995; Beckman et al. 2000; Meador et al. 2002). While in the estuary and nearshore environment, juvenile Chinook salmon are voracious consumers of invertebrates and small fish (Weatherley and Gill 1995), leading to a rapid increase in growth and accumulated lipid stores that will be beneficial for their first winter (Burrows 1969; Healey 1980; Biro et al. 2004). This stage in the life cycle of juvenile Chinook salmon is one of the most critical for determining its survival and reproductive success (Spromberg and Meador 2005) and even slight impedance in growth or lipid storage may have severe consequences for survival.

Even though PAHs are ubiquitous and abundant in the environment, very few studies have examined organismal-level responses to these compounds. Only a handful of studies have considered growth impairment or energetics as a response in fish and a few of these reported severe effects at low exposure concentrations (Carls et al. 1996; Rice et al. 2000). This study was designed to examine growth and a selected number of associated metabolic parameters in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) exposed to dietary PAHs. Our main focus was the juvenile stage that enters the estuary for several weeks to acclimate to seawater, increase in size, and accumulate lipid stores before migrating to open water. We selected fish growth, whole-body lipids, and several associated parameters in blood plasma as metrics for assessing toxic effects. The goal was to gauge the magnitude of effect on organismal properties such as body weight and whole-body lipid content that directly affect the survival of juvenile salmonids, in addition to several related blood chemistry parameters that may be useful as early indicators of adverse effects.

Materials and methods

Fish husbandry, experimental design, environmental parameters, and execution of these experiments followed the procedures described in Meador et al. (2005). The following is an abbreviated discussion of our methods.

Husbandry

Chinook salmon were cultured in our lab from eggs obtained from a local hatchery. Before the toxicity experiment, Chinook salmon fry were fed ad libitum with commercial food. Once large enough to consume pellets, juvenile Chinook salmon were fed at a rate of 1.5%–2.0% body weight (bw)·day⁻¹ with fish pellets from Rangen Inc. (Buhl, Idaho). When juvenile fish weighed approximately 3 g, they were switched to a specially prepared fish pellet (Rangen Inc.) that contained a total lipid content of 11% dry weight that was formulated to mimic the lipid content of prey for wild fish. Water quality parameters (pH, dissolved oxygen, alkalinity, and hardness) were measured at least once every week and adjusted if outside the accepted range (Meador et al. 2005). Fish were raised in freshwater and introduced to saltwater during the new moon in June. The rate of change from

freshwater to saltwater was approximately 5%·day⁻¹ until full-strength seawater was attained.

Experimental design

Individual fish were weighed and selected from a size window of 10.0 to 16.0 g wet weight (ww) (2.2–3.5 g dry weight (dw)), which was the range determined to provide the best achievable variance given the available pool of fish. Approximately 40% of the available fish fit the size selection criteria. Each tank contained 50 fish and the order of filling the tanks, as well as the assignment of treatment to each tank, was done randomly as determined with a random number generator.

Juvenile Chinook salmon were fed a mixture of PAHs intended to mimic those found in the stomach contents of field-collected fish both in magnitude and proportionality. Pellets were dosed using a stock solution of PAHs in dichloromethane (MeCl₂) (50 mg·mL⁻¹) that consisted of 21 PAHs (Table 1) at percentages similar to those found for stomach contents from field-collected fish (Varanasi et al. 1993) (Fig. 1). All fish food for a given treatment was made in one 3500 g batch. Fish pellets were placed in a stainless steel bowl, a calculated amount of stock solution was added to 4 L of MeCl₂, and the entire amount was added to the bowl covering all the pellets. The mixture was stirred occasionally and allowed to air dry. Once dried, the pellets were placed into plastic tubs with a tight-fitting lid and kept in a freezer at –20 °C until needed to feed fish.

An appropriate amount of food was weighed and supplied in two feedings (morning and afternoon). Fish in each experimental tank were fed 1.9% bw·day⁻¹ based on their average day 0 wet weight, and that amount was increased each week by a quantity determined with a growth equation that assumed a conversion efficiency of 20%. In a separate tank, 50 fish were weighed frequently to determine their rate of growth, which allowed us to keep the amount that was to be fed to all tanks at 1.9% bw·day⁻¹. In treatments where growth was inhibited (or enhanced), those fish actually received a higher (or lower) percentage of food to body weight because the amount fed to each tank was incremented according to the expected growth rate for control fish. Observations for each tank found that there was no aversion to pellets by fish in any treatment, and the entire allocation of pellets was consumed within a few seconds. Feeding was conducted 5 days per week; therefore, fish were fed 39 times over the 53-day dosing period starting on 27 July 2004 (day 0). The control fish were fed with pellets that were treated identically to those containing PAHs, including MeCl₂ soaking. The length of time for dosing was patterned after several studies showing estuarine residence time for juvenile salmonids ranging up to 60 days before migration to open water (Healey 1991; Thorpe 1994). The PAHs provided to fish are considered the administered dose. The administered dose is the amount given to an organism, whereas the acquired dose is the amount found within the tissues. Although food was delivered to the water in each tank, all pellets were consumed and therefore considered as the administered or ingested dose.

This study consisted of one control and five treatments, each with four replicate tanks. The Fiberglass tanks were round with a diameter of 1.3 m and contained 500 L of sea-

Table 1. Measured doses of each polycyclic aromatic hydrocarbon (PAH) in the experimental diet.

	Control	Dose ($\mu\text{g}\cdot\text{g fish}^{-1}\cdot\text{day}^{-1}$)				
		0.7	2.3	6.1	18	22.1
Naphthalene (NPH)	0.0016	0.018	0.049	0.12	0.28	0.34
2-Methylnaphthalene	0.0036	0.038	0.106	0.28	0.70	0.85
Dimethylnaphthalene	0.0094	0.057	0.161	0.40	1.11	1.34
Total naphthalenes	0.0146	0.112	0.315	0.80	2.10	2.53
Dibenzothiophene (DBT)	0.0019	0.036	0.113	0.28	0.85	1.02
Acenaphthene (ACE)	0.0004	0.021	0.066	0.18	0.49	0.60
Fluorene (FLU)	0.0004	0.087	0.283	0.79	2.27	2.83
1,8-Dimethyl(9H)fluorene	0.0004	0.002	0.007	0.02	0.05	0.07
Total fluorenes	0.0009	0.089	0.290	0.81	2.32	2.90
Phenanthrene (PHN)	0.0032	0.102	0.321	0.87	2.64	3.21
9-Ethylphenanthrene	0.0004	0.006	0.019	0.05	0.15	0.18
9-Ethyl-10-methylphenanthrene	0.0004	0.003	0.010	0.03	0.08	0.10
1-Methyl-7-isopropylphenanthrene	0.0004	0.002	0.007	0.02	0.06	0.07
Total phenanthrenes	0.0045	0.11	0.36	0.96	2.93	3.55
Anthracene (ANT)	nd	0.023	0.072	0.19	0.59	0.72
Fluoranthene (FLA)	0.0002	0.117	0.378	1.02	3.02	3.78
Pyrene (PYR)	0.0002	0.094	0.321	0.83	2.46	3.02
Methyl pyrene	0.0004	nd	nd	nd	0.003	0.003
Total pyrenes	0.0006	0.094	0.321	0.83	2.46	3.03
Benz[a]anthracene (BAA)	0.0004	0.032	0.111	0.28	0.91	1.15
Chrysene (CHR)	0.0005	0.057	0.185	0.49	1.53	1.89
Benz[a]pyrene (BaP)	0.0004	0.015	0.053	0.13	0.40	0.49
Benzo(k)fluoranthene (BKF)	0.0005	0.009	0.032	0.09	0.25	0.28
Benzo(g,h,i)perylene (BZP)	0.0004	0.002	0.007	0.02	0.06	0.07
Dibenzanthracene (DBA)	0.0003	0.003	0.009	0.02	0.07	0.09

Note: Each value is expressed in micrograms of PAH per gram of wet fish per day. Values in bold are totals for groups of PAHs. The top line is the summed dose for each treatment. nd is not detected.

water. The tanks were inside a building and arranged in a grid pattern. The flow-through seawater system provided 4 L·min⁻¹ of sand-filtered and ultraviolet (UV) sterilized water to each tank. Fish were maintained at 10–11 °C for the entire experiment. At the end of the dosing period (17 September 2004), the fish were not fed for 3 days before samples were taken for bile, blood chemistry, and tissue concentrations on day 56. Fish were fed uncontaminated food once on day 56 (after sampling) to prevent starvation conditions, but not again before weighing. All fish were weighed individually on day 58 on a Sartorius 4000 balance to 0.1 g accuracy. Fish length was not analyzed in this study because we consider fish weight to be a more important variable for assessing growth impacts resulting from toxicant exposure. This is supported by a few studies noting that fish weight was more affected than fish length for salmonids exposed to toxicants (Moles and Rice 1983; Heintz et al. 2000).

Samples

Chemical analyses were conducted with composite samples or a combination of individual fish and composites. For all bile, whole-body lipid, and plasma chemistry analyses, each value was determined on a composite of three fish. Whole-body lipids for each treatment were determined for three individual fish from one of the blood chemistry composites in addition to the two composites (three fish each) used for PAH determination for a total of five values. Tissue

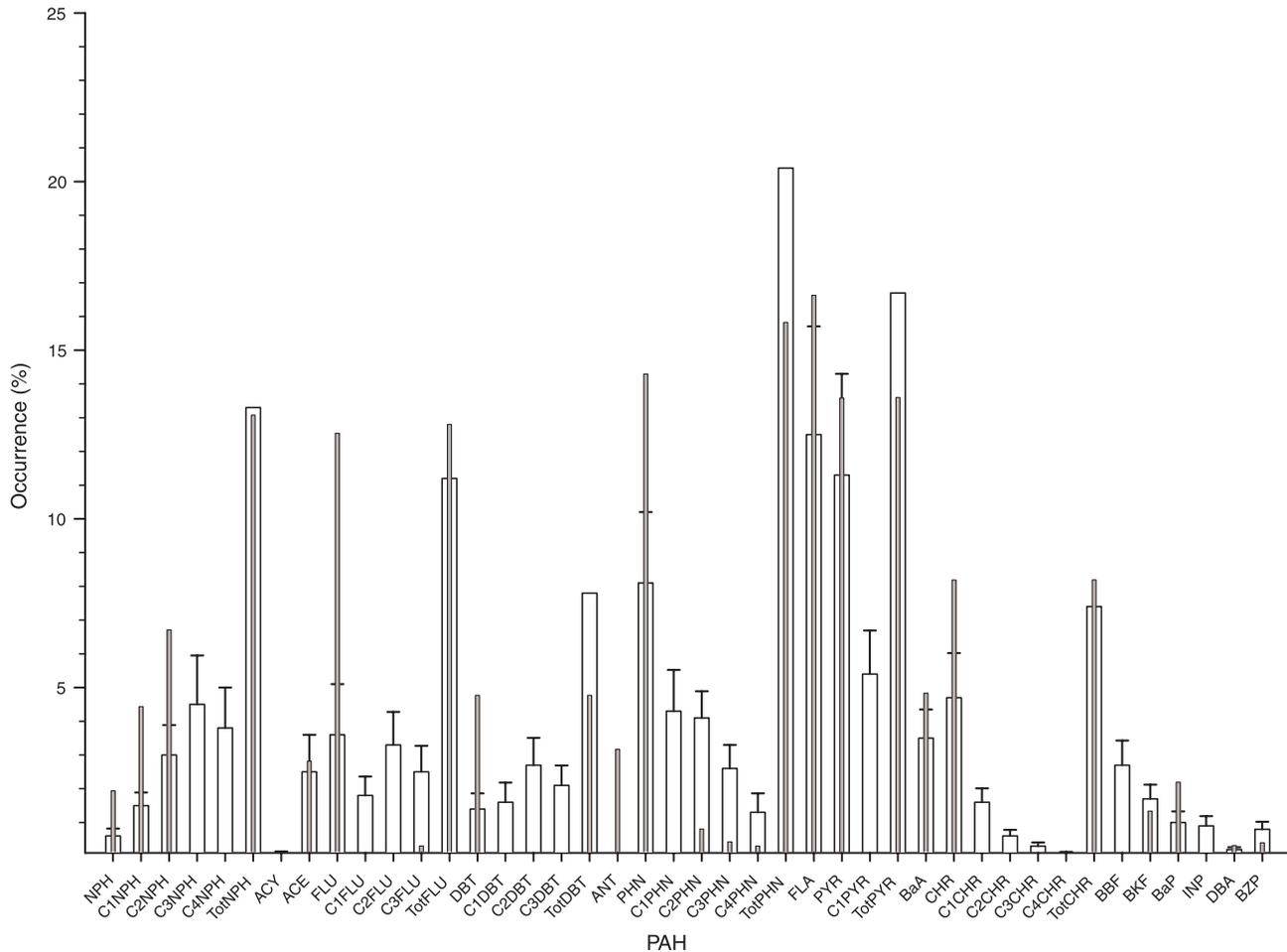
moisture content was determined for two of the PAH chemistry composites, and each was analyzed in duplicate with two separate aliquots from each composite. In all cases, each composite sample was composed of three fish from a given tank. Three of the four replicate tanks per treatment were chosen at random to make up the three separate composites.

Blood chemistry

Blood plasma samples were analyzed using an automated blood chemistry analyzer (Idexx VetTest 8008 Chemistry Analyzer; IDEXX Laboratories, Inc., Westbrook, Maine). Eleven blood chemistry parameters were measured simultaneously for each plasma sample. A quality control procedure (Idexx Vetrol control lot number J00951101) was run before samples to verify both the VetTest optics groups and the integrity of the test slides.

Blood was taken from stunned juvenile Chinook salmon by transection of the caudal peduncle and direct transfer of blood to heparinized 1.3 mL sample vials (heparin grade 1-A, 25 000 units). When approximately 1 mL was collected from three fish (one composite replicate), samples were immediately centrifuged at 18 800 rpm for 150 s in a StatSpin veterinary centrifuge (Iris Sample Processing, Norwood, Mass.). Plasma was transferred via pipette to a sample cup, which allowed for aliquots to be taken up with the VetTest auto pipette and injected into the machine. The minimum amount of sample required was 40 μL , which was used for initiation and the first parameter tested. Each additional parameter

Fig. 1. Percent occurrence of each polycyclic aromatic hydrocarbon (PAH) in stomach contents of field-collected Chinook salmon (*Oncorhynchus tshawytscha* (open bar)), and dosed fish pellets used in this experiment (shaded bar). Mean and standard error percentages (error bars shown) are for PAHs in stomachs of juvenile Chinook salmon collected from the Duwamish and Hylebos waterways near Seattle, Washington. Data are from Varanasi et al. (1993). Each value is the mean of 12 composite samples, each with 10 fish. Fish food PAHs are mean measured percentages for this study (no error bars). C1, C2, C3, and C4 designate the total number of carbons in the alkylated homologs. ACY, acenaphthylene; BBF, benzo[b]fluoranthene; INP, indenopyrene. See Table 1 for all other abbreviations. Bars for total NPH, FLU, DBT, PHN, PYR, and CHR show summed percentages for parent plus alkyl homologs. ANT was not measured in stomach contents.



required 10 μ L. The time lag between sampling plasma and analysis was less than 20 min. Blood plasma was analyzed for albumin (ALB), alanine aminotransferase (ALT), amylase (AMYL), calcium (CA), cholesterol (CHOL), creatinine (CREA), glucose (GLU), lipase (LIPA), inorganic phosphate (PHOS), total protein (TP), and triacylglycerols (TAG).

Lipid analysis for whole bodies

Whole-body fish were homogenized and analyzed for triacylglycerols (TAGs), cholesterol (CHOL), free fatty acids (FFA), sterol esters – wax esters (SE–WE), and polar lipids (PL). Total lipid concentrations (reported as percent total lipid) were calculated by summing the concentrations of the five lipid classes for each sample. Lipids were determined by a thin-layer chromatography – flame ionization detection (TLC–FID) method (Ylitalo et al. 2005) using an Iatron Mark 6 (Iatron Laboratories, Tokyo, Japan). In this method, fish whole bodies were mixed with sodium sulfate and mag-

nesium sulfate and extracted with dichloromethane using an accelerated solvent extractor (Sloan et al. 2005). Various classes of lipids were separated based on polarity, with the nonpolar compounds eluting before the more polar lipids (e.g., phospholipids). Duplicate TLC–FID analyses were performed for each sample extract and the mean value was reported.

Whole-body PAHs and metabolites in fish bile

Composite bile samples of Chinook salmon were analyzed by high performance liquid chromatography (HPLC) for PAH metabolites as described in Krahn et al. (1986). Biliary fluorescent aromatic compounds (FACs) are determined by fluorescence at excitation/emission (ex/em) wavelength pairs. For this study we analyzed for biliary metabolites fluorescing at PHN (ex/em 260/380 nm) and BaP (ex/em 380/430 nm) wavelengths. Whole-body concentrations of PAHs were determined according to the methods of Sloan et

al. (2005) using a gas chromatograph – mass spectrometer (GC–MS). Fish were not sampled for 3 days after the last dose to allow for gut purging. At the time of sampling, the stomach and alimentary canal were removed; however, it was difficult to assure total removal of these organs.

Because fish were not fed for 3 days before sampling for tissue concentrations, a large percentage of the PAHs were likely metabolized after the dosing period. From these data and the estimated half lives for selected PAHs found in the literature for salmonids (Niimi 1987; Niimi and Dookhran 1989), we calculated an expected whole-body tissue concentration for total PAHs (excluding metabolites) using eq. 1:

$$(1) \quad [\text{tissue}_{t=\text{end}}] = [\text{tissue}_{t=0}] / e^{-k_2 T}$$

where $[\text{tissue}_{t=0}]$ and $[\text{tissue}_{t=\text{end}}]$ are the beginning and ending tissue concentrations, respectively, for the time period T and k_2 (elimination rate constant) = half live in days/0.693.

Analysis of treatment effects

Fish weights, wet to dry weight ratios, whole-body lipids, and blood chemistry were analyzed by analysis of variance (ANOVA). To test for effects on fish growth, an ANOVA was used to examine treatment differences based on mean fish weights for replicate tanks. To avoid pseudoreplication (Hurlbert 1984), the tank, not individual fish, was treated as the experimental unit. Therefore, replicate tanks in each treatment were not pooled and individual fish were not part of the statistical analysis. The hypothesis of equal variance among tank means (homoscedasticity) was tested with Levene's test. Simple linear regression was also conducted for several parameters as a function of dose.

The ANOVA is generally used to test for differences in treatment means. Performing only this test misses important information that characterizes the growth response in these treatments. Testing for differences in variance heterogeneity can be informative and useful as evidence for adverse effects (Green and Montagna 1996). To test for treatment differences caused by variability in fish weight resulting from PAH exposure, a second ANOVA was performed using tank standard deviation (SD) of fish weights. For this ANOVA, the SD was computed among fish within a tank. The hypothesis we tested was that treatment had no effect on the variability of weight among fish. As with testing for mean fish weight, tanks are the experimental units and each tank yielded a single observation, in this case, the SD of fish weight. This procedure is not an alternative to Levene's test, which examines equality of variances across experimental units, in this case replicate tank means.

All comparisons were considered significantly different from the control at the $\alpha = 0.05$ level. Additionally, a number of p values in the range of $0.05 < p \leq 0.15$ were noted because these low values indicate a substantial response. The α value to determine the level of significance is not a bright line separating significance from nonsignificance. For example, a p value of 0.1 indicates that there is only a 10% chance that the observed treatment effect or one more extreme (i.e., a larger difference between treatment means) would be obtained if there were indeed no treatment effect (a true null hypothesis). This low probably indicates that it is likely that the null hypothesis is not true. Based on this, we

believe that these low p values ($p \leq 0.15$) provide a reasonable level of certainty for concluding that there are real treatment effects for many of these parameters.

Control versus treatment differences were determined with Fisher's protected least significant difference (PLSD) post-hoc test. Standard deviations were reported to show the range in the data, and the standard error of the mean (SEM, a statistic of the mean) was reported when comparisons of means were intended. Statistical analyses were conducted with SYSTAT (Systat Software Inc. 2004), JMP (SAS Institute Inc. 2004), and Statview (SAS Institute Inc. 1998).

Results

Chemistry

The measured concentrations in fish pellets were very close to nominal values. The mean (SD) for the ratio of observed to expected concentrations was 1.0 (0.08) for all five treatments. The percentage contribution of each compound in the experimental diet is shown along with the percentages found in stomach concentrations of field-collected fish (Fig. 1). For many PAHs, our measured percentages for pellets were very close to the percentages of PAHs in the stomach contents observed in field-collected fish. Because we could not obtain many of the alkylated homologs, we increased the amounts for some parent and alkyl compounds so that totals (e.g., TotNPH, TotFLU, TotPHN) were similar to the field values. The dose for each PAH is shown by treatment (Table 1), as are the total PAH concentration for fish pellets and total administered dose (as μg and $\text{nmol}\cdot\text{g fish}^{-1}\cdot\text{day}^{-1}$; Table 2). A few compounds were detected in control food, which resulted in an overall dose to fish of $0.026 \mu\text{g}\cdot\text{g fish}^{-1}\cdot\text{day}^{-1}$.

Overall, whole-body tissue concentrations were very low (Table 2). Based on eq. 2 and an average half-life of 2 days for PAHs in salmonids, the concentrations of total PAHs measured in the whole body on day 56 would have been approximately three times higher if the sampling had been done immediately after the dosing period ended. This would lead to adjusted whole-body concentrations ranging from 0.06 to $2.3 \mu\text{g}\cdot\text{g}^{-1}$ ww (0.4 to $12.4 \text{ nmol}\cdot\text{g}^{-1}$ ww) total PAHs (Table 2). The results for biliary FACs (Table 2) indicated a highly positive correlation with dose (PHN $r^2 = 0.98$ and BaP $r^2 = 0.97$). The proportional increases in biliary FACs over treatment indicate that fish in all treatments were consuming the dosed food, which was supported by behavioral observations.

Fish weights

Mean fish weight for each tank on day 0 (start of dosing) varied from 12.8 to 13.1 g ww (2.8 to 2.9 g dw) with an average SD of 1.5 g ww (0.31 g dw) (Table 3). The ANOVA using all individual fish weights on day 0 indicated that there were no differences among any of the tanks ($p = 0.99$). The post-hoc test (Fisher's PLSD) found no differences in mean weights between any of the tanks, when examined as pairwise comparisons for all tanks ($n = 276$; mean (SD) p value = 0.66 (0.21)). When replicate tanks were grouped by treatment, an ANOVA showed no difference between treatments ($p = 0.47$) for the day 0 mean weights (Table 3). The distribution is shown for day 0 fish by treatment ($n = 200$;

Table 2. Polycyclic aromatic hydrocarbons (PAHs) in food and fish tissue.

Treatment	Mortality (%)	Fish dose (μg [nmol]· g^{-1} · day^{-1})	Pellet concentration (μg [nmol]· g^{-1})	Whole-body concentration			Biliary FACs ($\mu\text{g}\cdot\text{mg}$ protein $^{-1}$)		
				$\mu\text{g}\cdot\text{g}^{-1}$ (obs)	$\mu\text{g}\cdot\text{g}^{-1}$ (adj)	nmol· g^{-1} (adj)	PHN	BAP	
Control	0	0.026 [0.15]	1 [7]	0.023	0.065	0.41	2.5 (0.3)	0.20 (0.03)	
1	1.5	0.7 [3.9]	38 [210]	na	na	na	3.5 (0.1)	0.11 (0.02)	
2	2.5	2.3 [12.3]	122 [650]	0.025	0.073	0.47	6.3 (0.6)	0.23 (0.03)	
3	2.0	6.1 [32.7]	324 [1730]	0.13 (0.10)	0.38 (0.28)	2.1 (1.5)	11.7 (1.2)	0.25 (0.02)	
4	0	18.0 [95.2]	951 [5040]	0.81 (0.46)	2.3 (1.3)	12.4 (7.0)	35.8 (5.5)	0.72 (0.11)	
5	0	22.1 [117.1]	1171 [6200]	0.67 (0.25)	1.9 (0.73)	9.9 (3.5)	36.8 (8.2)	0.85 (0.14)	

Note: Dose is expressed in μg [nmol] total PAH· g wet fish $^{-1}$ · day^{-1} that was ingested. Pellet concentration is the observed dry weight concentration of total PAHs for fish pellets. Observed (obs) whole-body PAH concentrations shown for day 56 along with the metabolism adjusted (adj) concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ and $\text{nmol}\cdot\text{g}^{-1}$). Whole-body concentration is the mean and standard error of the mean (SEM) concentration (wet weight) of total PAHs measured in one or two composites ($n = 3$ fish-composite $^{-1}$). Biliary FACs (fluorescent aromatic compounds) are the mean (SEM) phenanthrene (PHN) and benzo[a]pyrene (BaP) equivalents in bile ($\mu\text{g}\cdot\text{mg}$ protein $^{-1}$). na, not analyzed.

Fig. 2), which are expressed as dry weights using the wet to dry weight ratio for our control fish (Table 3).

The ANOVA for mean fish wet weight (by tanks) at the end of the experiment (day 58) was highly significant, with a p value of <0.0001 . Based on Levene's test ($p = 0.16$), the null hypothesis of equal variances was accepted. A Fisher's PLSD post-hoc test determined that only the $22.1 \mu\text{g}\cdot\text{g}$ fish $^{-1}$ · day^{-1} treatment was significantly lower than the control ($p = 0.0009$); however, the mean weight for the $18.0 \mu\text{g}\cdot\text{g}$ fish $^{-1}$ · day^{-1} treatment was substantially reduced ($p = 0.17$) (Table 3). When dry weights were considered, an ANOVA determined that the two highest treatments (4 and 5) exhibited significantly lower weights ($p < 0.0001$ and $p = 0.01$, respectively) (Table 3) when compared with the control.

An analysis of the moisture content of fish from each treatment revealed an increasing trend with treatment concentration on day 58. An ANOVA determined that fish from the two highest doses exhibited substantially elevated ratios of wet weight to dry weight compared with the control fish ($p = 0.16$ and $p = 0.04$ for the 18.0 and $22.1 \mu\text{g}\cdot\text{g}$ fish $^{-1}$ · day^{-1} treatments, respectively). Because of the extensive randomization during the experiment setup, there was no reason to suspect differences in ratios of wet weight to dry weight among treatments on day 0. This ratio was determined instead of moisture content because it is a useful multiplier for interconverting between wet and dry weight concentrations.

Although only the two highest doses produced significant reductions in overall fish weight, considerable variability among experimental units (tanks) occurred in all PAH treatments. The ANOVA using tank SD as an indicator of variability in fish weights was significantly different (overall ANOVA $p = 0.0098$). The result of Levene's test for this ANOVA was not significant ($p = 0.9$), indicating equal variances. Pairwise comparison of treatments with Fisher's PLSD determined that all control versus treatment comparisons were significantly different (Table 3) because the variability among replicate tanks was higher for each treatment compared with the control. An ANOVA using tank SD was conducted for day 0 fish weights and was not significant ($p = 0.75$).

Another indication of the increased variability for the day 58 fish is evident in Fig. 2. The cumulative distributions show that all treatments contained fish that were smaller than those in the control tanks when the lower percentiles were considered but were as large or larger in the higher percentile groups. Statistical tests were run for several percentile groups (Table 4), and the results show generally lower weights for those percentiles below the median and higher weights in the upper percentiles. In general, fish in the upper percentiles (e.g., 75th and 95th) of most treatments (except $22.1 \mu\text{g}\cdot\text{g}$ fish $^{-1}$ · day^{-1}) were larger than control fish. Treatment 3 ($6.1 \mu\text{g}\cdot\text{g}$ fish $^{-1}$ · day^{-1}) stands out with a significant increase in fish weight for the 95th percentile fish. Also shown is the treatment variance (Table 3), which was determined using the data for all individual fish from a treatment ($n \approx 200$).

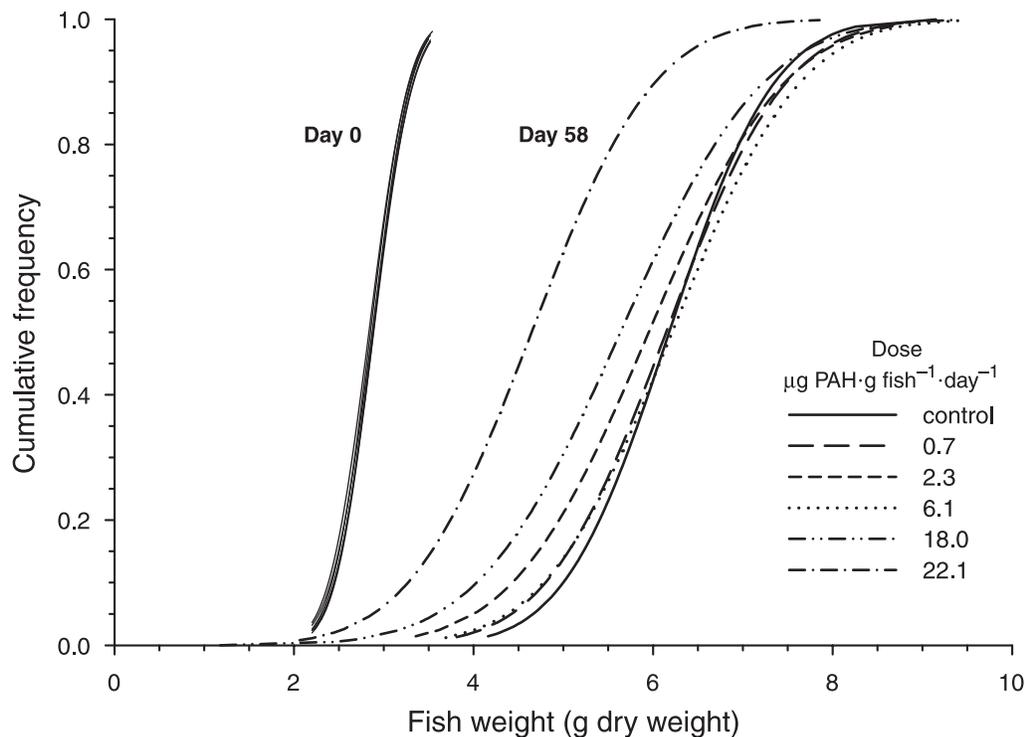
Whole-body lipids

Whole-body lipid content in our control fish was very similar to values observed in wild (and some hatchery) juve-

Table 3. Summary statistics for fish weights.

Treatment	Dose	Day 0	Day 58		Mean tank SD (dry weight)	Treat Var	Wet:dry
		Mean tank wet weight	Mean tank wet weight	Mean tank dry weight			
Control		13.1 (0.10)	27.6 (0.36)	6.18 (0.08)	0.91 (0.06)	0.85	4.47 (0.05)
1	0.7	13.1 (0.07)	28.2 (0.07)	6.15 (0.02)	1.09 (0.04)	1.1	4.59 (0.04)
2	2.3	12.8 (0.07)	26.9 (0.55)	5.95 (0.12)	1.19 (0.09)	1.41	4.53 (0.15)
3	6.1	12.9 (0.19)	28.3 (0.64)	6.20 (0.14)	1.10 (0.07)	1.25	4.47 (0.09)
4	18.0	13.0 (0.17)	26.3 (0.77)	5.63 (0.17)	1.28 (0.05)	1.56	4.67 (0.04)
5	22.1	13.0 (0.17)	22.3 (1.0)	4.66 (0.21)	1.09 (0.04)	1.16	4.80 (0.17)

Note: Dose is expressed in μg total polycyclic aromatic hydrocarbons (PAHs)-gram wet fish $^{-1}\cdot\text{day}^{-1}$. Table shows the mean fish weight for day 0 (wet weight) and day 58 (dry and wet weight) in addition to the mean tank standard deviation (SD) on day 58 (dry weight only) for each treatment ($n = 4$ experimental units per treatment). Treat Var shows the variance for all individual fish weights in a treatment (all tanks combined, $n \approx 200$ fish). Analysis of variance was conducted for each, except Treat Var. Wet:dry is the ratio of fish wet weight to dry weight. All bold values were significantly different from the control at $\alpha = 0.05$. Most values are presented as mean with standard error of the mean (SEM) in parentheses.

Fig. 2. Cumulative frequency distribution of fish weights by treatment. Plots are shown for day 0 and day 58 (end of experiment). All fish from a treatment were combined for each line ($n \approx 200$). All treatments are shown for day 0, which occur on the left side of the figure. Day 0 wet weights were converted to dry weights by a constant factor of 0.22.

nile Chinook salmon at this life stage (11.2% dry weight \approx 2.5% wet weight) (Meador et al. 2002). Whole-body lipids generally follow the same pattern as that seen for fish weights. Based on ANOVA, the two highest doses exhibit significant reductions in whole-body lipids and triacylglycerols (Table 5). Other noteworthy values are for CHOL and SE-WE for the $6.1 \mu\text{g}\cdot\text{g fish}^{-1}\cdot\text{day}^{-1}$ dose and the general downward trend for TAGs in all PAH treatments (Table 5). No trend was observed for whole-body CHOL. Also, the large increase in free fatty acids and elevated cholesterol for the $6.1 \mu\text{g}\cdot\text{g fish}^{-1}\cdot\text{day}^{-1}$ treatment are responsible for the boost in total lipid (TotLip). Significant regression equations were observed for total lipid and TAG in whole body, which were best fit by linear equations. TAG = $49.86 - 1.59\text{Dose}$,

$r^2 = 0.73$, $p = 0.018$. TotLip = $11.0 - 0.242\text{Dose}$, $r^2 = 0.49$, $p = 0.07$. Without the $6.1 \mu\text{g}\cdot\text{g fish}^{-1}\cdot\text{day}^{-1}$ dose, TotLip = $10.19 - 0.229\text{Dose}$, $r^2 = 0.89$, $p = 0.01$.

Plasma chemistry

Plasma lipids exhibited similar results to those observed for whole-body lipids (Table 6). A declining trend was observed over all treatments for both CHOL and TAGs in plasma, and linear regression equations were the best fit for each. The regression equations are TAG = $107.1 - 2.37\text{Dose}$, $r^2 = 0.72$, $p = 0.02$, and CHOL = $119.4 - 2.24\text{Dose}$, $r^2 = 0.96$, $p = 0.0004$. Several noteworthy patterns were seen in the other blood chemistry parameters analyzed, many significantly different from the control. A declining trend was

Table 4. Fish weights by percentile.

Dose	5th	10th	25th	50th	75th	95th
Control	4.72 (0.17)	5.00 (0.23)	5.55 (0.11)	6.16 (0.08)	6.76 (0.10)	7.71 (0.12)
0.7	4.53 (0.11)	4.78 (0.13)	5.35 (0.03)	6.10 (0.04)	6.93 (0.06)	7.82 (0.03)
2.3	4.34 (0.19)	4.52 (0.22)	4.98 (0.24)	5.84 (0.22)	6.85 (0.10)	7.87 (0.10)
6.1	4.51 (0.12)	4.91 (0.09)	5.51 (0.15)	6.06 (0.18)	6.90 (0.18)	8.18 (0.22)
18.0	3.71 (0.38)	4.18 (0.23)	4.90 (0.11)	5.56 (0.14)	6.37 (0.21)	7.84 (0.20)
22.1	3.17 (0.12)	3.35 (0.14)	3.96 (0.19)	4.66 (0.24)	5.30 (0.27)	6.41 (0.23)

Note: Dose is expressed in μg total polycyclic aromatic hydrocarbons (PAHs)-g wet fish⁻¹·day⁻¹. Values are presented as mean fish dry weight (grams) with standard error of the mean (SEM) in parentheses for all four replicate tanks per treatment. All bold values were significantly different from the control at $\alpha = 0.05$. Italicized values indicate $0.05 < p \leq 0.15$. Each Levene's test was not significant.

Table 5. Whole-body lipids.

Dose	Total lipid (%)	TAG	FFA	CHOL	PL	SE-WE
Control	11.2 (1.2)	58.6 (17.1)	31.7 (14.7)	15.9 (2.2)	5.2 (0.9)	0.35 (0.25)
0.7	9.8 (1.5)	49.9 (17.3)	27.0 (13.0)	16.9 (2.5)	3.8 (0.6)	0.40 (0.26)
2.3	8.7 (2.2)	32.9 (13.4)	28.8 (12.9)	20.0 (4.1)	4.9 (1.1)	0.35 (0.15)
6.1	12.9 (2.0)	43.4 (8.9)	53.8 (12.0)	25.4 (4.7)	5.6 (1.3)	1.1 (0.38)
18.0	6.8 (1.5)	20.5 (9.2)	25.4 (11.3)	16.4 (2.1)	5.2 (0.7)	0.43 (0.26)
22.1	4.9 (1.3)	16.5 (11.3)	14.0 (7.3)	14.7 (1.4)	3.4 (0.9)	0.46 (0.19)

Note: Dose is expressed in μg total polycyclic aromatic hydrocarbons (PAHs)-g wet fish⁻¹·day⁻¹. Values are presented as mean with SEM in parentheses for all five samples per treatment (six for control). TAG, triacylglycerols; FFA, free fatty acids; CHOL, cholesterol; PL, polar lipids; SE-WE, sterol esters and wax esters. Total lipids are expressed as percent of dry weight; all others are expressed in mg·g dry weight⁻¹. Bold values are significantly different from the control at $\alpha = 0.05$. Each Levene's test for these variables was not significant.

Table 6. Plasma chemistry.

Blood chemistry	Treatment					
	Control	0.72	2.3	6.1	18.0	22.1
Albumin (g·dL ⁻¹)	0.38 (0.07)	0.20 (0.06)	0.12 (0.06)	0.16 (0.09)	0.12 (0.07)	0.13 (0.10)
ALT (U·L ⁻¹)	25.3 (6.3)	26.7 (3.3)	30.3 (6.9)	30.3 (12)	21.7 (6.9)	16.3 (1.3)
Amylase (U·L ⁻¹)	599 (14)	561 (19)	526 (48)	614 (42)	568 (17)	507 (27)
Calcium (mg·dL ⁻¹)	12.1 (0.2)	11.6 (0.5)	11.6 (0.4)	12.1 (0.07)	11.7 (0.17)	11.3 (0.5)
Cholesterol (mg·dL ⁻¹)	125 (11)	118 (10)	110 (2)	104 (3)	79 (7)	72 (14)
Creatinine (mg·dL ⁻¹)	0.15 (0.02)	0.17 (0.04)	0.22 (0.03)	0.20 (0.03)	0.20 (0.05)	0.29 (0.03)
Glucose (mg·dL ⁻¹)	79 (2.9)	77 (3.2)	79 (5.4)	79 (2.1)	64 (1.1)	65 (4.0)
Lipase (U·L ⁻¹)	74 (12.5)	44.7 (11.5)	32.3 (5.3)	30.7 (8.8)	30.7 (21.1)	11.0 (11.0)
PHOS (mg·dL ⁻¹)	13.1 (0.3)	13.0 (0.5)	12.9 (0.5)	13.2 (0.14)	12.4 (0.4)	11.8 (0.5)
Total protein (g·dL ⁻¹)	3.1 (0.11)	2.7 (0.08)	2.7 (0.14)	2.7 (0.13)	2.6 (0.3)	2.5 (0.5)
Triacylglycerols (mg·dL ⁻¹)	122 (13)	105 (14)	81 (10)	95 (10)	72 (8)	52 (30)

Note: Mean and standard error of the mean (SEM) in parentheses for various parameters measured in blood plasma. Treatments are expressed in μg polycyclic aromatic hydrocarbon (PAH)-g wet fish⁻¹·day⁻¹. Composite samples ($n = 3$) are from different replicate tanks and each contained plasma from three fish. Bold values are statistically different from the control at $\alpha = 0.05$. Italicized values show those parameters that exhibited a p value between 0.05 and 0.15. ALT, alanine transaminase; PHOS, inorganic phosphate; U·L⁻¹, units per liter; dL, deciliter. None of the Levene's tests for these variables was significant.

generally observed for albumin, amylase, and lipase over PAH treatments; however, other blood parameters were relatively constant across treatments, declining only at the highest doses (ALT, phosphorus, and glucose) (Table 6). Only creatinine exhibited an increasing trend with dose (Table 6). Because the post-hoc power ranged from 0.2 to 0.5 for these ANOVAs, there was a higher probability of type II error, meaning that there was an increased likelihood of false negatives (i.e., accepting the null hypothesis of no treatment effect when in fact there is an effect). With additional statistical power, these parameters would have likely exhibited

much lower p values. Even with low power, several parameters exhibited low p values (0.05–0.15) that were considered noteworthy because of the trend observed for other doses (Table 6). Significant regression equations were observed for albumin and lipase, which were best fit with an inverse first-order polynomial:

$$\text{albumin} = 0.144 + (0.0047/\text{Dose}), r^2 = 0.89, p = 0.003$$

$$\text{lipase} = 29.44 + (0.898/\text{Dose}), r^2 = 0.69, p = 0.025$$

Discussion

PAH concentrations

The PAH concentrations tested here were environmentally realistic because of the similar proportions and magnitude of individual compounds observed in stomach contents for juvenile Chinook salmon in contaminated estuaries. This is based on the data in Varanasi et al. (1993) showing a median stomach contents concentration of 23.8 μg total PAH·g⁻¹ ww (range 3–365 $\mu\text{g}\cdot\text{g}^{-1}$ ww) for 12 composite samples (each with 10 fish) from two contaminated estuaries around Puget Sound, Washington. When based on this median concentration, the dose for field-collected fish would translate to approximately 3.8 μg total PAH·g fish⁻¹·day⁻¹, which is comparable to our middle dose. This dose was determined with the data from Varanasi et al. (1993) for mean fish weight (7 g) and PAHs in stomach contents, in addition to published growth rates of 3%–5%·day⁻¹ (Healey 1980; Weatherley and Gill 1995) for this life stage. The growth rates reported by these authors for Chinook salmon and the reported food conversion efficiency for ingested prey of $\approx 25\%$ (Weatherley and Gill 1995) would imply an average consumption rate of 16% body weight·day⁻¹ (based on wet weights), or more, for many salmonids (Weatherley and Gill 1995). In our study, the food conversion efficiency was 23% for the control fish.

The doses calculated above are based on dietary exposure only. Concentrations of PAHs in water also need to be considered when calculating the dose. For many species that have high ventilation rates, such as salmonids, a substantial dose can be obtained with relatively low water concentrations. Water concentrations of PAHs in the low parts-per-billion range, which are not unusual in urban environments and oil spills (Eisler 2000), can lead high doses of PAHs in fish (micrograms of total PAH per gram fish per day) (J.P. Meador, unpublished data). Therefore, it is necessary to consider both dietary and ventilatory sources for determination of dose to salmonids and other species of fish.

The PAH profile for the dosed food was very similar to that observed for PAHs in stomach contents from field-collected fish when most compounds and major classes were considered. Many of the compounds found in stomach contents were not represented in the dosed food. The missing compounds included many of the alkylated PAHs, which comprised from 12% to 88% (mean (SEM) = 48.5% (7.5%)) of the total PAH concentration in stomach contents from juvenile Chinook salmon residing in two different urban estuaries (Varanasi et al. 1993). Because the alkylated PAHs are generally more persistent and toxic than their parent compound (Irwin et al. 1997; Jonsson et al. 2004), our doses (mean alkylated component of 16%) likely underestimated the effects that would be observed in the fish from urban estuaries exposed to dietary PAHs with a higher percentage of alkylated homologs.

The expected concentrations for total whole-body PAHs indicate that more than 99% of the assimilated PAHs were metabolized. Based on the PAH values presented here and the high elimination rates determined by Niimi and Dookhran (1989), these adjusted values are likely close to the maximum that would be observed for this exposure. This

implies that the observed adverse responses occurred at very low tissue concentrations. As seen in the results section, whole-body tissue concentrations at low exposure concentrations provide very little information regarding toxic exposure to PAHs. A better approach may be to assess PAH toxicity in fish based on throughput (micrograms of PAH per gram fish per day) or biliary FACs, which shows promise as a metric for PAH exposure in salmonids.

Fish growth

Although the ANOVA and post-hoc tests for mean dry weight determined significant reductions only for the two highest doses, evidence of altered growth on day 58 was observed in most treatments with the distribution plots, ANOVA with SDs, and ANOVA of dry weight by percentile. The observation that fish dry weight was a more accurate indicator of reduced growth and adverse effects is an important observation for the higher doses in this study. In general, the moisture content increased with increasing dose, which was likely due to altered lipid metabolism (Navarro and Gutierrez 1995). At the highest doses (18 and 22.1 $\mu\text{g}\cdot\text{g}$ fish⁻¹·day⁻¹), the reduction in mean weight compared with that of control fish was magnified when dry weight was considered. This observation is noteworthy because the higher moisture content indicates that these fish would exhibit a lower energy content per gram wet weight, which is important physiological information that would not be readily apparent if wet weights were considered.

We consider the significant ANOVA using standard deviations and the post-hoc tests showing significant differences for each treatment compared with the control an important result. Although no significant differences in mean fish weights were observed for the low doses, the observed variability and general trend of small fish below the median and larger fish above the median is noteworthy. If exposure had continued for a longer period of time, it is likely that fish from these treatments would have further diverged from the control fish, leading to significant differences in growth. As noted by Green and Montagna (1996), heterogeneity of variance can be an indicator of impact. A very similar experiment in our lab with a different toxicant was also analyzed by ANOVA with SDs, and no significant differences were observed (J.P. Meador, unpublished data), indicating that PAHs are potent inhibitors of metabolic pathways relating to organismal growth.

Comparison to other studies

Only a few studies have examined the toxicity of PAHs to fish from dietary sources and the results are mixed. Wu et al. (2003) examined several responses to dietary exposure of one PAH, benzo[a]pyrene, and concluded that their high dose of 12 $\mu\text{g}\cdot\text{g}$ fish⁻¹·day⁻¹ did not affect growth or metabolic processes related to growth for the grouper *Epinephelus areolatus*. Another feeding study exposed polychaetes to creosote-contaminated sediment and then fed these worms to juvenile English sole (*Parophrys vetulus*; ≈ 1 g ww) (Rice et al. 2000). In one experiment from that study, fish exposed to a dose of 0.12 μg total PAH·g fish⁻¹·day⁻¹ exhibited a significant reduction in growth rate that was 10-fold lower than the observed rate for control fish (Rice et al. 2000; C. Rice,

NOAA Fisheries, 2725 Montlake Blvd. East, Seattle, WA 98112, USA, personal communication, 2006). Another dietary study by Carls et al. (1996) examined the responses in pink salmon (*O. gorbuscha*) exposed to oil-contaminated food. These authors reported growth inhibition at their lowest dose of $0.14 \mu\text{g PAHs}\cdot\text{g fish}^{-1}\cdot\text{day}^{-1}$, which is based on the published data and data from M. Carls (NOAA Fisheries, Auke Bay Laboratory, 11305 Glacier Highway, Juneau, AK 99801, USA, personal communication, 2006).

In another study on Chinook salmon, Palm et al. (2003) fed PAH-dosed pellets to juvenile fish for 28 days and reported no effects on growth. The doses in that study (approximately 0.33 , 1.3 , and $5 \mu\text{g}\cdot\text{g fish}^{-1}\cdot\text{day}^{-1}$) were similar to our low and midrange doses. The methods in Palm et al. (2003) differed substantially from those in our experiment in that no alkylated PAHs were included, a limited subset of fish were weighed, the exposure period was substantially shorter, and no attempt was made to start the experiment with statistically indistinguishable experimental units with low variance.

A field study (Willette 1996) found a significant decrease in growth for pink salmon that were released in moderately oiled areas compared with those released in a lightly oiled area. This reduction in growth was associated with an increase in cytochrome P4501A, which is used as an indicator of PAH exposure. This study ruled out prey density, prey abundance, and feeding rate as possible causes of reduced growth. Willette (1996) concluded that the reduction in growth was likely the cause of the observed reduction in survival to adulthood for these fish.

Plasma chemistry

Several of the blood chemistry parameters for our control fish were in the same range as those reported for migrating juvenile Chinook salmon by Wagner and Congleton (2004). These included lipase, total protein, TAGs, calcium, and total phosphorus. For example, the mean lipase values for our control fish ($74 \text{ U}\cdot\text{L}^{-1}$) were very similar to the values observed in migrating juvenile Chinook salmon ($64\text{--}93 \text{ U}\cdot\text{L}^{-1}$) (Wagner and Congleton 2004). A few parameters (glucose, cholesterol, and ALT) were lower in our control fish compared with the observed values in that study. Downward trends for cholesterol, TAGs, and lipase are all indicative of impairment to the pathways involved with lipid storage and utilization. Because several of these parameters are interrelated, many are expected to be altered. For example, albumin functions as a transport protein for free fatty acids in the blood stream (Tocher 2003) and will exhibit decline during starvation (Mommsen 1998; Wagner and Congleton 2004).

Very few studies have examined the effects of PAHs on fish by measuring parameters in blood plasma. A review by Folmar (1993) reported only two studies that had examined plasma chemistry as a result of PAH exposure and both had studied English sole. This review also lists control values for several serum and plasma parameters; however, very few values for salmonids are reported. As seen in the review by Folmar (1993), the responses of blood chemistry parameters to toxicant exposure are quite varied. This is no doubt a result of differences among researchers using a variety of tests, life stages, and exposure times. Additionally, the variability

in mechanism of action among the different toxicants will elicit increases in some parameters and declines in others. The results presented here are from one time point, which does not capture the potential dynamics of increases and decreases in blood chemistry parameters. Relative responses of the metabolic system to short-term exposure are expected to produce the pattern in blood chemistry parameters seen in our study, whereas longer-term exposure that results in tissue damage would likely produce increases in several blood parameters, especially enzymes.

Physiological condition and implications for the population

The high values for mean fish weight, fish weight by percentile, and total lipid for the $6.1 \mu\text{g}\cdot\text{g fish}^{-1}\cdot\text{day}^{-1}$ dose imply hormesis, which is defined as low dose stimulation (Calabrese and Baldwin 2003). Based on these parameters, it appears that fish in this treatment were in better health than the control fish. Examination of other parameters, such as lipid classes and plasma chemistry, indicate that fish from this treatment were strongly affected by PAHs and were not resistant to the toxic effects. The metrics that indicated no toxic effects were likely enhanced by physiological parameters (e.g., mobilized TAGs) or a shift in energy allocation that allowed a boost in growth that would likely not persist over time.

For the PAH-dosed fish, several of the lipid and blood chemistry parameters were depressed and exhibited similar patterns to those observed in fish undergoing starvation. For example, a number of studies report a reduction in lipids, glucose, lipase, and albumin in plasma, in addition to whole-body lipids, in salmonids that have been subjected to fasting (Navarro and Gutierrez 1995; Mommsen 1998; Kroghdal et al. 1999). In this experiment, many of these starvation-like alterations in physiology occurred even though these fish consumed all food supplied to them.

The proximal cause(s) of the starvation-like physiological parameters from PAH exposure in these juvenile Chinook salmon is not known. The physiological and biochemical factors that control and regulate growth and metabolism are diverse and only partially understood (Mommsen 1998). The enzyme lipase may be a key component for this "toxicant-induced" starvation syndrome. Several forms of lipase are known (Mommsen 1998; Tocher 2003) and these are regulated by transcription or phosphorylation. The enzyme responsible for triacylglycerol mobilization is hormone-sensitive lipase (HSL) (Tocher 2003). Under conditions of starvation, the activity of most forms of lipase is severely reduced (70%–90%) (Mommsen 1998).

Although the actual toxic mechanism(s) is not known, it is likely that these toxicants are acting on one or more components creating a cascade of interrelated abnormal physiological responses leading to toxicant-induced starvation. One hypothesis is that some or all of the PAHs absorbed from the diet are affecting lipase via the aryl hydrocarbon receptor (AhR). One study has shown that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and some PCB congeners reduce lipase activity as a result of interaction with the AhR and may be responsible for TCDD-induced "wasting syndrome" (Olsen et al. 1998). PAHs are also well-known agonists of the AhR (Varanasi et al. 1989) and may affect lipase activity via the

AhR. Barron et al. (2004) have published fish potency factors (FPF) for PAHs based on their function as AhR agonists, which are based on toxic equivalent factors (TEFs) indexed to TCDD potency.

The reduction in fish size and altered physiological parameters have important ramifications for survival of juvenile salmonids (Burrows 1969; West and Larkin 1987; Beamish and Mahnken 2001) and other species of fish (Cowan et al. 2000). A critical period in the salmonid life cycle occurs during their first winter in lakes, streams, or the marine environment. For many salmonid species, prey availability is greatly reduced during the winter months (Cooney et al. 2001) and fish often need to survive on lipid reserves. Additionally, predation is an important factor for first-year survival, which has been shown to be a function of size. According to Beamish and Mahnken (2001), juvenile salmon that do not attain a critical size during their first summer do not survive the winter.

Reduced lipid stores, which often occur in salmonids during winter months (Gardiner and Geddes 1980; Biro et al. 2004), can directly affect survival. For example, Burrows (1969) found that juvenile fall-run Chinook salmon with a lipid content of 4.1% (wet weight) exhibited a return rate to the hatchery that was approximately half of that for fish with a lipid content of 7.9%. As shown by Biro et al. (2004), the critical lipid level for *O. mykiss* is approximately 1% wet weight ($\approx 5\%$ dry weight), which was determined in lab and field studies. Below this critical level, mortality was very high. This value is supported by Finstad et al. (2004), who determined a critical body energy level of approximately 4400–4800 joules·g⁻¹ for winter survival of Atlantic salmon, which translates to a TAG value of approximately 23 mg·g⁻¹ dw. Some of the whole-body lipid and TAG values measured in our fish were at or below these critical levels determined by Biro et al. (2004) and Finstad et al. (2004).

The combination of reduced size and lipid content are critical for first year fish. For example, MacFarlane and Norton (2002) demonstrated a large increase in growth rate and a concomitant decline in TAGs when juvenile Chinook salmon exited the estuary to the open ocean. These high growth rates are likely beneficial to juvenile fish by decreasing predation and increasing their success for taking prey; however, they depend on ample prey for lipid accumulation before the winter months of low productivity. If juvenile fish exit the estuary with reduced lipid reserves, they will likely be unable to increase their growth rate and will be more susceptible to predation and less able to catch prey.

Based on the data from Biro et al. (2004) and Finstad et al. (2004), the rate of mortality for fish from our two highest treatments (18.0 and 22.1 µg·g fish⁻¹·day⁻¹) would likely approach 100%. Additionally, Biro et al. (2004) demonstrated that even small differences in fish size and lipid content led to increased mortality over winter months. Because of the reduction in size and lipid content observed for many of the fish in our experiment, especially from the lower percentiles, an increased probability for mortality would be expected for their first winter over that predicted for control fish. An increase in mortality for juvenile fish exposed to relatively low levels of PAHs would be an important stress for those populations that already experience myriad habitat alterations.

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