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The effect of food provisioning on persistent organic pollutant bioamplification in Chinook salmon larvae

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HIGHLIGHTS

• The scope for bioamplification in the critical period of Chinook larvae is reported.

• Trends in POPs bioamplification differed between feeding treatment groups.

• The highest bioamplification factors were in larvae without exogenous resources.

• Bioamplification still occurs under high food environments, but to a lesser extent.

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ABSTRACT

Fall spawning pacific salmon provision large amounts of yolk to their eggs to allow survival of larvae during under the ice winter conditions. This yolk provisioning leads to maternal offloading of persistent organic pollutants (POPs) to eggs and larvae. Previous research has shown that Chinook salmon larvae exhibit limited capacity to eliminate POPs during the cold water period resulting in bioamplification of POP residues. This study compared POPs bioamplification in Chinook salmon larvae under a high food provisioning treatment and a non-fed treatment to test whether or not food availability attenuates POPs bioamplification via growth dilution. Results demonstrate that larvae in the food provisioning treatment did not gain weight until after day 129. Between hatching and day 129, fed and non-fed treatments exhibited similar decreases in whole body lipid content, negligible POPs elimination and POPs bioamplification factors approaching 1.6. By day 184 of the study, POPs bioamplification factors in the non-fed treatment were as high as 5.3 across chemicals but ranged from non-detectable to approaching 1 in the fed group. This study demonstrates that POPs bioamplification occurs in Chinook salmon larvae even under ideal rearing conditions but peaks after day 129, following which growth dilution can attenuate bioamplification relative to starved individuals.

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1. Introduction

Fall spawning fish such as Chinook salmon (*Oncorhynchus tshawytscha*), provision large amounts of lipids (yolk) to eggs in order to allow incubation of eggs and survival of larvae post hatching (Wiegand, 1996; Urho, 2002; Kunz, 2004; Teletchea and Fontaine, 2010). These endogenous resources are critical to ensure their off-spring survive the extended winter period until food becomes available (Kaitaranta and Ackman, 1981; Wiegand, 1996; Russell et al., 1999). The maternal provisioning of yolk also deposits persistent organic pollutants (POPs) to eggs. As yolk utilization progresses, lipid normalized POP concentrations increase, because lipids are depleted faster than POPs, a process defined as bioamplification (Daley et al., 2012).

Bioamplification of POPs in Chinook salmon spawning in the Credit River, Ontario, was recently demonstrated in late staged post hatched larvae relative to fresh eggs (Daley et al., 2012). Bioamplification in larvae became pronounced after day 100 following significant depletion of lipid stores and limited chemical elimination. By the end of the study, bioamplification factors, expressed as lipid equivalent POPs concentration in larvae divided by lipid equivalent POPs concentration in fresh eggs, approached values of 5 for highly hydrophobic polychlorinated biphenyls (Daley et al., 2012). Furthermore, the increase in lipid equivalent PCB concentrations measured in larval fish at the end of the study resulted in tissue residues exceeding threshold effects concentrations for juvenile salmonids by factors ranging from 5 to 9 in individual fish (Meador et al., 2002; Johnson et al., 2007; Daley et al., 2012).

The Daley et al. (2012) study was performed by incubating larvae in a laboratory environment without provisioning food. This maximized the potential for bioamplification to occur but may re-





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flect an unrealistic condition compared to the wild where some, albeit variable, food resources are potentially available to over wintering and early spring fish. This raises an important question regarding the interaction between food availability and POPs bioamplification in natural populations of larval Chinook salmon and whether or not food provisioning causes an attenuation of the maximum bioamplification potential reported by Daley et al. (2012). Although fasting is considered a normal process for Chinook salmon larvae which rely on yolk lipids post hatch (Wiegand, 1996; Urho, 2002), information regarding the timing and abundance of food resources and when larvae first begin to feed under natural conditions is limited. Field-Dodgson (1988), studied the diet of emergent Chinook salmon in a New Zealand stream and reported that while yolk quantity was generally low for early emergent fry, up to 90% of fry sampled at the start of emergence (day 144) had empty stomachs and intestines, indicating very little feeding prior to emergence. Emergence is generally characterized in the literature as a critical period for fry, which occurs when their yolk-sacs are nearly depleted and they must leave the gravel bed to begin feeding exogenously (Jones et al., 2003; Skoglund and Barlaup, 2006; Gilbey et al., 2009). A laboratory study on Chinook larvae by Heming et al. (1982) indicated that while some "precocious feeding' had been initiated prior to emergence, this had no effect on growth. However, delaying exogenous food resources beyond a certain point reduces size and survival of fish. Studies involving other salmonids such as Atlantic salmon (Salmo salar) and Brook trout (Salmo trutta) linked first feeding by larvae to several factors including water flow, predator cues, substrate, and temperature (Hansen and Moller, 1985; Jensen et al., 1991; Metcalfe and Thorpe, 1992; Jones et al., 2003; Skoglund and Barlaup, 2006; Gilbey et al., 2009). A field study in Scotland, sampled Atlantic salmon from the earliest dispersal in late April until late June (García De Leániz et al., 2000). The authors reported that although complete re-absorption of the yolk sac generally leads to exogenous feeding, up to 35% of fry with no visible yolk had empty stomachs. It is therefore important to understand aspects such as the timing of feeding and the normal scope for weight loss and/or growth in order to place maximum bioamplification potentials into context of what occurs in nature. Growth may have significant impacts on the toxicological consequences of POPs in these young salmonids by attenuating bioamplification and even reducing chemical concentrations below those of fresh eggs via biodilution; the condition where an increase in lipid content occurs to a greater extent than an increase in chemical content in the organism.

In the present study, the scope for POPs bioamplification and growth of Chinook salmon larvae was characterized over a period of 184 d post fertilization. The scope for bioamplification and growth was determined by comparing larval size, lipid content and chemical bioamplification through time across two treatments consisting of a no-food provisioning and high food provisioning treatment. The study also sought to characterize when Chinook salmon begin using exogenous food resources and whether biodilution during the over-wintering period of larval salmon can attenuate or eliminate POPs bioamplification observed under prolonged starvation conditions.

2. Methods

2.1. Experiment

On October 5 2010, eggs were collected from a single spawning Chinook salmon female from the Credit River, Mississauga, ON, Canada (43°34′39.24″N, 79°42′8.19″W). The eggs were brought back to two aquaculture facilities (Great Lakes Institute for Environmental Research (GLIER) in Windsor and Leadley Environmen-

tal Inc. in Essex) in Ontario, Canada. Two separate aquaculture facilities were used in order to replicate the experiment and to test for consistencies across locations. The milt from three males was collected and mixed together and was used to manually fertilize eggs at each site. At the GLIER site, eggs were incubated in separate Heath trays with a flow through system receiving filtered water from the Detroit River at in situ temperatures. The same set up was used at Leadley Environmental Inc. but with eggs receiving filtered water from a large aquaculture pond at in situ temperatures. Six replicate eggs were collected post fertilization on day 1 and at a later egg developmental stage on day 21. Sampling days were strategically selected based on a previous study on Chinook salmon eggs and larvae (Daley et al., 2012). The majority of eggs were observed to hatch between days 30 and 38. On day 75, the larvae were split between fed and non-fed groups at GLIER and Leadley Environmental Inc. The non-fed larvae groups were not provisioned with food throughout the course of the study. The fed treatments were placed into three replicate flow-through aquariums (receiving the same Detroit River or aquaculture pond water at in situ temperatures). Black plastic mesh was attached in the middle of the aquarium, in order to allow uneaten food and feces to fall to the bottom of the aquarium to maintain high water quality. The fed larvae were rationed with Silvercup salmon starter #0 (Martin Mills, Almira, Ontario) for the first 60 d of feeding and were then introduced to a mixture of salmon starter and 1 mm food (Martin Mills, Almira Ontario). To account for potential food POP sources, comparisons of chemical mass balance in starved and fed fish were performed. The fed group were provisioned at a high feeding rate (roughly 5–10% body weight per day) to maximize the potential for growth dilution. Six replicate larvae were sampled from fed and non-fed groups on days 104, 129, 164 and 184 d. This study was performed in accordance with the local animal care committee at the University of Windsor, Windsor, ON.

2.2. Chemical analysis

Neutral lipid content, moisture content and POP concentrations were analyzed in individual eggs and larvae. A micro extraction technique described in Daley et al. (2009) and modifications in Daley et al. (2011) was used to analyze for POPs. Quality control procedures included spiking each sample with PCB 34 as a recovery standard and co-extracting a batch blank (equivalent mass of sodium sulfate) and reference homogenate (internal Detroit River fish homogenate) with each batch of six samples extracted. Neutral lipids were determined by gravimetric analysis according to Drouillard et al. (2004). This neutral lipid extraction which uses dichloromethane/hexane as the solvent system was selected over other methods such as the Bligh and Dyer method (1959), which extracts total lipids. Drouillard et al. (2004) showed that solvent systems like the dichloromethane/hexane method which extract neutral lipids, provide a better surrogate measure of partitioning capacity for POPs as opposed to total lipid extraction techniques. Sample cleanup was performed by Florisil chromatography as per Lazar et al. (1992). During Florisil clean-up, the first (50 mL hexane; ACP, Montreal, Quebec, Canada) and second fractions (50 mL; hexane/dichloromethane 85/15 v/v; Fisher Scientific, Fair Lawn, NJ) were collected. Following clean-up, extracts were concentrated to 1 mL by rotary-evaporator and analyzed for POP concentrations using a gas chromatograph/electron capture detector (GC-ECD) instrument (Daley et al., 2009). POP compounds were identified by retention time and quantified by comparing peak responses to the equivalent peak area in an external PCB standard (Quebec Ministry of Environment Congener Mix; AccuStandard, New Haven, CT) or certified organochlorine pesticide standard (AccuStandard, New Haven, CT) injected with each batch of samples. The following POPs were detected with sufficient frequency

and included in data analysis: polychlorinated biphenyl (PCB) congeners (IUPAC No.) 18/17, 31/28, 44, 49, 52, 70, 99, 101, 110, 118, 138, 149, 151/82, 153, 158, 170, 177, 180, 183, 187, 194, and 201, as well as pentachlorobenzene (QCB), hexachlorobenzene (HCB), octachlorostyrene (OCS), and mirex. Recoveries of PCB congeners from the in-house reference tissue were within 2 standard deviations from the mean laboratory database value derived from laboratory control charts. Recoveries of the internal standard (PCB 34) averaged $87 \pm 5.0\%$ (mean \pm standard deviation).

2.3. Data analysis

Persistent organic pollutant concentrations were expressed on a lipid equivalents basis calculated as follows:

$$C_{\rm org(lipid equiv)} = C_{\rm org(wet)} / (X_{\rm lipid} + 0.05 \cdot X_{\rm LDP})$$
(1)

where $C_{\text{org(lipid equiv)}}$ is the POP concentration normalized for lipid equivalents (ng/g lipid equivalents), $C_{\text{org(wet)}}$ is the wet weight POP congener concentration (ng/g) in the organism, and X_{lipid} and X_{LDP} are the fractions (g/g body weight) of neutral lipids and lean dry protein (LDP) in the sample. LDP was determined as the dry weight minus the lipid weight in the sample. The constant 0.05 was recommended by Debruyn and Gobas (2007) because lean dry protein has a partitioning capacity that is equivalent to 5% of lipids.

Dry weight and lipid equivalents in individual animals were compared by factorial ANOVA between treatment groups (feeding and non-feeding) and between the two study locations (Glier, Leadley). A *t*-test or one-way ANOVA was used to compare lipid equivalents POP concentrations or POP mass at different time intervals in the salmon eggs and post-hatched larvae, respectively. Normal probability plots were used to test for normality.

Elimination rate constants (k_{total}) for individual POPs were calculated using POP mass values (ng) derived by multiplying the wet weight POP concentration (ng/g wet weight) by the mass (g) of the individual larvae over the time period of days 104 (when elimination was first observed to occur) to 184. Elimination rate constants were calculated using a one-compartment first order model, where the k_{total} value was derived from the slope of the linear regression performed on a plot of the natural logarithm of chemical in the organism with time.

Bioamplification factors in eggs and larvae were calculated by expressing the ratio of POP lipid equivalent concentrations in the larvae to the mean fresh (newly fertilized) egg value. Unless otherwise noted, all values are reported as mean ± standard deviation and there were three samples per location and treatment group per time point (n = 3). Standard deviations for Fig. 2B which present bioamplification factors for the hydrophobic congener PCB 180, were generated using Monte Carlo simulations (Crystal Ball Software) to provide a measure of variability for the ratio which considered error in measured concentrations from both eggs and larvae. The mean and standard deviation values of larvae and egg PCB 180 lipid equivalent concentrations were used as inputs for Monte Carlo simulations. Simulations assumed concentrations followed a normal distribution (negative BaMFs were not permitted) and were run for 1000 iterations. Standard deviations were derived from the simulation output.

3. Results and discussion

There were no significant differences between locations (GLIER versus Leadley Inc.) for dry weight (p = 0.51) or whole body lipid equivalent contents (p = 0.37) within a given treatment group. The interaction terms (treatment × location) were also non-significant (p = 0.36; p = 0.98 respectively). There was, however, signifi-

cant differences between treatments (feeding versus non-fed) for both dry weight (p = 0.003) and whole body lipid equivalent contents (p = 0.006). Given that all eggs were taken from the same female and that there were no significant differences between grow out locations for dry weight and lipid equivalent contents, data for the two locations were combined for the remainder of analyses. The combined location mean dry weight of individual eggs and larvae over time is summarized in Fig. 1A. Egg weights, measured on a later day in egg development (day 21) exhibited no significant differences from fresh eggs (*t*-test, t = -0.05; p = 0.48). There was however a significant decline in organism dry weight over the larval period in the nonfed group (day 39–184) ($R^2 = 0.98$; n = 6; p < 0.000). Significant declines in dry total body weight over time were also observed in Chinook larvae from New Zealand (Kinnison et al., 1998). There were no significant differences in dry mass between treatment groups, until day 164 (*t*-test; t = 5.4; p = 0.001) where fed larvae were an average 2.3-fold higher than the nonfed group.

The mean percent lipid equivalents (expressed on a wet weight basis) in eggs and larvae over time are summarized in Fig. 1B. Similar to dry weight, there was no change in lipid equivalents content (*t*-test; t = 1.02; p = 0.17) during the egg incubation period. There was a strong negative correlation between % lipid equivalents in larval salmon for the non-fed group ($R^2 = 0.97$; n = 6; p < 0.000). The above trend in lipid equivalents is comparable to the trend observed in Daley et al. (2012). While, there were no differences in %



Fig. 1. Mean \pm standard deviation of panel A) whole body dry weight (grams) of individual eggs and larvae across time for both non-feeding (\blacklozenge) and feeding (\square) salmon, and B) mean (\pm standard deviation) of the % lipid equivalents individual eggs and larvae across time for both non-feeding (\blacklozenge) and feeding (\square) salmon (n = 6 observations for each point).

lipid equivalents between the fed and non-fed groups on days 104 (*t*-test; t = -1.16; p = 0.15) or 129 (*t*-test; t = 1.65; p = 0.08), a significant (*t*-tests; p < 0.05) increase in % lipid equivalents in the fed group was observed on days 164 and 184. Both the lipid equivalents and dry weight data suggest that feeding did not commence until after day 129.

The onset of exogenous feeding in Chinook salmon has been related to environmental factors such as available territory and migration distance (Beachman and Murray, 1990), temperature (Fleming and Gross, 1990; Jonsson and Jonsson, 1999), gravel size (Quinn et al, 1995), as well as physiological factors such as when the larvae reach their maximum wet body weight (Heming et al., 1982), when yolk constitutes less than 5% of the total larval wet weight (Thorpe et al., 1984; Jensen et al., 1991), and possible genetic factors (Heath et al., 1999). The study by Field-Dodgson (1988) examining emergent Chinook salmon in New Zealand observed that emergence occurred 144-201 d after fertilization. A lab study by Heming et al. (1982) examined the effects of temperature on the initial feeding of salmon, where fish were incubated at 4 constant temperatures throughout the experiment. They observed that temperature had no effect on the stage of development where Chinook were first able to feed, but the development of larvae was faster for warmer temperatures. The time when 50% of the larvae showed the presence of some ingested material was 905 \pm 14 thermal units among the four treatments (~150 d for the 6 °C treatment and 75 d at the warmest temperature of 12 °C). These studies, along with the present research, suggest that Chinook salmon do not commence feeding until a threshold has been reached whether it is related to remaining yolk resources, temperature or other factors.

Elimination rate constants were calculated for both treatment groups and a regression was performed to test if the slope was significantly different from zero. There was no significant loss of chemical mass between days 1 and 69 for all POPs. Elimination was first evident for the lowest hydrophobic chemicals on day 104. As a result, elimination rate constants were calculated between days 104 and 184 when elimination was observed to occur. Over this time period, elimination was slow to negligible for most POPs, and only the lowest K_{OW} POPs (18/17, 31/28, QCB) had slopes that were significantly different from zero (t-tests; p < 0.05). No significant elimination occurred for POP compounds having log K_{OW} values >5.6 in either treatment. There were also no consistent differences between k_{tot} values across feeding groups among chemicals where significant elimination occurred. This was counter to expectations, since the lower lipid content of the non-fed group was expected to contribute to higher PCB elimination by this group. Elimination rates are a function of temperature and physiological condition and since both temperature and lipid equivalents were changing throughout the study, variable patterns in elimination rates were expected over time. This contributed to additional variation that may have precluded detection of differences between groups.

The change in lipid equivalent PCB 180 concentration with time for the two treatments is presented in Fig. 2. PCB 180 was selected as a representative highly hydrophobic chemical which exhibited negligible elimination. There was no significant change in PCB 180 concentrations from the fresh egg value until day 104 at which point there was a significant increase (p < 0.05; *t*-test) in concentration in both groups (days 104: 1.4 ± 0.06 -fold higher and 129: 1.6 ± 0.09 higher than fresh eggs in both groups). Following day 129, differences between treatment groups were apparent. The non fed group closely resembled the trend presented for PCB 180 lipid equivalents in Daley et al. (2012). The fed group demonstrated biodilution, and by day 184, biodilution had attenuated concentrations back to levels observed in fresh eggs.

The scope for bioamplification and biodilution changed as a function of time as demonstrated in Fig. 2. In the case of the



Fig. 2. Mean ± standard deviation of panel (A) chemical concentration (ng/g lipid equivalents) of PCB 180 in eggs and larvae over time for both nonfeeding (\blacklozenge) and feeding (\Box) salmon (n = 6 for each point) and panel (B) mean ± standard error of bioamplification factors across three time points (day 104, 129, 164 and 184) for PCB 180 for both non-feeding (grey solid bars) and feeding (hatched bars) salmon. Bioamplification factors are presented across time. The double arrow represents the scope of bioamplification at day 184 for PCB 180 (n = 6 observations per point).

non-fed group, bioamplification increased with time whereas bioamplification in the fed group was maximized on day 129 and declined to a value of 1 or lower by day 184. Assuming the non-fed and fed groups represent the boundaries of bioamplification potential for this species (i.e. animals given excess food presumably exhibited the maximum growth rates possible, contrasted against starved larvae), the difference between BaMFs for the two treatments present the range over which natural BaMFs may be expected to occur depending on resource availability and other factors determining the onset of feeding and weight loss in natural populations. On day 129, the mean ± SD BaMF for chemicals having log K_{OW} > 5.5 for the non-fed and fed groups were 1.5 ± 0.2 and 1.6 ± 0.2 , respectively (Fig. 3). This demonstrates that a wide variety of POP compounds exhibit a positive potential for bioamplification in Chinook salmon larvae even under ideal situations with unlimited food resources and no predation.

By day 184, differences in BaMFs between the groups were maximized across the chemicals. For the non-fed group, BaMFs were positively associated with chemical hydrophobicity and ranged between values of 2.4 and 5.3 over the $\log K_{OW}$ range of 5.8–7.8 (Fig. 3). The magnitude and hydrophobicity trends of BaMFs for the day 184 time point was comparable to the results of Daley et al. (2012) who reported BaMFs above 4.9 for the most hydrophobic POPs after 168 d of starvation. These trends of no food provisioning likely represent the maximum bioamplification potential in Chinook larvae. In the fed group, day 184 BaMFs were also dependent on hydrophobicity, but showed BaMF values of <1 for chemicals



Fig. 3. Persistent organic pollutant (POP) bioamplification factors comparing both non-feeding and feeding salmon for panel A) day 129 larvae/fresh egg, as a function of chemical K_{OW} where (\blacklozenge) represents non-fed salmon and (\Box) is the fed group and for panel B) day 184 larvae/fresh egg, as a function of chemical K_{OW} where (\blacklozenge) represents non-fed salmon and (\Box) is the fed group.

having $\log K_{OW} < 6.5$ and approached an asymptotic value of 1 for more hydrophobic chemicals (Fig. 3). Thus, food provisioning attenuates bioamplification via growth dilution at later stages of development after commencement of feeding. However, for any given population of fish, it is expected that individual variation in the timing of first feeding and access to food resources will result in a distribution of individuals achieving BaMFs within the range of those reported in the present study.

Studies on natural Chinook juveniles have shown that some fish experience highly variable temperatures and stomach contents in these fish declined to near empty in the winter (Gardiner and Geddes, 1980; Beckman et al., 2000; Larsen et al., 2001). Higher bioamplification factors, approaching the non-fed group, could therefore be expected to occur under cold temperate and arctic conditions where it is unrealistic to assume that the fish are able to feed to their maximum satiation even in early March as many northern rivers are still frozen over (Cunjak, 1988). The Credit River, where eggs were collected in the present study has extensive periods of ice accumulation and ice jams up until March and this could alter food availability. A natural study on the Credit River of 1+ year old wild brook and brown trout showed lipids were rapidly depleted in early winter and suffered a second depletion in February-March (Cunjak, 1988). Similar results were observed in the Yakima River on naturally reared juvenile Chinook salmon where lipid levels fell below 2% in the winter (Beckman et al., 2000). The above studies demonstrate that even in the more advanced 1+ year old juveniles, feeding can be extremely limited in the winter period due to unpredictable environmental conditions.

In the present study on day 129, both feeding and non-feeding treatment groups had lipid levels between 2% and 4%. For young fish, winter mortality is often the result of starvation, as they frequently reach the point-of-no return in which a critical lipid level is attained and mortality due to starvation is possible (Heming et al., 1982; Steinhart and Wurtsbaugh, 2003; Biro et al., 2004). Steinhart and Wurtsbaugh studied age-0 kokanee (Oncorhynchus nerka) and found this critical lipid threshold level was approximately 2%, in which animals often died from starvation. Therefore even in the fed treatment group, the combination of approaching lipid levels near the point of no return and the enhanced toxicological stress due to bioamplification, could lead to augmented mortalities during this critical mortality period in the life history of this species. Daley et al. (2012) reported that the elevated sum POP concentrations in the late yolk-sac stages of Credit River Chinook salmon due to bioamplification were approximately 5–9-fold higher than the estimated threshold level used to protect juvenile salmonids from adverse health effects (Meador et al., 2002; Johnson et al., 2007). The authors also noted that this protection benchmark was placed on juvenile salmonids, but a study on newly hatched rainbow trout estimated that larvae may be 20-40 times more sensitive to POP mortalities than one year old juveniles (Walker and Peterson, 1994). These toxicological consequences would likely be highest in the most nutritionally compromised larvae. While bioamplification in a real population would likely be captured between the two extremes of maximum food provisioning and the starved group, other stressors could interact with the toxicokinetics of POPs. Based on the observations from the current study, it is predicted that multiple stressors such as resource limitation, habitat quality and climate change could potentially push bioamplification factors closer to the non-fed state observed in the present research.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/ j.chemosphere.2013.03.036.

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