

## Do chemically contaminated river estuaries in Puget Sound (Washington, USA) affect the survival rate of hatchery-reared Chinook salmon?

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Abstract: This study examined the rate of survival for hatchery-reared, ocean-type juvenile Chinook salmon (*Oncorhynchus tshawytscha*) to the adult life stage in relation to contamination status for estuaries where they temporarily reside. The hypothesis tested here is that juvenile Chinook from Puget Sound (Washington, USA) area hatcheries exhibit differential survival as categorized by the state of contamination in their respective natal estuaries. Data were examined from 20 hatcheries that released fish to 14 local estuaries in the Greater Puget Sound area over 37 years (1972–2008). A parallel analysis was also conducted for coho salmon (*Oncorhynchus kisutch*) outmigrating from many of the same hatcheries. For all years combined, juvenile Chinook transiting contaminated estuaries exhibited an overall rate of survival that was 45% lower than that for Chinook moving through uncontaminated estuaries, which was confirmed when tested year by year. The results for coho originating from the same hatcheries and sharing a similar marine distribution indicated no substantial differences among estuaries. These observations have important implications for wild juvenile Chinook that spend more time in the estuary compared with hatchery-reared fish.

**Résumé**: L'étude se penche sur le taux de survie jusqu'au stade de vie adulte de saumons quinnats (*Oncorhynchus tshawytscha*) juvéniles de type océanique élevés en écloserie par rapport à l'état de contamination des estuaires dans lesquels ils résident provisoirement. L'hypothèse testée veut que les saumons quinnats juvéniles issus d'écloseries de la région du Puget Sound (État de Washington, États-Unis) présentent des taux de survie distincts selon l'état de contamination de leurs estuaires natals respectifs. Des données ont été examinées pour 20 écloseries ayant relâché des poissons dans 14 estuaires de la grande région du Puget Sound pendant une période de 37 ans (1972–2008). Une analyse parallèle a également été réalisée pour le saumon coho (*Oncorhynchus kisutch*) migrant vers la mer à partir de bon nombre des mêmes écloseries. Pour toutes les années combinées, les quinnats juvéniles ayant transité par des estuaires non contaminés, une observation également avérée à l'échelle annuelle. Les résultats pour les saumons cohos issus des mêmes écloseries et présentant une répartition marine semblable n'indiquent aucune différence notable entre estuaires. Ces observations ont d'importantes conséquences en ce qui concerne les saumons quinnats juvéniles sauvages, qui passent plus de temps en estuaire que les poissons élevés en écloserie. [Traduit par la Rédaction]

## Introduction

Ocean-type Chinook salmon (Oncorhynchus tshawtyscha) that rear naturally or are released from a hatchery migrate in the spring and summer to the estuary as subyearlings (age 0+) and reside there for several weeks (Simenstad et al. 1982; Healey 1991; Thorpe 1994) as they adjust physiologically to seawater and increase in size and lipid content before moving offshore to marine waters. Puget Sound Chinook are of special concern because wild and some hatchery-produced populations are listed as threatened under the US Endangered Species Act (USDOC 2005). Conversely, juvenile coho salmon (Oncorhynchus kisutch) spend their first year in freshwater and migrate to the estuary in the spring or summer as yearlings (age 1+), generally spending only a few days in the local estuary before proceeding to more open waters (Simenstad et al. 1982; Thorpe 1994). This major difference in life history can have a large effect on the degree of toxicant exposure in contaminated estuaries, which can affect fish in several ways, including impaired growth, altered behavior, higher rates of pathogenic infections, and changes to physiological homeostasis, all of which can lead to increased rates of mortality.

Even though Puget Sound is considered one large estuary, there are many local estuaries formed by numerous rivers that empty into the Sound, and these exhibit various degrees of physical and chemical alteration. Many of these local estuaries have been highly modified over the past 100 years through dredging, channelizing, armoring, and diking for agriculture. They have been used as shipping ports, sites for industry, and as receiving water for waste treatment plants (Bortleson et al. 1980; Thom and Hallum 1990). Diking for agriculture has reduced the area of native marshlands by 25%-95% (Thom and Hallum 1990; Simenstad et al. 2011), especially in the Lummi, Samish, Skagit, Nisqually, Stillaguamish, and Snohomish river deltas. Higher percentages of loss for original habitat occurred in the Duwamish and Puyallup systems (Thom and Hallum 1990). The Bortleson et al. (1980) data show extensive reductions for subaerial and intertidal habitat for the more urban sites (e.g., Duwamish and Puyallup), but also relatively high losses for the Samish (79% of subaerial habitat) and modest losses for the Nisqually ( $\sim$ 22%–28% of both subaerial and intertidal). Only one of the estuaries in this study, the Nooksack, is considered relatively undisturbed in terms of areal extent of subaerial and intertidal wetlands (Bortleson et al 1980; Thom and

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Hallum 1990). Of course the local estuaries of Puget Sound continue to change with development and restoration; therefore these values may change with more recent evaluations. Several of the rearing estuaries are considered highly degraded owing to anthropogenic activity, and the degree of contamination is confounded with urbanization and loss of prime habitat. Many of these local estuaries occur near human-impacted (urban or agricultural) areas and have been contaminated by industrial waste, stormwater effluent, chemical spills, wastewater treatment facilities, and runoff from impervious surfaces and other modified areas (e.g., farms, ranches, and logged areas). Historically, concentrations of several contaminants were low, as measured in sediment, but rose to high levels as urbanization increased (Crecelius et al. 1985). Even though levels of some contaminants have declined over the past few years, others have increased recently, and many of the legacy contaminants are still elevated in sediment and fish (Puget Sound Ambient Monitoring Program 2007), especially during the time frame of this analysis.

The main characteristics of a local estuary that are necessary to enhance the probability of survival for juvenile salmonids have been addressed by several authors and include refuge from predation, freshwater-seawater transitional areas, and productive foraging allowing increased growth (Simenstad et al. 1982; Healey 1982; Macdonald et al. 1988; Thorpe 1994). Additional parameters to consider include water quality (oxygen, temperature, and salinity), water velocity, physical habitat, pathogen occurrence, competition, and chemical contamination. By considering a large number of hatcheries and estuaries within the Puget Sound area over several years, many of the important parameters that determine survival to the adult stage can be minimized or accounted for in the analysis. Data were available or generated for a few of these factors such as growth rate, prey abundance, fish density, predation, and distance from the hatchery to Puget Sound and were examined in light of their potential effect on survival. The quality of freshwater habitat is also a crucial factor for salmonid vitality (Myers et al. 1998); however, this factor was considered not critical for these comparisons because I examined only hatchery fish, which quickly move downstream to the estuary (Nelson et al. 2004; Seattle Public Utilities 2008; Chittenden et al. 2008)

The primary metric to assess life-cycle success is the smolt-toadult return rate (SAR), which provides a percent value based on the number of juvenile salmon (smolts) released and the number of adults enumerated and estimated from fisheries and hatchery returns. Survival for first-year ocean-type Chinook in the Pacific Northwest has been estimated at 0.4% (compiled by Spromberg and Meador 2005). Rates of survival over successive years are considerably higher for 2-, 3-, 4-, and 5-year-old fish at 60%, 70%, 80%, and 90%, respectively (Pacific Salmon Commission Chinook Technical Committee 2002). Clearly, first-year survival is important for Chinook, and most of the mortality for first-year oceantype Chinook is attributed to predation, poor growth, pathogens, starvation, and toxicants.

#### Hypothesis

The goal for this analysis was to examine the SAR as an indicator of survival for outmigrating ocean-type Chinook from hatcheries within the Greater Puget Sound area and to examine the influence of contamination in the estuaries where fish rear before migrating to open water. The main hypothesis was that contaminant exposure for outmigrating juvenile Chinook was sufficient to affect the probability of survival during their first year in marine water. SAR values for coho salmon, a species that spends little time in the estuary, were also assessed, and the comparison to those for Chinook was used as another line of evidence to test the hypothesis that contaminated estuaries are one of the main factors determining the rate of survival for Chinook. Additionally, coho SAR values were useful for identifying hatcheries that practiced poor husbandry, as both coho and Chinook SAR values would likely be lower than the mean.

Salmonid survival is dependent on a large number of factors, many that co-occur. The analysis presented here is simplistic, but highlights an important relationship between hatchery Chinook survival and contaminated estuaries. Because this analysis examined the smolt-to-adult survival rate in fish from a large number of hatcheries and estuaries over several years in one geographical location, many of these factors were likely accounted for and therefore had less of an effect on the overall results.

## Methods

Only hatchery-released Chinook and coho salmon were considered in this analysis. All releases over the years 1972-2008 were included for both species over all areas of Puget Sound and the northern Washington State portion of the Salish Sea (Table 1; Fig. 1). The Skagit River hatchery was not included because of several factors, including the limited availability of data that met the criteria listed below, the high probability of densitydependent mortality and emigration (Greene and Beechie 2004), and the fact that this system is dominated by wild fish, whereas all other systems in this study are hatchery dominated. Estuaries in the Hood Canal were also excluded because of persistent low dissolved oxygen levels (Brandenberger et al. 2011) that would likely confound the analysis. All major estuaries within Puget Sound and northern Washington (Thom and Hallum 1990) were included except for the Skagit (mentioned above) and the Lummi (no data) in addition to several minor estuaries.

#### Fish data

Data for hatchery-released juvenile salmon were obtained from the Regional Mark Information System (RMIS), which is maintained by the Regional Mark Processing Center (RMPC) as part of the Pacific States Marine Fisheries Commission (PSMFC) (Nandor et al. 2010). Coded wire tag (CWT) data, release masses, dates of release, and SARs were obtained from the online RMPC database (http://www.rmpc.org) (Regional Mark Information System 2006). The SAR was obtained by running SA1 queries for a given tag code group from all available years (release years 1972-2008). The recoveries are estimated based on the observed number of CWT fish captured in all fisheries and the number of hatchery returns. All fisheries were selected in the SA1 query, which included all adult fish landings from troll, gill net, purse seine, sport fishing, and others. The number of fish returning to the hatchery (escapement) is variable but usually averages in the 25%-50% range of the total recoveries for ocean-type Chinook (Pacific Salmon Commission Chinook Technical Committee 2002), indicating that a high percentage of the SAR was determined by fish returning to their natal hatchery. The SAR values represent survival for the entire cohort and are not year specific. Survival for a tag code group was estimated by comparing the total number of CWT fish released with the number of adult fish found with CWTs retrieved at the hatchery (100% sampling rate) and from commercial and sport fisheries (20% sampling goal) (Nandor et al. 2010). Final estimated recoveries from the various fisheries were estimated according to specific algorithms (Pacific Salmon Commission Chinook Technical Committee 2002). I assumed that the available CWT recovery information based on escapement and fisheries did not contain values for juveniles, because there is no fishery for this life stage, except for the small number taken for research.

For Chinook and coho, a number of criteria were applied to the data to ensure adequate statistical power and to reduce variability when possible. Only tag code groups with more than 10 000 CWT fish were selected; however, most groups contained 50 000 to over 200 000 tagged fish. For most hatcheries and years, several tag code groups were released (mostly groups of two, but occasionally three to five groups per year). All values from a hatchery for a given year were reduced to mean values. The variability for

Table 1.	General	information on	hatcheries and	their loc	al estuary.
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	Main freshwater		Distance to	Area of	Fish growth and	
Hatchery	system	Estuary	estuary (km)	estuary (km²)	estuary prey*	Fish∙m⁻
Northern Washing	gton					
Skookum Creek	Nooksack River	Nooksack	77.3	7.9		0.95
Kendall Creek	Nooksack River	Nooksack	74.1			
Samish	Samish River	Samish Bay	18.5	17.5		0.23
North Puget Sound	1					
Bernie Gobin	Tulalip River	Tulalip Bay	0.25	1.36		1.84
Harvey Creek	Stillaguamish River	Stillaguamish	27.8	25.2	BA high (10)	0.01
Whitehorse Ponds	Stillaguamish River	Stillaguamish	47.5			
Wallace River	Skykomish River	Snohomish	63.4	12.5	High % SF (1)	0.09
Mid-Puget Sound						
Grovers Creek	Grovers Creek	Miller Bay	1.9	1.65		0.26
Issaquah	Lakes Washington and Sammamish	Shilshole Bay	52	0.54	BA high (5)	4.7
Portage Bay	Ship Canal	Shilshole Bay	8.5			
Puyallup Tribal	Clarks Creek, Puyallup River	Puyallup	15.5	5.9	>3% body mass·day <sup>-1</sup> (2); BA high (8, 11)	0.51
Voights Creek	Puyallup River	Puyallup	35.7			
Soos Creek	Green River	Duwamish	55.7	2.6	>2%–3% body mass day <sup>-1</sup> (3, 4, 7); BA adequate to high (12, 13)	2.2
Keta Creek	Green River	Duwamish	66.0			
Crisp Creek	Green River	Duwamish	64.4			
Gorst Creek	Gorst Creek	Sinclair Inlet	0.6	2.7	1.3%−4.1% body mass∙day <sup>-1</sup> (14); high % SF (14), BA high (11)	0.74
South Puget Sound	d					
Capitol Lake	Capitol Lake	Budd Inlet	0.8	5.0	Adequate (zooplankton + benthos) (9, 11)	2.1
Tumwater Falls	Deschutes River	Budd Inlet	3.2			
Garrison	Chambers Creek	Chambers Bay	1.6	0.28		3.0
Minter Creek	Minter Creek	Henderson Bay	1.6	0.44		7.3
Clear Creek	Nisqually River	Nisqually	10.1	7.5	≈2.5% body mass·day <sup>-1</sup> (6)	0.81
Kalama Creek	Nisqually River	Nisqually	14.8			

Note: Area of local estuary includes the intertidal and subtidal area of the river outlet and immediate nearshore habitat. If available, stomach fullness (SF) and growth rate (% body mass-day<sup>-1</sup>) are listed for juvenile Chinook. Prey availability based on intertidal or subtidal benthic abundance (BA) is considered low, adequate, or high, and is based on data for density and biomass (see online Supplementary data<sup>1</sup>). Fish-m<sup>-2</sup> was determined with the number of outmigrating ocean-type Chinook and coho for each system.

\*Citations are shown in parentheses next to data: (1) Cordell et al. (2001b); (2) Shreffler et al. (1990); (3) Meador et al. (2010); (4) Cordell et al. (2011); (5) Simenstad (2003); (6) Ellings and Hodgson (2007); (7) Nelson et al. (2004); (8) Meyer and Vogel (1978); (9) Giles and Cordell (1998); (10) Heatwole (2006); (11) Puget Sound Ambient Monitoring Program (1994); (12) Cordell et al. (2001a); (13) Windward Environmental (2010); (14) Fresh et al. (2006).

survival among these tag code groups for a given year was very low, and release masses and dates were usually identical among groups. For example, the Soos Creek hatchery released 56 qualifying Chinook tag code groups over all years (11 single and 18 multiple releases), and the mean coefficient of variation (CV) for the 18 multiple SAR values was very low at 18.4%. Over the time period of this study, 390 tag code groups for Chinook, and 476 tag code groups of coho qualified for inclusion in this analysis. For coho, all hatcheries with Chinook data were included. Also, coho data from two additional hatcheries were included (Crisp Creek and Keta Creek) to increase the number of replicates. Coho and Chinook releases overlapped for 10 hatcheries, and the most recent release year for each species was 2008.

The specific criteria for Chinook included release masses ranging from 3 to 12 g, release dates between 15 April and 30 June, and only fall or summer run ocean-type fish that were released at age year 0+. Only fish released from a given hatchery or nearby stream were considered, and those that were released in another watershed or netpen were excluded. For coho, tag code groups were included for fish released between 23 March and 30 June, and fish ranged from 15 to 40 g. All coho were released at age 1+ years. Very few (<5%) tag code groups were excluded for either species based on the above criteria. The McAllister Creek hatchery SAR values for Chinook were not used (n = 3, 1992, 1999, and 2001), because this facility was closed in 2000 as a result of severe problems with parasites (Hatchery Scientific Review Group 2002).

The final dataset for Chinook consisted of releases from 20 hatcheries into 14 different local estuaries over 37 years (Fig. 1). For hatchery-year combinations, there were eight hatcheries releasing fish into contaminated estuaries (80 mean SAR values) and 12 hatcheries for uncontaminated estuaries (164 mean SAR values). These are mean values for hatchery-year combinations, hence the total releases (tag code groups) were much higher. For coho, data were available for releases from 12 hatcheries into eight estuaries. Overall for coho there were 226 releases, 106 to contaminated estuaries and 120 to uncontaminated estuaries, when based on mean values for hatchery-year combinations.

The SAR values for coho from the Kalama Creek (mean SAR = 1.4%) and Clear Creek (mean SAR = 0.56%) hatcheries were from 5 to 10 times lower than values for all other coho hatcheries in this study. Based on the anomalous values, these hatcheries were excluded from the analysis for coho. SAR values for Chinook from these two hatcheries, which pass through the Nisqually estuary, were generally as high as or higher than the mean value for all other uncontaminated estuaries when examined by release year. The low coho SAR values may have resulted from poor water quality or pathogens due to extended time spent in freshwater. It is unknown whether hatchery practices contributed to these

Fig. 1. Map of Puget Sound and the Salish Sea in northern Washington. Boxes indicate hatcheries releasing salmon to contaminated estuaries, and circles denote hatcheries upstream of uncontaminated estuaries.



differences, and no explanation or theories for these anomalous values were found in the literature. Noteworthy are the relatively high SAR values for coho released from nearby marine net pens on Squaxin and Fox islands. The mean SAR for netpen coho (1974–2006) fitting the same requirements listed above for release date, mass, and tag code group size was 6.7% (n = 32 mean SARs, 68 total tag code groups), indicating no unusual problems with the marine phase of their life cycle in this area of Puget Sound.

Implicit within this analysis is that there were no major differences in husbandry practices for juvenile fish, including CWT procedures, disease, and rearing conditions among the hatcheries examined that would differentially affect salmonid survival in the estuary or marine environment. The only highly disparate values observed were for the Kalama Creek and Clear Creek hatcheries mentioned above. It was also assumed that there was no relationship between the SAR and hatchery location within Puget Sound, and differences in fish physiology and genetics were inconsequential. Additional assumptions for the open-water phase (marine) include similar conditions for prey availability, predation, and all others factors that would determine survival during this phase of their life cycle.

#### Determining the state of contamination in a local estuary

Multiple lines of evidence were used a priori to categorize estuaries as either contaminated or uncontaminated, and data were available for most locations. For one estuary (Chambers Bay) the determination was based on a narrative analysis (qualitative) (see online Supplementary data<sup>1</sup>). Four independent factors were used to categorize a local Puget Sound estuary as clean or contaminated: (i) tissue concentrations of contaminants in juvenile Chinook and other species determined from animals collected in the estuary; (ii) results of sediment toxicity bioassays; (iii) evaluation of numerical criteria from the Washington State Sediment Management Standards (SMS) and Effects Range Medium (ERM) values; and (iv) the siting of Superfund sites and Puget Sound Initiative and Washington Department of Ecology (Washington Department of Ecology 2012) cleanup sites. Even though the determination of contamination could be due to only a few toxicants, most contaminated estuaries contain myriad chemicals at concentrations known to cause adverse effects in biota.

## **Tissue contaminants**

Fish and invertebrate tissue concentrations were available for 8 of the 14 estuaries. A number of studies reported contaminant concentrations in tissue for outmigrating juvenile Chinook in many of the local estuaries of Puget Sound and from the hatcheries that raise those fish. Most of the data were for polychlorinated biphenyls (PCBs) in liver, whole-body, and stomach contents and polycyclic aromatic hydrocarbons (PAHs) in stomachs. Local estuary data were compared with concentrations determined in juvenile Chinook from several hatcheries, including Soos Creek, Puyallup, Kalama Creek, McAllister Creek, and Wallace River. Several studies (Mac et al. 1979; Varanasi et al. 1993; Easton et al. 2002; Maule et al. 2007) were used to determine concentrations of contaminants in stomach contents for hatchery fish based on measured values for stomach contents and hatchery food. For a given contaminant and tissue, the mean concentration +1 standard deviation (SD) for fish from the estuary was selected to represent the degree of exposure. This value was then compared with the mean value for all hatchery data and shown as a factor difference (Table 2). Differences between hatchery and estuary Chinook greater than two-fold indicated that outmigrating salmon had bioaccumulated or been exposed to contaminants, supporting the conclusion that an estuary was contaminated. Data for English sole liver, fillet, and stomach contents were also included in addition to values for clams. These additional data were compared on a relative basis between contaminated and uncontaminated estuaries.

## Sediment

The first sediment index is based on bioassay results, which were obtained from a series of reports to gauge the potential toxicity of sediments in local Puget Sound estuaries. These studies conducted bioassays with sediment from a large number Puget Sound sites, including an amphipod mortality bioassay and a sea urchin fertilization test with sediment pore water (Long et al. 1999, 2000, 2002, 2003). If one of these tests indicated toxicity for a given local estuary, then the sediment was considered toxic.

The second sediment index was based on sediment standards and guidelines. For this index, the Washington State SMS (Washington Department of Ecology 1995) and ERM values (Long et al. 2003) were used to determine whether a local estuary was contaminated. The SMS comprise different levels of criteria for Puget Sound sediment, including the Sediment Quality Standards (SQS) and Cleanup Screening Levels (CSL). The SQS are numeric values for a large number of contaminants that are designed to protect biological resources and human health and are considered to be "no effect levels". The SQS serve as the cleanup objective for all cleanup actions; however, these are based on severe responses (amphipod mortality, polychaete growth inhibition, and large reductions in the number of benthic invertebrates). The CSL values include Minimum Cleanup Levels (MCUL) and Maximum Chemical Criteria (MCC). These are higher for a given contaminant than their corresponding SQS value and are based on sediment quality that may result in minor adverse effects. The ERM is the 50th percentile sediment concentration determined from a database of matched bioassay and sediment concentrations where adverse biological responses were observed. In many cases these adverse effects were severe (e.g., mortality). Based on the data presented in Long et al. (2003), a failure for any one of these metrics (SQS, CSL, or ERM) led to the conclusion of sediment contamination. Contaminated sediment is likely reflective of high concentrations of harmful chemicals in salmon prey species and is usually correlated with elevated water concentrations that fish ventilate.

## Listed sites

If any of the estuaries contained US Environmental Protection Agency Superfund sites or Puget Sound Initiative and Washington Department of Ecology cleanup sites, then the estuary was considered contaminated. Listed sites are considered severely contaminated and are studied in depth to consider options for remediation.

#### **Estuary description**

The hatcheries and rivers used by outmigrating juvenile salmon to their local estuary are listed in Table 1. Areal size of each local river estuary and distance from the hatchery to the estuary was calculated using Google Maps with the program provided by Daftlogic (http://www.daftlogic.com/projects-google-maps-areacalculator-tool.htm). Bortleson et al. (1980) provided estimates of the area for subaerial and intertidal habitat and the percent loss from the mid- to late 1880s to 1980 for 7 of the 14 river estuaries examined in this study, which can also be found in Thom and Hallum (1990). These two references were used as a guide for most calculations. In some cases the areal values were similar to those in Bortleson et al. (1980), as was the case for Samish Bay, indicating a predominance of intertidal habitat. These data along with the distance from each hatchery to the local estuary are shown in

<sup>&#</sup>x27;Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2013-0130.

Table 1. Additional information for each estuary is provided in the Supplementary data<sup>1</sup>.

Total square metres for each estuarine exposure area was determined without considering differences in habitat type or quality. These values (converted to km<sup>2</sup>) represent only the intertidal and subtidal areas of the local estuary plus the immediate nearshore habitat, which in some cases resulted in different values from those of Bortleson et al. (1980), who calculated total surface area of the estuary. Depths were limited to 10 m as shown on National Oceanic and Atmospheric Administration charts, the range that juvenile Chinook are known to utilize (Carter et al. 2009). The area of subtidal habitat, which can support prey for juvenile salmon, was not included in the Bortleson et al. (1980) analyses. Prey species are more abundant in some types of habitats; however, it was assumed that invertebrates from the benthos and associated water column would be available for consumption by juvenile Chinook. In many systems juvenile salmon feed on the benthos (Higgs et al. 1995; Cordell et al. 2001a; Fresh et al. 2006), which can contain very high densities of common prey species, even in those systems that are considered contaminated and lacking prime habitat (e.g., the Duwamish).

To determine the number of fish per square metre in local estuaries, the number of ocean-type Chinook and coho released into these systems was estimated and divided by the calculated area (Table 1). Data for the number of fish released was obtained from the Hatchery Scientific Review Group (2002), Washington Department of Fish and Wildlife (2000–2005), and RMIS database. Only ocean-type Chinook and coho were included in this analysis because they would likely compete for resources in the estuary and represent the majority of juvenile salmon in these systems. Mean values were used for fish release estimates taken from the RMIS database, which were often variable over years.

Many of the estimated density values for outmigrating fish are likely overestimates, because some hatcheries have not operated for all years of this study and many of the release estimates are current levels and were lower in previous years. The Green River – Duwamish estimate is the only one that includes both hatchery and natural production for both species. Hatchery fish likely predominate in most systems, as indicated by Rice et al. (2011) for Chinook in many of these local estuaries; however, the actual contribution of wild fish to most systems is unknown. It should be noted that these density values include outmigration for fish over several months (April–July) and are system-wide means that do not account for location-specific abundance, which can be higher (Cordell et al. 2011).

#### Analyses

In this analysis, hatchery was treated as a replicate (Ryding and Skalski 1999). One-way analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were run on various combinations of factors. The SAR data were not normally distributed, which violates the assumption of normality for ANOVA. The Chinook data were best fit to a log-normal distribution, and the coho SAR values were arcsin square-root transformed to achieve a normal distribution. The nonparametric Wilcoxon signed-rank test (Sokal and Rohlf 1995) was also performed, which allowed year-wise comparisons and a reduction in the large variability for survival that occurs from year to year. The Wilcoxon test uses the sign and magnitude of the differences to determine trends. Also, a Mann-Kendall test was performed on SAR values over years to test for a temporal trend. Regressions were performed to test for relationships between survival, release mass, and release day of year (DoY). These were performed for combinations of hatcheryyear data and for all tag code groups over different time periods. One period (release years 1985-2008) was selected because of the general decline in SAR after 1985 and to capture the most recent data. Another period selected was for the release years 1997-2008, to more closely match the analysis from Duffy and Beauchamp

(2011). Standard deviations (SD) show the range in data for a parameter, and the standard error (SE) was reported when comparison of means was intended.

## Results

The mean (SD) SAR, release mass, number of fish released (total and with CWT), and years of data for each hatchery are shown in Tables 3 and 4. This dataset comprises  $2.3 \times 10^8$  total Chinook (21% with CWT) and  $1.1 \times 10^8$  coho (30% with CWT) released over 37 years. A few hatcheries were represented most years of the analysis; however, many yielded data for a subset of this time period.

For 5 of the release years (1974, 1977–1978, and 1983–1984), Chinook SAR values were available for only one of the categories (contaminated or uncontaminated estuary) and were therefore not used in the analysis. For 9 of the years, data were available for only one hatchery (no replicates) in one of the categories. Coho SARs were represented in all release years (1973–2008) for both groups.

#### **Estuary contamination**

A comparison of concentrations in whole-body, liver, and stomach contents for fish from several hatcheries indicated that juvenile Chinook were exposed to high levels of contaminants in some estuaries (Table 2). Mean (SD) concentrations of PCBs in stomach contents were relatively high for hatchery fish (60 (39)  $ng \cdot g^{-1}$  wet mass) because of a few high values in the 1980s. Over the past 20 years, hatchery feed concentrations have declined substantially to very low levels, which may be due to changes in the oil added to fish pellets (Maule et al. 2007). When available, contaminant concentrations in salmon and other species, in conjunction with sediment toxicity bioassays, sediment criteria, and the number of listed sites, all agreed (except for one minor case), supporting the designation of contaminated or uncontaminated for each estuary. For contaminated estuaries, these different lines of evidence all support the expectation of adverse effects for outmigrating salmon.

Few data exist on contaminant concentrations in juvenile coho. One study examined whole-body and stomach content concentrations in juvenile Chinook and coho from five Oregon estuaries over several years. Estuary-matched mean concentrations for whole-body total PCBs and DDTs were higher in juvenile Chinook ( $\sim$ 2.5-fold for PCBs and  $\sim$ 3.2-fold for DDTs) (Johnson et al. 2007). Differences this large, and greater, for these two species were also seen for fluorescent aromatic compounds in bile (phenanthrene wavelengths) as a result of PAH exposure and for total DDTs and PCBs in stomach contents for those sites exhibiting elevated levels. The largest differences were observed for total PAHs in stomach contents, which ranged from  $\sim$ 10 to 200 times higher in Chinook over coho. These results are supported by another study showing that the concentrations of whole-body total PCBs in juvenile Chinook were 6.6 times higher compared with values for juvenile coho collected in Commencement Bay near the Puyallup River estuary (Olson et al. 2008). These data support the hypothesis of higher levels of toxicant exposure for Chinook compared with coho outmigrating through contaminated estuaries.

#### Smolt-to-adult survival

When all data were considered (all hatchery–year combinations; n = 244), the mean survival for juvenile Chinook released from hatcheries into contaminated estuaries was 45% lower than for fish outmigrating through uncontaminated estuaries (SAR values 0.48% versus 0.87%; p < 0.0001) (Table 5). The ANOVA for Chinook release masses was not significant (p = 0.28), indicating no difference among hatcheries and years. The ANCOVA also determined that there was no interaction between estuary contamination status and fish mass at release (p = 0.27). The more appropriate analysis by the nonparametric Wilcoxon signed-rank test

<b>Table 2.</b> Contaminants in outmigrating salmon, other species, and local estuaries.
------------------------------------------------------------------------------------------

			Chinook							
Hatchery	Estuary	Status	PAHs (stomach)	PCBs (stomach)	PCBs (liver and whole body)	Other data	Other species (ng·g <sup>-1</sup> )	Toxic sediment	Listed sites and contaminants of concern	References*
All hatcheries (ng·	g <sup>-1</sup> )		99 ( <i>n</i> = 22)	60 ( <i>n</i> = 35)	53 (liver, <i>n</i> = 32); 19 (whole body, <i>n</i> = 36)	Hg: 17.4 (whole body, <i>n</i> = 8)				1–3
Northern Washin Skookum Creek, Kendall Creek	n <b>gton</b> Nooksack	UC					Clams ( <i>n</i> = 4) PAHs: 6.7, Hg: 10, Pb: 80; crab ( <i>n</i> = 8) PCB: 20	NT, 1	Relatively pristine	5, 9, 13
Samish	Samish Bay	UC						NT, 1	Used as reference site	5, 13,14
North Puget Sour Bernie Gobin	<b>1d</b> Tulalip Bay	UC						ND	Relatively pristine area	
Harvey Creek, Whitehorse Ponds	Stillaguamish	UC						NT, 1	Used as reference site	5, 13
Wallace River	Snohomish <sup>†</sup>	С	$12.7 \times$ ( <i>n</i> = 4)	$2.4 \times (n = 4)$	4.3× (liver, $n = 5$ )			Tox, 5	CU-10, TBT, As, PAHs, Hg, dioxin	5, 13
Mid Puget Sound Grovers Creek	Miller Bay	UC					Clams PAHs: 22	NT, 1	Used as reference site	6, 13
Issaquah, Portage Bay	Shilshole Bay	UC			1.6× (whole body, $n = 2$ )	Hg: 0.5×, <i>n</i> = 2; TBT: BD	English sole (fillet) PCB: 42 $(n = 3)$	ND		1, 11
Puyallup Tribal, Voights Creek	Puyallup†	С	980× ( <i>n</i> = 8)	6.1× ( <i>n</i> = 6)	5.4× (liver, <i>n</i> = 8)		English sole (liver) PCB: 800 ( $n = 17$ ); English sole (stomach) PCB: 110 ( $n = 7$ ); English sole (stomach) PAH: 3000, $n = 7$	NT, 5	Metals, DDX, HCBD, phthalates, dioxin, organics	2, 4, 12, 13
Soos Creek	Duwamish <sup>†</sup>	С	943× (n = 19)	8.5× ( <i>n</i> = 30)	6.3× (liver, <i>n</i> = 15); 11× (whole body, <i>n</i> = 111)		Clams PAHs: 220	Tox, 6	CU-33, TBT, As, Cd	2, 3, 4, 10, 13
Gorst Creek	Sinclair Inlet†	С						Tox, 6	TBT, PCBs, PAHs, metals	4, 13
South Puget Sour Capitol Lake, Tumwater Falls	nd Budd Inlet†	С					Clams PAHs 100–1100, <i>n</i> = 3	Tox, 2	CU-7, dioxin, TBT, PCP, phthalates	6–10, 13
Clear Creek, Kalama	Nisqually	UC	0.3× ( <i>n</i> = 11)	1.4× (n = 11)	2.3× (liver, <i>n</i> = 10)		Engish sole (liver) PCB: 160 ( $n = 14$ ); English sole (stomach) PCB: 30 ( $n = 4$ ); English sole (stomach) PAH: 30 ( $n = 4$ )	ND	Used as reference site, low contamination <sup>‡</sup>	2, 12, 13

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Table 2 (concluded)

Chinook

							1		Listed sites and	
			PAHs	PCBs	PCBs (liver and		Other species	Toxic	contaminants	
Hatchery	Estuary	Status	(stomach)	(stomach)	Status (stomach) (stomach) whole body)	Other data	$(ng \cdot g^{-1})$	sediment	sediment of concern	References*
Garrison	Chambers Bay (	C						QN	Narrative	
Minter Cr	Henderson Bay UC	UC						NT, 1	Used as reference site 6, 13, 14	6, 13, 14
Note: Status de clams, and crabs.	notes estuary as contam All Chinook values are f	inated (C) o rom compo	or uncontamina sites of several	ated (UC). For fi l individuals. A	ish concentrations, fill Il concentrations are I	let refers to a fillet v recorded as wet mas	vith skin on, and stomach re is. Juvenile Chinook from So	fers to stomach os Creek, Puyal	Note: Status denotes estuary as contaminated (C) or uncontaminated (UC). For fish concentrations, fillet refers to a fillet with skin on, and stomach refers to stomach contents. Other species include English sole, clams, and crabs. All Chinook values are from composites of several individuals. All concentrations are recorded as wet mass. Juvenile Chinook from Soos Creek, Puyallup, Kalama Creek, McAllister Creek, and the	ude English sole. rr Creek, and the
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shows the results of toxicity bioassays conducted in estuaries or nearby (Tox, toxic; NT, not toxic). Also in this column are values indicating pass or fail for sediment concentrations compared with ERM values (effects range-medium) (Long et al. 2003) and WA State Sediment Standards (1, full pass; 2, fail SQS; 3, fail SCM; 5, fail BRM; 5, fails two of three; 6, fails all three). Listed sites include Puget Sound Initiative (PSI) and Washington for a given estuary. Also shown are estuaries used as control or reference (ref.) were determined by dividing the mean + 1 standard deviation (determined for the site) by the hatchery mean value. n is sample size. Values for Shilshole are from samples taken in the estuary downstream of the locks. Toxic sediment sites. CoC lists contaminants of concern occurring at elevated concentrations. ND, no data; BD, below detection. See text and Supplementary data<sup>1</sup> for details. actor differences number of such sites the multiplication sign are t because of contamination; CU denotes the 2 values followed Department of Ecology sites designated for cleanup and source control determine mean Wallace River hatcheries were used

\*References: (1) D. Houck, personal communication, 2011; (2) Varanasi et al. (1993); (3) Meador et al. (2000); (4) Long et al. (2000); (5) Long et al. (2002); (6) Long et al. (2002); (7) Norton (1999); (8) USEPA (1988; (9) Cubbage (1991); (10) Norton (1986); (11) Era-Miller 2004; (12) McCain et al. (2000); (13) Long et al. (2003); (14) PTI (1991). PSI sites from Washington Department of Ecology (2012) Superfund site.

Data from Kerwin (1999)

indicated that survival was higher for Chinook transiting uncontaminated estuaries for 28 out of 32 years (p < 0.0001) (Table 5). When compared year by year, the mean difference in survival for Chinook transiting uncontaminated estuaries was 2.5-fold higher (n = 32 years) and for coho was essentially neutral (0.98-fold) (n = 32 years)36) (Table S11). The Wilcoxon test for fish mass at release returned a *p* value of 0.25, also indicating no pattern among years. The Chinook SAR over years as grouped by estuary contamination status is highlighted in Fig. 2. For the past 10 years (1998-2008), the SAR was on average 2.1-fold higher for fish transiting uncontaminated versus contaminated estuaries (Table S11).

The regression between SAR and release mass for Chinook over all tag code groups indicated a weak relationship ( $R^2 = 0.03$ , p = 0.002, n = 390), which was also observed when analyzed separately by contamination group. A similar result was obtained when all tag code groups (n = 290) were included from 1985 to 2008  $(R^2 = 0.01, p = 0.09)$ . The regression between the SAR and release DoY for all tag code groups (n = 390) in this time period exhibited a low *p* value (p < 0.001); however, the R<sup>2</sup> (0.08) was too low to be predictive. A regression with all qualifying tag code groups (n = 57) for the same years as those analyzed by Duffy and Beauchamp (2011) yielded a relatively strong negative correlation (log SAR =  $-0.134 - 0.015 \times \text{DoY}$ ;  $\mathbb{R}^2 = 0.38$ , p < 0.001), which supports the importance of this factor for select time periods.

A generalized linear model (GLM) was constructed with Chinook SAR as the dependent variable and release mass and DoY as independent variables using all tag code groups (n = 390). The overall model exhibited a low p value (p = 0.002) using only DoY due to the large sample size. The Akaike information criteria (AIC) changed less than 1.7% for each parameter added (release mass and the interaction term), and the R<sup>2</sup> was always below 0.1 for all models, indicating that these parameters explained only a low percentage of the SAR variance. This was expected, given the high degree of interannual variation.

The ANOVA for survival among coho for all years and hatcheries exhibited a low p value (p = 0.07) because the rate of survival was slightly higher for fish transiting contaminated estuaries (6.9% versus 8.1%, n = 226) (Fig. 3; Table 5). However, when compared year by year (n = 36), the mean for differences in survival was 0.98, indicating no difference overall even though the data were variable (Table S2<sup>1</sup>). The release masses for coho were on average larger (26.1 versus 25.0 g) for fish from contaminated estuaries (p = 0.05), although regression analysis for all years determined no relationship between release mass and SAR ( $\mathbb{R}^2 \approx 0$ ). The Wilcoxon analysis by year indicated that for most years (23 of 36 years), coho SAR values were on average higher for fish that outmigrated through a contaminated estuary (Table 5). A similar pattern was observed for coho release masses (Table 5). Without coho data from the Wallace Falls hatchery, the difference in SAR values between contaminated and uncontaminated estuaries was greatly reduced (ANOVA p = 0.34, Wilcoxon p = 0.12), indicating that this hatchery exhibited a strong influence on the results. Without including that hatchery, the differences for fish release mass did not change, as the overall mean increased slightly to 26.6 g for coho transiting contaminated estuaries.

The Chinook data were examined to determine whether any hatcheries may have had an undue influence on their respective group. The results clearly show that among the hatcheries where fish entered an uncontaminated estuary, one hatchery (Portage Bay) stood out because of its very high rate of survival (Table 3). This hatchery contributed data for only 6 of the 37 years and was restricted to the early 1970s, 1981-1982, and 2001, so its influence on the overall pattern was minor. Kendall Creek also exhibited unusually high survival, but only for the early 1970s. These high survival values overlapped with other hatcheries also exhibiting high survival in the 1970s and early 1980s, including Samish and Soos creeks. For Portage Bay and Kendall, no survival values exceeded 2.0% after 1979, except for Portage Bay in 2001. The ANOVAs

Estuary	Hatchery	Location	SAR (%)	Release mass (g)	CWT total	Total no. of fish released	Total years of data	Release years
UC	Kendall Creek	North	3.40 (2.60)	7.08 (1.35)	5.20×10 <sup>5</sup>	3.50×10 <sup>6</sup>	4	1972, 1975–1976, 1986
UC	Skookum Creek	North	0.37 (0.29)	5.47 (1.75)	$2.97 \times 10^{5}$	6.68×10 <sup>6</sup>	5	1975, 1976, 1980–1982
UC	Samish	North	0.89 (1.06)	4.92 (0.81)	$7.00 \times 10^{6}$	$2.88 \times 10^{7}$	27	1973, 1975–1976, 1980, 1986–2008
UC	Bernie Gobin	North PS	0.58 (0.25)	5.57 (0.58)	$2.43 \times 10^{6}$	$1.70 \times 10^{7}$	15	1987–1992, 1999–2006, 2008
UC	Harvey Creek	North PS	0.90 (0.68)	5.78 (0.82)	7.92×10 <sup>5</sup>	9.22×10 <sup>5</sup>	9	1987–1995
UC	Whitehorse Ponds	North PS	0.46 (0.21)	6.14 (1.31)	$2.09 \times 10^{6}$	$2.23 \times 10^{6}$	12	1995–2000, 2003–2008
С	Wallace River	North PS	0.25 (0.18)	6.22 (1.12)	$3.59 \times 10^{6}$	$3.83 \times 10^{6}$	10	1973, 1986, 2001–2008
UC	Grovers Creek	Mid-PS	1.02 (0.73)	7.42 (1.77)	5.86×10 <sup>6</sup>	$1.28 \times 10^{7}$	24	1982, 1985–1994, 1996–2008
UC	Issaquah	Mid-PS	0.75 (0.63)	4.74 (1.25)	$2.45 \times 10^{6}$	$1.42 \times 10^{7}$	14	1972-1973, 1979-1982, 1985-1988, 2003-2007
UC	Portage bay	Mid-PS	2.26 (1.35)	7.42 (1.53)	5.18×10 <sup>5</sup>	5.29×10 <sup>5</sup>	6	1975, 1979–1982, 2001
С	Puyallup Tribal	Mid-PS	0.19 (0.13)	7.14 (2.01)	6.92×10 <sup>5</sup>	$1.47 \times 10^{6}$	7	1985–1987, 1998, 2000–2002
С	Voights Creek	Mid-PS	0.38 (0.29)	5.67 (1.71)	1.94×10 <sup>6</sup>	6.39×10 <sup>6</sup>	13	1972, 1979–1982, 1998–1999, 2003–2008
С	Soos Creek	Mid-PS	0.65 (0.71)	5.56 (1.07)	8.28×10 <sup>6</sup>	$5.20 \times 10^{7}$	29	1972, 1973, 1975, 1976, 1979, 1980, 1982,
								1986-2005, 2007, 2008
С	Gorst Creek Rearing Pond	Mid-PS	0.50 (0.22)	7.39 (2.91)	7.71×10 <sup>5</sup>	$6.48 \times 10^{6}$	4	1973, 2002–2004
С	Capitol Lake Rearing	South PS	0.77 (1.06)	5.54 (1.32)	6.77×10 <sup>5</sup>	1.19×10 <sup>7</sup>	5	1972, 1980, 1986–1988
С	Tumwater Falls	South PS	0.20 (0.10)	5.08 (0.44)	5.98×10 <sup>5</sup>	6.01×10 <sup>5</sup>	4	2001, 2003–2005
С	Garrison	South PS	0.50 (0.46)	9.01 (2.46)	7.81×10 <sup>5</sup>	$3.21 \times 10^{6}$	8	1980–1982, 1988, 1990–1992, 2004
UC	Minter Creek	South PS	0.46 (0.36)	5.62 (1.68)	1.09×10 <sup>6</sup>	$4.37 \times 10^{6}$	7	1973, 1979, 1980, 1982, 2003–2005
UC	Clear Creek	South PS	0.86 (0.55)	8.66 (1.07)	4.89×10 <sup>6</sup>	$3.45 \times 10^{7}$	17	1991–1994, 1996–2008
UC	Kalama Creek	South PS	0.54 (0.41)	7.71 (1.85)	$3.07 \times 10^{6}$	$1.95 \times 10^{7}$	24	1980–1982, 1985–1995, 1999–2008

Note: Values for smolt-to-adult return rate (SAR) and release wet mass are means and standard deviation for all years. Estuary indicates contaminated (*C*) or uncontaminated (UC). Locations are North (northern Washington portion of Salish Sea), north Puget Sound (PS), mid-PS, and south PS. Total years of data, years of release, total number of fish released, and fish with coded wire tags (CWT) are shown for each hatchery. Total number of fish released was  $2.3 \times 10^8$ , and the number with CWT was  $4.8 \times 10^7$  (21% of the total).

Table 4. Hatcheries, location, SAR, release mass, and number of fish years released for coho salmon.

-					5			
				Release	CWT	Total no. of	Total years	
Estuary	Hatchery	Location	SAR (%)	mass (g)	total	fish released	of data	Release years
UC	Kendall Creek	North	6.14 (4.99)	26.38 (1.82)	1.86×10 <sup>6</sup>	2.15×10 <sup>7</sup>	29	1975–1976, 1982–2008
UC	Skookum Creek	North	6.43 (5.45)	23.04 (4.02)	1.39×10 <sup>6</sup>	2.92×10 <sup>7</sup>	30	1975–1981, 1986–2008
UC	Samish	North	6.90 (0.52)	32.07 (3.98)	$5.98 \times 10^{4}$	9.30×10 <sup>5</sup>	2	1975–1976
UC	Bernie Gobin	North PS	7.95 (4.06)	25.23 (1.77)	$1.38 \times 10^{6}$	2.06×107	29	1975, 1980–1982, 1984–2008
С	Wallace River	North PS	9.63 (5.43)	24.68 (3.40)	$1.62 \times 10^{6}$	6.45×10 <sup>6</sup>	28	1973, 1975–1976, 1983–2007
UC	Issaquah	Mid-PS	7.63 (3.23)	25.96 (2.44)	6.48×10 <sup>5</sup>	5.74×10 <sup>6</sup>	9	1973–1976, 1979–1980, 2002–2004
UC	Portage Bay	Mid-PS	8.35 (3.93)	17.17 (2.04)	$2.06 \times 10^{5}$	2.12×10 <sup>5</sup>	6	1975, 1977, 1981, 1983, 1985–1986
С	Voights Creek	Mid-PS	8.17 (5.13)	26.54 (3.21)	$2.41 \times 10^{7}$	$2.37 \times 10^{7}$	33	1974–1976, 1978–1989, 1991–2008
С	Soos Creek	Mid-PS	7.08 (4.53)	25.79 (3.41)	$2.70 \times 10^{6}$	1.39×107	33	1973, 1975–2005, 2007
С	Crisp Creek	Mid-PS	6.19 (3.88)	29.98 (8.31)	4.36×10 <sup>5</sup>	1.10×10 <sup>6</sup>	7	1993–1995, 2003, 2005–2007
С	Keta Creek	Mid-PS	8.36 (3.91)	32.38 (4.09)	$2.38 \times 10^{5}$	1.15×10 <sup>6</sup>	5	1998–2001, 2008
UC	Minter Creek	South PS	6.66 (4.73)	27.71 (7.18)	$7.53 \times 10^{5}$	8.76×10 <sup>6</sup>	15	1973, 1975–1976, 1979–1985, 2001–2005

Note: Values for smolt-to-adult return rate (SAR) and release wet mass are means and standard deviation for all years. Estuary indicates contaminated (C) or uncontaminated (UC). Locations are North (northern Washington portion of Salish Sea), north Puget Sound (PS), mid-PS, and south PS. Total years of data, years of release, total number of fish released, and fish with coded wire tags (CWT) are shown for each hatchery. Total number of fish released was  $1.1 \times 10^8$ , and the number with CWT was  $3.4 \times 10^7$  (30% of the total).

were rerun for the uncontaminated group without Portage Bay and Kendall, and the results indicated that no one hatchery influenced the overall pattern of high survival for this group. Several hatcheries (Clear Creek, Grovers Creek, Harvey Creek, Issaquah, and Samish) all exhibited relatively high survival for Chinook (>0.75%). These hatcheries represented a large portion of the total hatchery-year data points and covered all four geographic areas (Table 1). The same analysis was conducted for hatcheries that released Chinook into contaminated estuaries and no undue influences were found.

The SAR values for Chinook (Fig. 2) indicate a general decline over the past several years; however, this pattern was not strong for either group. Trend analysis with a Mann–Kendall test returned high p values (p > 0.8), indicating no trend over this time frame. For coho, survival rates among all Puget Sound hatcheries declined in the late 1980s through the late 1990s, then improved in the late 1990s but at levels below earlier values (Fig. 3). There was no relationship between Chinook versus coho SAR values where they overlapped for hatchery and release year (n = 107,  $R^2 \approx 0$ ). This comparison helped to assess potential differences among hatcheries, in that problems at a given hatchery would likely lead to anomalously low SAR values for both species.

#### **Estuary characteristics**

The density of fish (Table 1) was variable among local estuaries, and no relationship was observed between this value and mean adult survival ( $R^2 \approx 0$ , n = 20). There was also no relationship between Chinook SAR and distance to the estuary ( $R^2 \approx 0$ , n = 20), which may be a surrogate for differential freshwater mortality. Accurate residence times for fish in each estuary were not available; therefore the role of fish density on survival or reduced residence time is unknown. In some watersheds a high percentage of hatchery released fish may spend only a few days in the estuary before moving offshore (Ruggerone and Weitkamp 2004), which may be caused by high numbers of fish in the system (density-dependent migration) or estuary size. It is unknown

	ANOVA (all years)			Wilcoxon (year by y	
	SAR (%)	Mass × contamination	Mass (g)	SAR	Mass
Chinook					
Mean uncontaminated	0.87 (0.07), <i>n</i> = 164		6.49 (0.15)	28 years	21 years
Mean contaminated	0.48 (0.06), n = 80		6.21 (0.21)	4 years	11 years
p value	<0.0001	0.27	0.28	< 0.0001	0.25
Coho					
Mean uncontaminated	6.9 (0.42), <i>n</i> = 120		25.0 (0.39)	13 years	11 years
Mean contaminated	8.1 (0.48), <i>n</i> = 106		26.1 (0.40)	23 years	25 years
p value	0.07	0.75	0.05	0.013	0.08

**Note:** Mean and standard error (SE in parentheses) values for smolt-to-adult return rate (SAR) and release mass (g wet mass) for all years. p values from ANOVAs for SAR and mass as the dependent variable. n shows the number of hatchery releases for each group. The p value for the interaction term mass × contamination from an ANCOVA (with SAR as the dependent variable) also shown. Under "Wilcoxon" values are the years of dominance for each categorical group (contaminated or uncontaminated estuary). For Chinook, the SAR was higher for fish from uncontaminated estuaries for 28 out of 32 years of data.

Fig. 2. Mean and standard error smolt-to-adult return rate (SAR) for groups of hatcheries releasing juvenile Chinook to contaminated and uncontaminated estuaries.



whether this is true for smaller estuaries with fewer fish but relatively high densities. Fish that reside for days instead of weeks can still accumulate high concentrations of contaminants in a short period of time through high rates of ingestion and ventilation. To test the hypothesis that observed SAR values were influenced by estuary size, an ANOVA was run for hatcheries releasing only into large estuaries (>2.5 km<sup>2</sup>; see Table 1). The results were essentially identical to that determined for all estuaries (mean SAR values 0.48 for contaminated estuaries versus 0.83 for uncontaminated estuaries; p < 0.007), indicating that estuary size is likely not an important factor for this analysis.

## Analysis and discussion

This analysis supports the conclusion that Chinook from contaminated estuaries have a lower probability of completing their life cycle compared with fish transiting estuaries that are considered uncontaminated. The overall pattern of reduced survival for ocean-type Chinook that migrate through contaminated estuaries in Puget Sound is robust because of the large number of fish, hatcheries, and estuaries spanning more than 37 years of data. Because this dataset encompasses decades of data and myriad factors are known to influence salmonid survival, high variability was 172



Fig. 3. Mean and standard error smolt-to-adult return rate (SAR) for groups of hatcheries releasing juvenile coho to contaminated and uncontaminated estuaries.

expected. It is important to note that these results held even when blocking by time, estuary size, release mass, and geographic location. The Wilcoxon test allowed for a year-by-year comparison that provided even stronger patterns compared with the analysis of all years together. Also, a focused evaluation for the last 10 years revealed essentially identical results as that for the overall analysis. Estuary size may affect the residence time for Chinook and limit their exposure to contaminants if they move through quickly; however, this factor was found to be unimportant in this analysis, as the results were the same when only large estuaries (>2.5 km<sup>2</sup>) were considered. Also noteworthy is the absence of a pattern for high and low SAR values in the four geographic regions and the lack of correlation with distance from hatchery to estuary.

The observation of a substantially higher rate of survival for Chinook from uncontaminated estuaries over all years and the lack of such a strong difference for coho supports the hypotheses that contaminated estuaries decrease the probability of a successful life cycle. Outmigrating coho experience relatively similar freshwater and oceanic conditions as Chinook, but spend little time in the local estuaries and generally move quickly to marine waters. Surprisingly, the data show that survival is slightly higher for coho transiting contaminated estuaries, which is mostly due to the Wallace River hatchery, as shown in the Results section. For Chinook, the aggregate data often appear contradictory, in that mean SAR values for some hatcheries releasing fish to uncontaminated estuaries are comparable to or even lower than those for hatcheries releasing to contaminated systems (Table 3), which is due in part to relatively high SARs in the 1970s and 1980s for some hatcheries. Even though the overall pattern does show some important correlations, it is more appropriate to conduct such an analysis year by year to mitigate the high interannual variability

in survival. For example, even though Soos Creek fish exhibited a relatively high overall SAR value (Table 3), survival was higher than the mean value for fish from hatcheries transiting uncontaminated estuaries for only 7 of 29 years examined, and 4 of the 7 years were less than 20% higher.

# Non-contaminant factors potentially affecting smolt-to-adult survival

A number of authors have addressed the importance of estuarine residence for salmonids, especially juvenile Chinook. The crucial factors that are commonly listed include refuge from predation, freshwater-seawater transitional areas, and productive foraging allowing increased growth (Simenstad et al. 1982; Healey 1982; Macdonald et al. 1988; Thorpe 1994). Adjunct to these are such factors as flow rate, water temperature, intraspecific and interspecific competition, hatchery practices and husbandry, fish stock (genetic differences), contamination, habitat quality (e.g., sediment type, prey availability, cover, predator densities), and numerous minor attributes. Hatchery practices are multifaceted and include a number of factors such as age of the hatchery, disease accumulation, and genetic changes, all of which were ruled out as important by Coronado and Hilborn (1998) in their analysis of Chinook and coho survival in the Pacific Northwest. Often an estuary is considered in terms of its natural or "pristine" state, which encompasses many of the factors listed above. As acknowledged by Magnusson and Hilborn (2003), factors that covary with the degree of pristine habitat may be important in determining survival for outmigrating Chinook. Some of the more important factors that could potentially affect the success of juvenile Chinook reaching the adult phase are addressed below, such as fish density, migration distance to the estuary, availability of prey, and growth rate. The conclusion here is that contamination

is an important factor for Chinook survival; however, this cannot be easily distinguished from the same covariates mentioned by Magnusson and Hilborn (2003). There are few, if any, situations where contamination and a high percentage of pristine or unaltered habitat occur together. As mentioned by other authors, the determinants of survival are numerous and complex, and few studies address all of them simultaneously.

## Prey availability and growth rate in the estuary

A major consideration for evaluating juvenile survival in these local estuaries is the availability of prey for growth. Few data were available for these estuaries; however, adequate prey species may have been available for many of those considered contaminated (Table 1 and Supplementary data<sup>1</sup>). It is important to note that contamination will also affect invertebrates in the estuary; however, there are numerous pollution-tolerant taxa, and these will often increase in abundance over sensitive species and contain higher concentrations of contaminants. Even though invertebrate communities may be impaired, as indicated by various metrics such as species diversity and taxa dominance, benthic biomass and suitable prey for salmonids may not be substantially impacted.

Juvenile Chinook in an estuary are capable of growing at rates of 3%-5% body mass day-1 (Healey 1982, 1991; Brett 1995). This very high rate of growth is due to an ingestion rate of 12%-20% body mass day-1 (Brett 1995), which is noteworthy because these fish are likely accumulating contaminants at a high rate. Healey (1982) reported growth rates of 3.5% body mass-day-1 in the relatively pristine Nitinat estuary and up to 5.5% body mass day-1 in the less pristine Nanaimo estuary on Vancouver Island, British Columbia. Interestingly, the Nitinat does not contain intertidal areas, indicating that relatively high growth rates can be achieved without this type of habitat. As noted by Healey (1991), this technique of estimating growth by assessing mean fish size in the estuary over time likely underestimates actual growth by 50%, implying that many of the estimated growth rates may be higher. A number of authors have concluded that food was not limiting or that density-dependent growth effects were not observed for some Pacific Northwest estuaries (Duwamish, Nanaimo, and Campbell rivers) (Healey 1980; Levings et al. 1986; Cordell et al. 2011); however, these factors may be occurring during the peak migration of hatchery fish (Reimers 1973; Nelson et al. 2004) (see Supplementary data<sup>1</sup>).

After juvenile Chinook leave their local estuary for more open water in Puget Sound and beyond, it appears that prey abundance is sufficient to allow continued growth. Duffy (2009) found that juvenile hatchery Chinook during their first summer in open water exhibited consumption rates that were between 50% and 100% of the expected maximum value. These estimates varied by year and location (north, central, or south Puget Sound) for 2 of the years examined and were also within the predicted range for fish in Puget Sound. Growth was estimated to be between 0.9% and 2.3% body mass day-1, which varied by year and season. Also, Brennan et al. (2004) noted very low percentages of empty stomachs (2%-5%) in Chinook captured at 16 sites in mid-Puget Sound in 2001 (n = 410) and 2002 (n = 409). These data indicate that juvenile Chinook likely encountered sufficient prey. Therefore any differences in growth or survival for hatchery fish was possibly due to factors other than prey availability in estuaries or open marine waters of the greater Puget Sound area where fish comingled

Even though habitat quality was quite variable, it appears that juvenile Chinook within estuaries often increased in mass at rates that were comparable among contaminated and uncontaminated estuaries (Table 1). Although the data are sparse, a few of the more contaminated estuaries (Duwamish, Puyallup, Sinclair, and Budd Inlet) indicated relatively high densities of prey and high percentages of full stomachs in juvenile Chinook (see Supplementary data<sup>1</sup>). Based on the available data, it was concluded that growth for juvenile Chinook residing in local estuaries was not a function of the state of contamination; hence this factor may not be important for determining the differential rate of survival. It is important to note that inhibited growth due to contaminant exposure in local estuaries may be delayed and not manifest until fish leave these systems for open water.

#### Physical habitat alterations

Most of the small delta estuaries in Puget Sound are considered degraded compared with their preindustrial state, and some have lost more than 99% of their intertidal and subaerial habitat (Bortleson et al. 1980). A recent compilation for several local estuaries examined in this study, including the Duwamish, Nisqually, Nooksack, Puyallup, Samish, Snohomish, and Stillaguamish river estuaries, reported large reductions in the amount of tidal freshwater and oligohaline transition habitat (Simenstad et al. 2011). The residence time and areal extent needed for the salinity transition zone is difficult to quantify and is dependent on species, life stage, and stock. Ocean-type Chinook are known to tolerate seawater at an early stage (Healey 1980) and juveniles from the hatchery likely transition quickly to full-salinity seawater. In some of the more degraded estuaries, the salt wedge and mixing zone extends several kilometres upstream from the river mouth, as noted for the Duwamish and Puyallup river estuaries (Supplementary data1), thereby providing some habitat for salinity acclimation.

While several of the Puget Sound estuaries categorized as contaminated also exhibit poor habitat quality for juvenile Chinook rearing, this is not always the case. The estuaries for Gorst Creek and the Snohomish are not as highly altered as the Duwamish, but do exhibit a high degree of contamination. Even though the Puyallup and Duwamish river estuaries have lost essentially all their intertidal habitat, their shallow subtidal areas provide rearing habitat and prey for juvenile Chinook. Healey (1991) noted that subtidal habitat is desirable for this life stage, and as mentioned above, juvenile Chinook will prey on benthic species.

One interesting comparison is between the Nisqually and Snohomish river estuaries. The Nisqually has lost 22% of its intertidal habitat, which is similar to the 32% loss for the Snohomish River estuary (Bortleson et al. 1980). The Nisqually is considered uncontaminated, whereas the Snohomish River estuary was judged contaminated, and the SAR values for these two estuaries reflects that categorization. Another noteworthy observation is for fish released in Gorst Creek that outmigrate through Sinclair Inlet (contaminated) and those from Grover's Creek transiting Miller Bay (uncontaminated). The Gorst Creek fish are spawned, incubated, and reared at Grover's Creek hatchery (Hatchery Scientific Review Group 2003). When considered by year when these hatcheries overlapped (2002–2004), the SAR for Gorst Creek Chinook ranged from 40% to 60% lower than the mean SAR for fish released from the Grover's Creek hatchery.

#### Fish size and release day

Juvenile fish size has been strongly associated with survival to the adult phase (Cowan et al. 2000; Beamish and Mahnken 2001; Duffy and Beauchamp 2011). In this analysis there were no discernible differences in juvenile fish mass at release among hatcheries and years, and there was no correlation with the SAR, which was also reported in other studies (Quinn et al. 2005; Duffy and Beauchamp 2011). Fish release dates are also considered an important factor but were limited in this analysis to 10–12 weeks during the spring for both species. There was no correlation between Chinook SAR and release DoY, which is likely due to high interannual variability. As shown in Duffy and Beauchamp (2011), DoY release can be an important factor for survival when considered within a short time frame.

#### Habitat area and fish density in delta estuaries

Based on growth rates (Table 1), it appears that densitydependent growth may not be an important factor in these Puget Sound estuaries, which is supported by Levings et al. (1986), who did not observe density-dependent growth rates in a small estuary (0.5 km<sup>2</sup>, 1.4 fish·m<sup>-2</sup>) and by Healey (1980) for the Nanaimo estuary. No correlation was observed for estuary size and Chinook SAR, which was also the case for juvenile Chinook from coastal estuaries (Magnusson and Hilborn 2003). Area is not as important as the density of fish rearing in the estuary, and the present study found no relationship between this factor and Chinook survival. Density-dependent mortality, growth, and emigration is thought to occur in some Pacific Northwest estuaries (Reimers 1973; Greene and Beechie 2004). Reimers (1973) found that ocean-type Chinook exhibited reduced growth during the peak migration, which was hypothesized to be related to the high density of conspecifics. The focus for those growth studies was a very small (0.08 km<sup>2</sup>) and shallow (mostly <1 m) area in lower Sixes estuary in Oregon. Fish density ( $\approx 2$  fish·m<sup>-2</sup>) was based only on Chinook and is a minimum estimate based on a monthly census that does not account for fish entering or leaving the lower estuary. This density is comparable to those of contaminated estuaries in this study with the highest fish densities for both species.

#### Predation

Predation is another factor that may influence interestuary differences in Chinook SARs. Very few studies have quantified predatorprey interactions in Pacific Northwest estuaries; however, the available data indicate that this interaction is not important. One study quantified predation on juvenile salmon in Puget Sound by cutthroat trout and determined that the number consumed were minor compared with the total number of outmigrating fish (Duffy and Beauchamp 2008). Another study examined the impact of bird predation, specifically mergansers, on juvenile salmonid mortality. For juvenile Chinook (6 g) the mortality was less than 1.3% in Big Qualicum Creek on Vancouver Island, British Columbia, Canada (Wood 1987). Based on the high abundance of outmigrating juvenile salmon from hatcheries and the relatively low abundance of some predators, predation is likely not an important factor in local Puget Sound estuaries. This is supported by Simenstad et al. (1982) and Macdonald et al. (1988), who also noted low rates of predation and suggested that estuaries may be a sanctuary from predators. Predation can be significant depending on the species, life stage, and estuary (Wood 1987) in addition to increased predation rates in the marine environment (Brodeur et al. 2003). Predation was not examined for the freshwater portion of the migration to the estuary. Distance to the estuary (Table 1) was considered a surrogate for potential source of mortality, assuming that all such systems in Puget Sound contain similar types and densities of predators. There was no correlation between Chinook SAR and distance to the estuary. Once in open water, size-selective predation is an important factor for juvenile Chinook and may account for a high percentage of the early marine-phase mortality (Beauchamp and Duffy 2011).

## Spatial distribution within marine waters

All available data indicate that Chinook in open water comingle and are not likely to exhibit differential survival as a function of their natal hatchery location. The total number of subyearling Chinook released into Puget Sound from hatcheries has been relatively consistent, ranging from 45 to 55 million per year since the early 1970s (Ruggerone and Goetz 2004). For the first several months after leaving the estuary, juvenile Chinook from many of the hatcheries in this study appear to mix within Puget Sound. One study (Brennan et al. 2004) sampled juvenile Chinook from May through December in 2001 and 2002 and found that fish exhibited a variety of movement patterns. For this period, it appears that fish from hatcheries all over Puget Sound comingle, and in many cases appear to move south after leaving their local estuary. This was noted for fish from the Soos Creek, Samish, Wallace River, and Lummi sea ponds. This was confirmed by Rice et al. (2011), who observed substantial movement and mixing of juvenile fish from hatcheries all over Puget Sound, with the most co-occurrences in mid- to northern Puget Sound. Fresh et al. (2006) also noted high percentages of juvenile Chinook from the Nisqually, Soos Creek, Wallace, and Grovers, in addition to the local fish from Gorst Creek in outer Sinclair Inlet in June and July 2001 and 2002.

A high percentage of ocean-type Chinook appear to spend their entire life in coastal British Columbia and the Salish Sea, which includes Puget Sound, the Strait of Georgia, and the Strait of Juan de Fuca as based on CWT recovery (Healey 1991; Weitkamp 2010). As noted by Weitkamp (2010), salmon released from a common freshwater area (e.g., Puget Sound watershed) have a similar marine distribution. The marine distribution for coho is similar to that for Chinook as a function of their freshwater release location (Weitkamp 2010). Fishery catch records indicate that most adult fall and summer run Chinook (85%–90%) were captured in Puget Sound, the Strait of Georgia, or southern Vancouver Island (Quinn et al. 2005; Weitkamp 2010), indicating that they likely experienced relatively similar ocean conditions.

#### **Other factors**

Chinook from all hatcheries in this analysis are considered part of the Puget Sound evolutionarily significant unit for this species. All juvenile Chinook released from these hatcheries come from stocks originating within Puget Sound, and many of the hatchery programs were founded with, or utilized fish from, the Green River stock, resulting in a similar genetic background for many of the hatchery fish released into this evolutionarily significant unit (Myers et al. 1998).

No information was found regarding potential hatchery problems. This study included many comparisons of Chinook and coho from the same hatchery, which allowed some insight. As shown in the Results, there was no correlation for the 107 cases where Chinook and coho SAR values co-occurred by release year and hatchery. A noteworthy example is the Wallace River hatchery, where the SAR values for coho were the highest for all groups, but were among the lowest for Chinook (Tables 3 and 4).

This analysis does not explicitly consider the El Niño – Southern Oscillation and Pacific Decadal Oscillation cycles or other oceanic conditions such as upwelling and salinity that are considered relevant for salmonid survival. Even though these cycles are known to have a significant impact on juvenile growth and survival (Brodeur et al. 2003), the focus of this study is on annual comparisons among hatcheries and therefore incorporates such impacts, because all fish for a given release year experience similar oceanic conditions. Of course, these cycles may magnify the effects. For example, fish from contaminated estuaries may be at a greater disadvantage when prey abundance is impacted by an adverse El Niño – Southern Oscillation cycle.

## Potential effects due to contamination

A number of contaminants in these estuaries are known to affect growth, reproduction, immune function, physiological homeostasis, and the behavior of salmon, which may explain the reduced survival observed for Chinook. Even though growth appears to be relatively unaffected for fish captured within contaminated estuaries, growth impairment would likely be delayed for several weeks until they had accumulated toxic levels and exited to open water. Another possibility is that some of the contaminants can lead to increased susceptibility to pathogens, also leading to delayed mortality. Altered behavior is another important consideration that would certainly impact the ability of juvenile fish to catch prey and avoid predation, especially outside the estuary.

Myriad contaminants can be found in these estuaries. There are legacy compounds such as PCBs, DDTs, and endrin, several metals (As, Cu, Cd, Hg, Pb, and Zn), PAHs, pesticides (organotins, organophosphates, carbamates, triazines, pyrethroids, and chlorophenols), a large number of industrial chemicals (e.g., phthalates, bisphenol A, flame retardants, and perfluorocarbons), pharmaceuticals and personal care products from wastewater treatment plants and septic systems, and dozens of new and emerging contaminants. We have exposure and toxicity information for a few of these chemicals; however data are lacking for most, especially with regard to fish health. Additionally, there are almost no data on the toxicity of mixtures for these contaminants. In the majority of cases, toxicants act by additive effects (see Meador 2006) and therefore should be considered the default condition. Synergism has been reported for some mixtures of pesticides (Laetz et al. 2009), which should be considered along with potential antagonist interactions. It is likely that a complex mixture of contaminants found in urban estuaries is responsible for the reduced survival experienced by juvenile Chinook that rear in these estuaries.

Over the past several decades, a number of contaminants in urban and industrial areas have increased, or remain elevated, in terms of frequency of occurrence and magnitude including polybrominated diphenyl ethers (PBDEs), bisphenol A, phthalates, organotins, PAHs, pesticides, metals, and pharmaceuticals and personal care products (Puget Sound Ambient Monitoring Program 2007; Daughton and Brooks 2011). All these contaminants are capable of causing adverse metabolic, endocrine, immune, and behavioral responses that may jeopardize the chances of juvenile fish surviving to reproductive maturity. It is worth noting that some contaminants, such as PCBs, were higher in the 1970s–1980s in Puget Sound (O'Neill and West 2009), which corresponds to many of the years exhibiting large differences in SAR values for Chinook between contaminated and uncontaminated estuaries (Table S1<sup>1</sup>).

Most bioaccumulative contaminants increase quickly in fish tissues. This is especially true for salmonids, because they exhibit very high rates of feeding and gill ventilation. It is not uncommon for juvenile Chinook to consume 20% of their body mass per day and to ventilate 0.5 L·kg<sup>-1</sup>·min<sup>-1</sup>, allowing rapid increases in toxicant tissue concentrations. Also, because the rates of uptake and elimination follow first-order kinetics, a high percentage of a steady-state concentration can occur rapidly. Even for compounds such as PCBs that may exhibit steady-state concentrations after 28 days, fish can achieve 50% of that level within 6.5 days. Rapid uptake and high rates of consumption and ventilation allow juvenile salmon to accumulate high concentrations even if their residence time in a contaminated estuary is limited. One study (Meador et al. 2010) that analyzed 111 whole-body juvenile Chinook samples (many as composites of several individuals) found that most (53%) contained high concentrations of total PCBs (>50 ng·g<sup>-1</sup>), indicating that these fish had been accumulating contaminants for an extended period of time. When considered in terms of increased growth and total amount accumulated in the estuary, the median increase for all 111 samples was 11 times over that for fish from the hatchery. Because of the low lipid content for outmigrating juvenile Chinook, almost all fish with total PCBs >50 ng·g<sup>-1</sup> exceeded the predicted threshold concentration for toxicity (Meador et al. 2002). It is important to note that toxic effects can occur in fish without detectable increases in tissue concentrations. This has been reported for PAHs (Meador et al. 2006) and is also expected for other highly metabolized organic compounds and some metals, indicating that throughput (e.g., µg·g fish<sup>-1</sup>·day<sup>-1</sup>) or metabolite determination (Meador et al. 2006, 2008a; McElroy et al. 2011) are more appropriate metrics for exposure and toxicity for these chemicals.

#### **Growth effects**

Most of the mortality for juvenile salmon during their first year in open water is due to predation, which has been shown to be a function of size (Beamish and Mahnken 2001; Brodeur et al. 2003; Duffy and Beauchamp 2011). A dietary toxicity study of a mixture of PAHs fed to juvenile Chinook salmon found altered metabolic parameters as low as 2  $\mu$ g·g fish<sup>-1</sup>·day<sup>-1</sup> and severe reductions in growth and lipid content for higher doses ( $\approx 20 \mu$ g·g fish<sup>-1</sup>·day<sup>-1</sup>) (Meador et al. 2006). These doses are consistent with expected rates of uptake and observed PAH concentrations for stomach contents found in outmigrating Chinook in the Duwamish and Puyallup river estuaries. A large number of frequently occurring contaminants can affect organismal growth, such as tributyltin (Meador et al. 2011), copper (Marr et al. 1996), dioxin (Eisler 1986), and numerous other metabolic disruptors (phthalates, bisphenol A, perfluorooctane, and pesticides).

## Physiological changes

Lipid content is also an important factor determining the probability that juvenile salmon will survive their first winter in open water (Gardiner and Geddes 1980; MacFarlane and Norton 2002; Biro et al. 2004), which is related to growth potential and having energy reserves when prey availability is reduced. One study demonstrated that juvenile Chinook experience a growth spurt once they leave the estuary, which is fueled in part by their lipid reserves (MacFarlane and Norton 2002). This rapid increase in size is advantageous for avoiding predation. Normal lipid content for juvenile Chinook can average 2%-3% (wet mass), depending on the analytical method, as they exit the estuary to open water (MacFarlane and Norton 2002; Johnson et al. 2007; Sloan et al. 2010), which can contribute to the increase in growth and serve as a reserve for the winter when prey are less abundant. Related to this, one study found that juvenile ocean-type Chinook salmon with a higher lipid content (7.9% wet mass) exhibited a SAR that was twice as high as those with a lower lipid level (4.1%) at the time of release from the hatchery (Burrows 1969).

A critical lipid content of 1% was determined in lab and field studies for rainbow trout (Oncorhynchus mykiss), with high mortality resulting when lipid content fell below this level (Biro et al. 2004). The concept of a critical lipid content for winter survival was also established by Finstad et al. (2004), who showed that Atlantic salmon (Salmo salar) needed 4400-4800 J·g-1 for winter survival, which translates to a value of approximately 0.46% wet mass for triacylglycerols (TAGs). Numerous toxicants are metabolic disruptors affecting metabolic processes, growth, and lipid homeostasis. Meador et al. (2006) reported a substantial reduction in whole-body lipid content in juvenile Chinook from 2.5% wet mass for control fish to 1.0% for high-dose fish (TAGs below 0.4% wet mass) exposed to environmentally realistic concentrations of dietary PAHs. Alterations to related physiological parameters (plasma TAGs, lipase, and albumin) were also observed at low exposure doses. Another critical aspect is the lipid-normalized tissue concentration for hydrophobic compounds. As lipid levels decline in fish, a given concentration of poorly metabolized contaminants, such as PCBs, will increase in bioavailability within the animal and result in increased toxicity (Lassiter and Hallam 1990). This is especially relevant for juvenile fish during their first winter, as lipid content declines and effective toxic concentrations rise internally.

#### Immunotoxicants

A number of studies demonstrate that common urban contaminants such as PAHs and PCBs are immunotoxicants in juvenile salmon at environmentally low concentrations (Arkoosh et al. 1991, 1998, 2010; Bravo et al. 2011). When the immune system is compromised by these chemicals, juvenile salmon are more susceptible to fatal infections from common pathogens found in the environment. Karrow et al. (1999) found that a number of immune parameters were altered in rainbow trout exposed to creosote PAHs at concentrations as low as 0.6 ng·mL<sup>-1</sup>. Bravo et al. (2011) noted a number of biochemical alterations in juvenile rainbow trout fed relatively low concentrations of PAHs in their diet. This study also demonstrated a reduction in survival for fish from low dose (0.66  $\mu$ g·g fish<sup>-1</sup>·day<sup>-1</sup>) and high dose (7.8  $\mu$ g·g fish<sup>-1</sup>·day<sup>-1</sup>) PAH treatments exposed to the pathogen *Aeromonas salmonicida*. As noted by these authors, the dietary concentrations were modeled after those observed in field-collected fish from contaminated Puget Sound estuaries. Another study on flame retardants found that dietary concentrations of PBDEs in field-collected fish compromised the immune system in juvenile Chinook, resulting in increased mortality when salmon were exposed to a common marine pathogen (Arkoosh et al. 2010).

## **Behavioral effects**

Altered behavior in juvenile salmon can result in mortality. Many chemicals are known to affect fish behavior, including copper, cadmium, mercury, several organochlorine and current-use pesticides, organotins, and pentachlorophenol (Scott and Sloman 2004), and low levels can result in high rates of mortality (McIntyre et al. 2012). A number of mechanistic and behavioral studies support the observation of altered behavior in salmon exposed to low concentrations of copper and pesticides (Scholz et al. 2000; Sandahl et al. 2004; Laetz et al. 2009).

#### **Field observations**

There are very few field studies that have examined impacts on salmonids exposed to contaminants. One relevant study found a negative correlation between catch data for Atlantic salmon and the spray application of a pesticide to various tributaries within a river basin during smolt development for a 1-year period (Fairchild et al. 1999). Similar effects were also noted for spray events over several years and for another species (blueback herring (Alosa aestivalis), which is also anadromous. The authors concluded that 4-nonylphenol, a component of the spray mixture, likely affected smoltification, leading to excess mortality for this life stage. Another study confirmed the ability of the endocrine disruptors 4-nonylphenol and estradiol (E2) to impair physiological processes related to smoltification and demonstrated delayed downstream migration and increased mortality for exposed fish (Madsen et al. 2004). These results have important implications because endocrine disruptors are commonly found in contaminated estuaries.

Some of the strongest evidence supporting adverse effects in outmigrating juvenile salmon from contaminated estuaries comes from Puget Sound area field studies when viewed in light of the abovementioned laboratory studies of individual compounds or classes of compounds. One study calculated growth rates for juvenile Chinook from the Soos Creek hatchery that were captured in nearshore areas of Puget Sound within 2-4 weeks of release (Brennan et al. 2004). Of the juvenile Chinook assessed from all hatcheries (22 hatcheries, n = 86 fish captured in 2001 and n = 107 captured in 2002), fish from the Soos Creek hatchery accounted for most of the fish that exhibited negative growth (95%, 18 of 19 fish) or exhibited zero growth (100%, 4 of 4 fish) when compared with their respective release masses. Similar results were observed in 2002, with 91% of all negative growth fish (by length) coming from the Soos Creek hatchery (21 of 23 fish) (70% of all fish based on mass). Even though juvenile Chinook within the Duwamish estuary exhibit acceptable growth rates (Table 1), once they have accumulated contaminants and exit to open water they appear to be growth inhibited.

To test the observed patterns described here and the hypothesis that first-year factors are important for survival, this model was applied to the data presented in Duffy and Beauchamp (2011). In that study, juvenile Chinook were collected during their first year in open water with a mid-water tow net in three regions of Puget

Table 6. Analysis of Duffy and Beauchamp (2011) data for
juvenile Chinook captured in offshore waters of Puget
Sound.

	SAR (%)	Release mass (g)	July mass (g)	SGR
Contami	nated			
Mean	0.34	6.7	12.7	0.013
SE	0.09	0.5	1.3	0.001
n	11	11	11	11
Unconta	minated			
Mean	0.86	7.6	22.6	0.017
SE	0.12	0.5	1.9	0.001
n	14	14	14	14
<i>v</i> value	0.007	0.18	0.0001	0.04

Note: Data grouped by contaminated or uncontaminated rearing estuaries for juvenile Chinook. Mean and standard error (SE) are shown for smolt-to-adult return rate (SAR, %), release wet mass at hatchery, and fish wet mass in July. The specific daily growth rate (SGR) was also calculated. n is the number of hatchery-year combinations for each group over the release years 1997–2002. ANOVA p values shown for each variable. See text for details.

Sound over 4 years (1997, 1999, 2001, and 2002) during July and September. I divided the data in their table 1 by hatcheries that released into contaminated and uncontaminated estuaries (all except Hupp Springs, which release stream-type Chinook). Only hatcheries represented by more than three fish (25 of 28 possible data points) were selected, and ANOVA was performed on the groups. Survival was 2.5 times higher for fish transiting uncontaminated estuaries compared with the survival of fish migrating through contaminated estuaries (Table 6), which was consistent with the overall results of the present study. Mean release masses were higher for fish from the uncontaminated estuaries, but the differences were modest, especially when compared with the observed mass in July for these tag code groups (Table 6). Also, the specific growth rate was significantly lower in fish from contaminated estuaries. A focused examination of only the 2002 data from Duffy and Beauchamp (2011) shows the same large differences between fish from uncontaminated versus contaminated estuaries for SAR values (2.1-fold), fish mass in July (1.4-fold), and specific growth rate (1.5-fold), but with equal release masses (0.99-fold), supporting the contention that release mass was not an important factor. The results of this analysis (Table 6) are consistent with the conclusion in Duffy and Beauchamp (2011) that survival is strongly linked to fish mass in July and the conclusion of the present study that contaminated estuaries strongly affect juvenile survival. These data also support the hypothesis of this review that fish outmigrating through contaminated estuaries are likely affected by contaminants for the first phase of their marine residency. Also, if fish had experienced reduced growth in the estuaries because of prey limitations, compensatory growth (Johansen et al. 2001) would have likely allowed them to increase quickly once they encountered more favorable conditions as experienced by juveniles from other estuaries.

#### Laboratory studies with field-collected fish

Another strong line of evidence can be found in laboratory studies with fish from specific estuaries. One study collected juvenile Chinook from three hatcheries (Soos Creek, Kalama Creek, and Puyallup) and their respective estuary (Duwamish, Nisqually, and Puyallup). Fish were held in the lab for 40 days (1990) or 84 days (1991) (Varanasi et al. 1993). The percent survival of fish from the Duwamish River (56%) and Puyallup River (58%) estuaries was significantly less than for fish from their respective hatchery (86% and 88%). Survival for fish from the Kalama hatchery (88%) was not different from that for fish collected in the Nisqually River estuary (81%). This experiment was repeated in 1991 for the Soos Creek Hatchery – Duwamish River Estuary combination, yielding a similar result (77% for Soos Creek hatchery and 59% for the Duwamish River estuary fish).

Three experiments in 1993 and 1994 examined susceptibility of juvenile Chinook to a common marine pathogen (Vibrio anguillarum) (Arkoosh et al. 1998). The 1993 experiments were conducted in two phases whereby one group was tested 1 month after capture and the second group was allowed 2 months to depurate contaminants. Fish were collected from the Nisqually delta and Duwamish River and their respective hatchery (Kalama and Soos Creek) and exposed to the pathogen for 1 h. For both years, fish from the Duwamish waterway exhibited substantially higher mortality at 4 and 7 days post pathogen exposure compared with fish from their respective hatchery. There was no difference in mortality for these time points or sampling years for fish from the Nisqually hatchery and estuary. Mean concentrations of PAHs and PCBs in the stomachs and liver of fish from composited samples (n = 60fish per composite) from these estuaries and hatcheries at the time of collection were very similar to those reported in Table 2 (current study), except for the PAH concentrations, which were lower, ranging between 5 and 10 µg·g<sup>-1</sup> (wet mass) for the Duwamish fish. These results are supported by a laboratory study, with fish from these same hatcheries and estuaries demonstrating altered immune parameters (Arkoosh et al. 1991).

## **Contamination in adult Chinook**

Any adverse responses resulting from exposure to contamination are likely to occur in juveniles and not adults. Because water and prey in open marine waters contain less contamination than found within estuaries, concentrations of most toxicants likely decline because of growth dilution, which has been demonstrated for PCBs in salmon from Puget Sound (O'Neill and West 2009). Therefore, the focus for this analysis is on factors that may affect first-year survival when contaminated fish are most susceptible. Theoretically, returning adult salmon could accumulate contaminants to adverse levels; however, there is no evidence to support this hypothesis. One recent study assessed PCB concentrations in returning adult Chinook salmon to Puget Sound rivers and found relatively low concentrations in terms of lipid normalized concentrations (mean 53 ng·g<sup>-1</sup> (wet mass fillet), lipid 5.4%) in fish from the Nooksack, Skagit, Duwamish, Nisqually, and Deschutes rivers (O'Neill and West 2009). Also, returning adult Chinook do not feed prior to, or within, the local estuary when in their reproductive mode (Higgs et al. 1995), and therefore it is unlikely that potential contamination at this life stage can explain the SAR values.

#### Implications for wild fish

If contamination is indeed the causative factor limiting the SAR for hatchery Chinook, then the extended time expected for naturally reared Chinook may lead to even more dramatic impairment. This is also relevant for any other salmonid at this life stage that may reside in an estuary for an extended time. If this level of reduction in survival for wild fish outmigrating through contaminated estuaries is occurring, it will likely manifest in large changes to population abundance and structure as demonstrated with life history modeling (Spromberg and Meador 2005). As shown by Spromberg and Meador (2005), first-year survival is the most important period of the Chinook life cycle, and increases in mortality as low as 10% can result in a substantially reduced population growth rate for this species, given that impacts (e.g., reduced growth or elevated mortality) occur over several generations.

Five or more life history trajectories have been identified for Chinook salmon (Ruggerone and Weitkamp 2004), including the numerous smolts that migrate in May and June and spend several days to weeks in the estuary. Also identified are fry migrants that can spend weeks to months in the estuary before migrating out to marine waters. One study determined that wild juvenile Chinook spend approximately twice as long in the estuary as do hatchery fish (Levings et al. 1986), which would likely increase their exposure to harmful chemicals. The degree of unaltered habitat is an important factor to consider for naturally reared Chinook, which are likely more susceptible to habitat modifications compared with hatchery fish.

### Next steps

Considering the large effort and resources devoted to understanding the factors that affect salmonid success in an attempt to rebuild depleted stocks, contamination of their natal estuaries receives very little attention. The impact of environmental toxicants on aspects of fish health such as growth rate, lipid stores, susceptibility to pathogens, altered behavior, and physiological changes both in the laboratory and field should be considered for any evaluation of population vitality. Defining toxicity for myriad contaminants found in urban estuaries and studies that evaluate the health of juvenile salmon will provide much-needed information for source control and remediation of contaminated estuaries, which is expected to improve cohort survival. Concentrations of chemicals in fish tissue can be quite valuable for assessing toxic effects (Meador et al. 2008b), and there are data available now for several chemicals (Beckvar et al. 2005; Meador 2006; McElroy et al. 2011).

It is clear that a simple binary designation for estuary status in terms of chemical contamination is insufficient for focused risk assessments and determining when harm is reduced. Unfortunately, there are few data available that can be employed for such evaluations. A concerted effort to characterize toxicant exposure is required to allow for finer scale categorization of estuary status. Extensive datasets on fish tissue concentrations of suspected harmful chemicals are needed for characterizing exposure, toxicity evaluation, and determining the degree of remediation success. Studies highlighting water and sediment concentrations are also necessary for gauging exposure and determination of cleanup levels. Primary consideration should be given to tissue (fish and stomach contents) and water concentrations, which can be more easily linked to toxic effects. Additional metrics to consider in future studies include the location of wastewater treatment plants and combined sewer overflows, runoff from impervious surfaces, and potential inputs from pesticide applications and waste from industrial animal production. As more data become available, successive analyses should include weighting factors for each metric and possibly a summation style index describing the state of contamination for each estuary.

The results of this analysis in no way diminish the conclusions of other studies and their findings relating salmonid survival to habitat characteristics (e.g., shoreline armoring, loss of intertidal habitat, and reduced flow) or biological interactions (e.g., effects of competition), but instead raise the possibility of yet another potentially important factor that should be considered in conjunction with all other known determinants. Remediation efforts for compromised estuarine areas usually consider myriad factors during design and implementation, and as shown in this study, contamination should be included as one of those important determinants. Understanding and characterizing chemical contamination in our estuaries is just one crucial and necessary aspect of advancing efforts for the recovery of salmon populations.

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