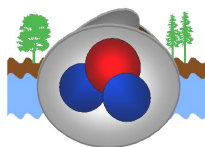


ORIGINS OF JUVENILE CHINOOK IN SAN JUAN COUNTY, WASHINGTON



**Final Report to the
San Juan County Marine Resources Committee**

September 2007 (Revised)



KWÍÁHT

Center for the Historical Ecology of the Salish Sea
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Origins of juvenile Chinook in San Juan County

Final Project Report

EXECUTIVE SUMMARY

Microsatellite genotyping of nine juvenile Chinook found in WRIA2 waters between May and August in 2005-2007 identified five Fraser River fish and four Puget Sound fish. Two of the Puget Sound juvenile Chinook originated in the Suiattle (Skagit) River, one in the Stllaguamish River and one in a WDFW hatchery at Shelton. Juvenile Chinook from Puget Sound and the Fraser were both found in northern as well as southern WRIA2 bays and in San Juan Channel, suggesting that juvenile Chinook spend weeks to months in WRIA2 moving from island to island. This supports the assertion that ESA-listed Chinook populations rely on WRIA2 habitats and prey for a significant portion of their early life history.

A considerably larger number of tissue samples was purchased from Dr. Tina Wyllie Echeverria for this study but genotyping determined that the majority of them were not in fact Chinook but either coho or other salmonid species. This calls into question the species composition data in a number of previous WRIA2 studies. In particular, our genotyping data suggest that the islands are utilized by considerably more juvenile coho, and possibly also juvenile steelhead, than has previously been reported.

Juvenile coho in our sample were, on average, 22 percent *larger* than juvenile Chinook utilizing the same beaches at the same time, suggesting the possibility of competition for nearshore prey between these species.

The results of this study are preliminary, but they underscore the need for a larger, systematic assessment of stocks-of-origin of juvenile Chinook in WRIA2 waters. New genotyping technology developed by Eric LaHood of the Northwest Fisheries Science Center and tested by this study should make it possible for future genotyping to be carried out quickly and inexpensively within San Juan County.

Origins of juvenile Chinook in San Juan County

Final Project Report

Russel Barsh and Madrona Murphy¹

The San Juan Archipelago is the oceanographic crossroads of the Salish Sea, daily washed by the Fraser River and by tides from Puget Sound and the Gulf of Georgia. Not surprisingly, a SRFB-funded study found large numbers of juvenile Chinook, chum, pink, and coho in San Juan County nearshore waters from early spring through late summer (Barsh and Wyllie-Echeverria 2006). The largest aggregations were observed along the beaches of Waldron Island and President Channel; the beaches of south Lopez; and rocky shorelines of north San Juan Island.

Juvenile salmon use of coarser, higher-energy beaches distinguishes the San Juan Islands from most of Puget Sound, where smolts congregate preferentially in much more protected delta environments and pocket estuaries (Beamer et al. 2003; Fresh et al. 2006). It is not yet certain that all juvenile salmon shift to higher-energy nearshore habitats as they move seaward; or what prey attract them to higher-energy environments. First, it is necessary to determine the origins of juvenile Chinook utilizing San Juan County waters, so that we connect their foraging behavior in the islands with their behavior in their natal streams, deltas and estuaries. This report is a first step in that direction.

With over four hundred miles of shoreline, furthermore, San Juan County must set priorities for protection and restoration that have the highest likelihood of contributing to the recovery of ESA-listed salmon. Identifying the habitats used by ESA-listed stocks as opposed to juvenile Pacific salmon generally will help focus county action.

Partners

This study was originally planned as collaborative effort of KWIAHT; Paul Moran and his salmon genetics team at the NOAA Northwest Fisheries Science Center, Seattle; and Terry Beacham and colleagues at the Canadian Department of Fisheries and Oceans Pacific Research Station, Nanaimo. The Moran and Beacham labs are founding members of the genetics consortium recently formed under the auspices of the Chinook Technical Committee of the Pacific Salmon Commission. KWIAHT is working towards certification, with the assistance of the Moran lab; and had agreed to use this study to help the Moran lab test a new “ladder” designed to simplify and speed the process of genotyping samples of Chinook tissue (described under Methods, below). After the project got underway, the Beacham lab advised us that other obligations made it impossible for them to participate.

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Previous research

The importance of the San Juan Islands to inbound adult Pacific salmon has been known to fishermen for centuries, as evidenced by the antiquity of Native American reef-net sites and the great value placed on them by Native communities throughout the Salish Sea (Barsh 2005; Suttles 1974). Salmon migration paths through the islands were first investigated using acoustic tags more than 30 years ago (Stasko et al. 1976), but were not pursued further systematically, nor did they determine what resources adult migrants used in the archipelago.

A 1985 State tag-and-recapture study found 16 coho and 5 Chinook stocks among salmon recaptured in the San Juan Islands (Stohr and McGowan 1987), including three ESA-listed Chinook stocks (Nooksack, Lower Skagit, Dungeness River). More recent unpublished data from the Pacific States Marine Fisheries Commission is set out in the WRIA2 *Handbook of Salmon Recovery in San Juan County* (June 2004), Table 2, 19-20. Coded wire tag recoveries in the San Juan Islands included coho and Chinook stocks from the Fraser River, Georgia Strait, the west side of Vancouver Island, the Strait of Juan de Fuca, Hood Canal, all parts of Puget Sound, and much of the Columbia River. It is certain that adult salmon linger in the San Juan Islands each summer, but it remains unclear whether they feed, and if they do, what prey they target and where.²

Juvenile salmonids are also present in the archipelago throughout the year (Rice et al. 2004; Barsh and Wyllie-Echeverria 2006). Juvenile chum, juvenile pinks, and adult Chinook were amongst the ten species most commonly found in beach seine collections made by students at Friday Harbor Laboratories in the San Juan Islands (Miller et al. 1977). Data from several decades of FHL student projects are of limited value, however, because the methods, sampling locations, and times of year have varied from year to year (Moulton 2004). A more comprehensive multi-year approach is essential to ascertain, not only which WRIA2 beaches juvenile Chinook utilize, but also where these fish originate, since WRIA2 has no natal Chinook stocks of its own.³

Determining stock origins is crucial for public policy insofar as ESA-listed stocks must be given priority under federal and state management plans. To the extent that there are stock-specific preferences regarding habitat types or prey selection, moreover, greater focus can be given to local protection and restoration measures. For example, if juvenile Chinook from a particular endangered ESU are found feeding on smelt eggs on President Channel beaches, there is far more than a theoretical basis for protecting and enhancing smelt sands in that part of the county.

² In August 2003 we analyzed the stomach contents of 10 sockeye taken by purse seined on the west side of San Juan Island. Seven stomachs contained prey items including insects, euphausiids, hypolittid shrimp, isopods, fish scales, and octopus, which suggest both pelagic and nearshore feeding within 24 hours of their capture (consistent with Higgs et al 1995, for sockeye harvested in Georgia Strait).

³ A study currently underway by Barsh and Glasgow (Wild Fish Conservancy) has, thus far, identified five WRIA2 streams with native cutthroat populations and two with chum and coho. Chinook are produced in great numbers by the Glenwood Springs hatchery on East Sound, Orcas Island, operated by Long Live the Kings, but these tagged fish have not been found in Wyllie-Echeverria's beach seine or tow samples.

Methods

1. Sampling strategy

We began with 17 tissue specimens from fish previously collected and identified by Dr. Tina Wyllie-Echeverria using a beach seine (described below) at various locations in San Juan County in 2005-2006. Seine sites included the two “hot spots” where Barsh and Wyllie-Echeverria had observed very large seasonal aggregations of juvenile salmon, including Chinook, for several years (*i.e.*, Waldron and south Lopez), as well as several intermediate points on San Juan Channel (Figure 1). We felt that it was most important to understand these large aggregations: are they relatively homogeneous, or composed of a wide variety of stocks; and from where?

Ideally, we would have sampled the two “hot spots” frequently over the course of several years, to capture seasonal and inter-annual variation. This was not possible given the time and financial constraints of our contract, which was approved in mid-August for completion by June the following year: that is, from the end of the 2006 juvenile Chinook migration to the beginning of the 2007 juvenile Chinook migration. Contract constraints therefore left us with two small time windows to collect fish. In previous studies we had used beach seines to sample fish populations in shallow water in different habitat types, but rarely brought more than 2-3 juvenile Chinook to hand together. To achieve a larger sample in such a very short time, we decided to use a larger net in deeper water where we believed we would be able to collect larger and more representative samples. However, it was conceivable that different salmon stocks frequent shallower and deeper waters in the San Juan Islands, so that beach seines and tow-nets might produce different results.

2. Beach seine sampling

Our beach seine sampling followed the method of Beamer et al. (2003). An 80-foot seine is deployed by hand in 1-4 feet of water from a floating tub or dinghy, and then pulled ashore by a crew of four. Three successive seine sets are carried out at each site as replicates, typically at the speed of one set every 15-20 minutes, moving up the current to minimize catching the same fish. Processing the catch from each set may take an hour or longer depending on the number of fish landed, which in our experience may include over a thousand chum or pink salmon, but rarely more than a few Chinook.

The beach-seined Chinook in our genetic sample were brought to hand in 2005 and 2006 in the course of our earlier exploratory study aimed at identifying the kinds of shoreline habitats most attractive to juvenile salmon (Barsh and Wyllie-Echeverria 2006). More than 20 exposed and more sheltered beaches in WRIA2 were seined at least once in the course of the study; those where salmon were found were seined repeatedly in spring and summer to determine the timing of different Pacific salmon species and their overlap with other seasonal fish populations such as herring, surf smelt, and shiner perch. This is an “opportunity sample” in the sense that it was not collected for the present study and is not ideal for the purposes of identifying all of the Chinook stocks that may seasonally use WRIA2 shorelines.

3. Tow net sampling

We accordingly contracted with Dr. Wyllie-Echeverria to collect 100+ additional Chinook specimens by tow-net in August 2006 and June 2007. A long pursed net was towed behind the vessel *Coral Sea* with the assistance of a skiff in the deeper waters of Cowlitz Bay (Waldron) and Hughes Bay (Lopez) on August 22 and August 24, 2006, and May 26 and June 18, 2007, using the methods of Fresh (1979) and Parr (1972). The duration of each tow was ten minutes, with the net maintained just below the surface, *i.e.*, in that portion of the water column where other studies suggested the greatest likelihood of encountering juvenile salmon. A single tow was also conducted in Shoal Bay (Lopez) on May 24, 2007 in connection with a different project, and yielded a single specimen for the present study.

Dr. Wyllie-Echeverria delivered 46 specimens from her August 2006 towing but only 6 from tows in late May and early June 2007. It is conceivable that juvenile salmon were late arriving in WRIA2 due to this year's cooler and wetter than average spring weather. However, "leapers" were observed along WRIA2 shorelines during the same weeks that the tow net was deployed. As discussed in greater detail below, poor early-summer tow results may also be due to previously unreported changes in juvenile salmon behavior during their summer residence in the San Juan Archipelago: more nearshore feeding in early summer when they are still relatively small, and more pelagic feeding as the summer continues and they grow larger.

We note that contractual constraints prevented us from repeating our late summer 2006 tows in 2007 in order to increase sample size and test the possibility of a seasonal shift in habitat use. Our study was approved on August 10, 2006, with a final reporting deadline of June 30, 2007, preventing us from towing at the same time for both summers as we originally had proposed.

4. Fish identification

Dr. Wyllie-Echeverria identified to species, and measured the fork lengths of all seined and tow-netted fish at the time of capture on beach or boat. See Figures 2 and 3 for a summary of all of the specimens provided to us as Chinook.

5. Tissue preservation

After sedation in seawater dosed with Finquall (MS-222), juvenile salmon were weighed, measured, and checked for marks and coded wire tags. A small part of anal fin tissue was then clipped with surgical scissors and transferred to an Eppendorf tube with 95% ethanol as a preservative and a waterproof paper slip with the site name, date, and a field number. At Friday Harbor Labs, tissue specimens larger than 5 mg were carefully divided (using sterilized disposable scalpels and trays to prevent cross-contamination) so that tissue not needed for DNA extraction could be preserved for future study. Extracted DNA not expended in microsatellite analysis (see below) was also preserved at Friday Harbor Laboratories by freezing at -80° Celsius.

6. DNA extraction

We extracted DNA from Chinook tissue samples using a DNAeasy Tissue and Blood Mini kit from QIAGEN. The tissue was rinsed in filtered TBE prior to extraction to remove any residual alcohol. Extractions were eluted twice using 200 μ l of AE buffer, and the eluates were collected in separate microcentrifuge tubes. The concentration of DNA in a subset of six extractions (including two extractions that consisted of the second elution) was measured by spectrophotometer (absorbance at 260 nm). Concentrations of DNA varied from 7.5 ng/ μ l to 77.5 ng/ μ l, with an average of 29.58 ng/ μ l. In most cases, amplification was carried out on the first eluate.

7. Microsatellite analysis

We amplified 13 microsatellite loci from each sample—the so-called “CTC loci” as approved by the Chinook Technical Committee of the Pacific Salmon Commission (PSC) for determinations of Chinook origins—for comparison with the CTC database of Pacific Coast Chinook populations.

Microsatellites are small sequences of non-coding (*i.e.*, non-functional) DNA that are susceptible to relatively frequent duplication mutations called “short tandem repeats” or STRs (Banks et al. 1999; Nelson and Beacham 1999). Since they are non-coding, they are not subject to selection pressure to remove them from the population gene pool, hence they accumulate within populations, and reproductively isolated populations accumulate different patterns of greater or fewer repeats (different microsatellite alleles) that can be distinguished using DNA amplification (PCR), gel electrophoresis and fluorescent tags in a DNA sequencer. Patterns of microsatellite variations can be used to determine whether Chinook populations are reproductively distinct, and to assign individual fish to stocks of origin (e.g. Nelson et al. 2001). The fineness of scale of geographic distinctions, and the certainty or statistical power of the identification of individual specimens is a function of the number of microsatellites compared (Kim et al. 2004; Kinnison et al. 2002). The 13 CTC loci identify Chinook stocks to watersheds with 95+ percent confidence. (This is, coincidentally, the number of microsatellite loci used in human DNA “fingerprinting”.) Several microsatellites are typically “multiplexed,” *i.e.* tagged with different colors and run simultaneously on the same gel (Olsen et al. 1996).

As part of this project, KWIAHT tested the prototypes of a microsatellite “ladder” designed by Eric LaHood and Paul Moran at the Northwest Fisheries Science Center to reduce the time and expense of genetic stock identification considerably. Multiplexing identifies 3 to 8 different microsatellite alleles per run by comparing each of them with a size standard, while ladders sort and identify any number of alleles by weight, thereby reducing several-fold the number of runs needed to identify a specimen. While ladders are already used in human “DNA fingerprinting,” their use in salmon stock identification is novel, and San Juan County will have one of the first laboratories to use this new tool.

Samples were first amplified with 5'-end labeled (6-FAM, HEX and NED) custom primers constructed for our use by Applied Biosystems, MGW DNA and Integrated DNA

Technologies, as well as primers supplied by Paul Moran and Eric LaHood of the NOAA Northwest Fisheries Science Center in the form of a single amplification “cocktail”. The Moran lab also provided us with the CTC primer sequences. The 13 primers amplify the microsatellite loci of the CTC (Chinook Technical Committee) set standardized between 12 labs identifying Chinook (Moran et al 2005). Amplifications were performed using a Biometra thermal cycler in 10 µl reactions with 0.13-0.26 µl taq polymerase (Promega), 200µM dNTPs, 0.4µM labeled primer and 3µl sample or ladder or control.

Amplified loci were then run on an Applied Biosystems 377 automated slab gel sequencer. Fifty lanes were run on each gel, by loading with a paper membrane comb from The Gel Company (Toonen and Hughes, 2001). Where appropriate, PCR products with different colored labels were multiplexed by mixing prior to the addition of the size standard. 0.27-0.80µl of each PCR product was mixed with 0.9µl size standard-dye mix (2:1:6 ROX-500 size standard from Applied Biosystems comprising dextran blue EDTA:formamide); 1µl of this mix was loaded onto the comb using a loading tray from The Gel Company. Combs containing samples were loaded by filling the well with 5% ficoll + dextran blue and sliding the comb into it before filling the upper buffer chamber with 1x TBE. After the first few minutes of the run the membrane comb was removed, and the ficoll was rinsed out of the well. Each gel had a collection time of three hours. Two amplifications of an allele ladder (developed and provided by Eric LaHood) were run for each locus on each gel as well as two samples of known genotypes (also provided by Eric LaHood). Alleles were scored by comparison with the ladders and the control genotypes. STRand software developed by the University of California—Davis was used to identify alleles in chromatograms.

Paul Moran and his student Eric LaHood then analyzed the allele frequencies of our specimens, compared them with the genotypes of known source populations (Moran et al 2005) and assigned them to their stocks of origin. For reasons described below, they re-sequenced samples at the Northwest Fisheries Science Center and then, for additional confirmation, they sent aliquots of DNA to David Kulikowski at the NOAA laboratory at Manchester, WA, for re-sequencing and further corroborative analysis. These additional steps were not foreseen in our original proposal, but lend a very high level of confidence to our results.

Results

As summarized in Figure 3, the large majority of specimens supplied to us by Dr. Wyllie-Echeverria were not Chinook. This included roughly half of the seine specimens, and nearly all of the tow-net specimens. As a consequence, our sample was insufficient to establish with statistical certainty the *mix* of Chinook populations that utilize WRIA2 as a nearshore nursery. However, we were able to identify the origins of the Chinook in Dr. Wyllie-Echeverria’s collection (N=9) with great certainty, and thus establish without doubt that WRIA2 is utilized by at least some ESA-listed Chinook populations.

1. Origins of juvenile Chinook

We have grouped stream-of-origin results by geographic domains and collection methods (Figures 4, 5, and 6). As noted above, Waldron and south Lopez are “hot spots” where juvenile salmon congregate seasonally in the nearshore. One of our questions was whether different populations of Chinook utilize these “hot spots”—for example, it would seem plausible geographically for Fraser-origin fish to dominate the “northern” Waldron aggregation, and Puget Sound-origin fish to dominate the “southern” Lopez aggregation. Our limited results suggest the opposite: Fraser-origin fish were collected at Waldron and south Lopez as well as San Juan Channel, the sea corridor connecting these two “hot spots”. Puget Sound-origin fish were also collected both at Waldron and south Lopez, as well as San Juan Channel. Once juvenile Chinook reach WRIA2, they circulate between islands, utilizing high-energy outer shorelines as well as the protected passes.

We also planned to compare the beach seine and tow-net results, since these gears sample different habitats: inter-tidal shorelines (beach seines) and the neritic zone beyond the deepest sub-tidal eelgrass meadows (tow-nets). Different stocks of Chinook may feed in the inter-tidal and neritic zones; or different size classes or life history stages of the same stocks; or the same stocks may simultaneously exploit both zones, following daily tides and currents. A Fraser fish and a Hood Canal fish were in the tow-net collection; but the size of this sub-sample was too small to draw reliable conclusions.

When interpreting these data, it is important to bear in mind that juvenile Chinook tissue was collected over three seasons (2005-2007). Habitat choices may vary from year to year, especially if juvenile Chinook are attracted by the abundance of particular prey—rather than stock fidelity to particular shorelines or classes of shoreline morphology. Our current sample is too small to explore inter-annual variation in the habitat associations of different Chinook populations, but this question should be pursued in future studies.

Furthermore, our sampling strategy was seriously constrained by the timing of our funding, as described above under Methods. We can say with confidence that the stocks identified in Figure 4 were present in WRIA2—and, in the case of the beach seine sample were feeding along WRIA2 shorelines—during the narrow time windows in which they were collected: May-August 2005 and 2006, and late May 2007. We cannot say with any confidence how representative these data may be of WRIA2 as a whole, year-round. The importance of conducting a more comprehensive, multi-year sampling exercise for stock identification cannot be over-emphasized.

It should be noted that all juvenile Chinook included in this study were examined for external tags or marks and checked for coded wire tags with an electronic “wand” by Dr. Wyllie-Echeverria, who reported no marked or tagged individuals. Genetic analysis traced one fish to the George Adams state salmon hatchery near Shelton, however; all of the other Chinook in our sample were from wild stocks. There is always the possibility that some hatchery fish were released untagged. In any case, this was the first juvenile Chinook of hatchery origin reported from WRIA2.

2. Identity of non-Chinook specimens

What, then, were the fish that Dr. Wyllie-Echeverria mistook for Chinook? All of them were Pacific salmon. Based on their microsatellite alleles, most of the misidentified seined fish were probably coho. This is highly significant for two reasons. First, juvenile coho were previously reported to be infrequent visitors to WRIA2 (e.g. Barsh and Wyllie-Echeverria 2006). This was apparently the result of misidentification. Second, the coho we have identified in Dr. Wyllie-Echeverria's collection were as large as the Chinook she brought to hand. This confirms that like Chinook, coho stay longer in mainland delta and pocket estuary habitats than chum or pink salmon, before migrating to WRIA2 waters. It also suggests that like Chinook, coho may have longer residence in WRIA2 waters than juvenile chum or pink salmon.

We were unable to identify the other misidentified salmon using microsatellites alone. They will be re-sequenced again with species-specific primers, and be the subject of a supplemental report. About half of them have allele patterns suggestive of "trout"—*Oncorhynchus mykiss* (rainbow and steelhead) and *Oncorhynchus clarki* (cutthroat). Use of WRIA2 nearshore habitats as nurseries by these species was not previously reported, and is significant in view of the fact that steelhead have now been ESA-listed.

3. Analysis by size (fork length)

An interpretation of size (Fork Length) of the specimens collected by Dr. Wyllie-Echeverria is confounded by the fact that three or more salmonid species are represented in the collection, and we are only certain about the identity of two of them (Chinook and coho). Some interesting issues are nonetheless raised by the aggregated data.

There was no aggregate size difference between juveniles caught in beach seines (chiefly in May and June) and juveniles caught in deeper water by our tow net in August. There was a greater range of size in shallower water, however, and the variance was quite high (Figure 2). In part, the wide range appears to have been a product of the species mix in the seines: individuals that genotyping confirmed as Chinook averaged 105.8 mm fork length, whereas individuals that genotyping indicated were probably coho averaged 128.8 mm fork length. Contrary to widespread assumptions, then, the juvenile coho collected in beach seines were 22 percent *larger*, on average, than the juvenile Chinook found with them. Do these large juvenile coho compete with Chinook for nearshore prey resources? The question merits further investigation.

Juvenile salmon taken in Hughes Bay by tow-net were clearly smaller than those taken by tow-net in Waldron waters, a difference in means of 13 mm or 10 percent of the combined mean size of our tow-net sample. The two areas were towed within 48 hours of each other. Why were the juveniles in Hughes Bay smaller, and therefore presumably younger? Did they leave their natal streams for the islands at a younger age and smaller size? Or, had the Waldron fish arrived earlier in the season and grown more than a centimeter longer whilst residents in WRIA2? Again, the problem of misidentification of

fish by Dr. Wyllie-Echeverria is a confounding factor because the species composition of juvenile salmon in the two tow-net collections may differ.

The seasonal difference between our beach seine and tow-net samples suggests an alternative possibility: as the summer progresses, juvenile salmon feeding along WRIA2 shorelines either continue their seaward migration, or move to deeper WRIA2 waters and continue to feed on more pelagic prey. This predicts mixed stock aggregations of widely varying size along shorelines in the early summer, but relatively few juvenile salmon in deeper WRIA2 waters until late summer, when only some salmon stocks remain behind. Our poor tow-net results in late May and early June, during a time when shore observers on Waldron and Hughes Bay reported large numbers of “leapers”, is consistent. In future studies it would be useful to pair beach seine and tow-net collections of juvenile Chinook during the summer months—or to maintain systematic shore observations for comparison with tow-net results.

Results from our 2007 tow net sampling are also suggestive, although the sample size is small and from different years, therefore non-comparable with 2006 data. Juvenile salmon taken by our tow net in early summer 2007 were about 15 percent smaller (fork length) than juvenile salmon taken by tow net in the same areas in late summer 2006. It is plausible that many juvenile salmon spend the summer in the islands and grow by that much or more before continuing their seaward migration.

Discussion

Adaptive genetic diversity has enabled Pacific salmon to survive the vagaries of a dynamic geology and volatile climate in the Pacific Northwest, marked by frequent rapid changes in the distribution of habitat patches and prey populations (Hutchings 2004). To help salmon survive the additional rapid changes brought about by industrialization of the Puget Trough, we must maintain habitat diversity and foraging options. Protecting only a part of the habitat mosaic collectively utilized by Pacific salmon would have a pernicious selective effect of selecting for particular life-history types and favoring genetically more homogenous populations. But first we must better understand the extent of the diversity of life histories and habitat uses of existing salmon stocks.

There is a growing body of evidence that juvenile salmon utilize a wide range of littoral and neritic habitats such as floodplains (Sommer et al. 2001), delta channels and pocket estuaries (Beamer et al 2003), and river plumes (Cooney et al. 2001; Fukuwaka and Suzuki 1998). A simple distinction between freshwater behavior and ocean behavior is no longer tenable. As Beamer emphasizes, salmon have evolved various complex life histories that involve different patterns of exploitation of the diverse aquatic ecosystems that lay along salmon migration paths. Nearshore habitats are probably a major factor in the total life-span survival of Puget Sound Chinook and presumably other Pacific salmon (Beamish et al. 2004; Greene et al. 2005; Greene and Beechie 2004; Quinn et al. 2004).

Diversity of life histories implies some diversity in prey selection as well. On the Columbia River, juvenile coho and Chinook consistently prefer pigmented invertebrates, feasting on hyperiid amphipods, crab megalopae and euphausiids, as well as some larval and juvenile fish, with larger Chinook accounting for most of the fish consumption; small copepods dominate the zooplankton community but are a small part of the salmonid diet (Schabetsberger et al. 2003). Juvenile salmon are not passively opportunistic. Studies of pink and coho salmon have found that they are attracted to dense clouds of zooplankton (Willette 2001; Cooney et al. 2001), which they will attempt to exploit even if associated with dangerous conditions such as high temperatures (Birtwell et al. 2001).

Beamer et al. (2003) have shown important diversity in the life history strategies of wild fry migrant chinook of Skagit origin. Based on otolith data, they distinguish *fry migrants* (fry migrate directly into Skagit Bay); *tidal delta users* (fry remain in the tidal delta where they rear to c. 70 mm before proceeding into Skagit Bay); and *parr migrants* (fry remain in fresh water to c. 70 mm before proceeding into Skagit Bay). Once in the bay, furthermore, at least some juvenile Skagit Chinook congregate in “pocket estuaries” where they continue to grow for several weeks before migrating to deeper waters. What proportion of Chinook migrants utilize pocket estuaries, and where they go to feed after leaving Skagit Bay (perhaps to the San Juan Islands) was unclear until the present study.

Habitat use is at least partly a function of the prey preferences of salmon and the abundance of preferred prey in particular types of habitat. In an exhaustive review of the literature, Higgs et al (1995) reported that the prey items most frequently identified in juvenile Chinook whilst they are rearing in estuaries have been gammarid amphipods, calanoid copepods, euphausiids, mysids, and insects. As Chinook transition to marine habitats, they add juvenile fish (including other salmonids) and decapod larvae to their diet. Estuarine sockeye consumed insects, copepods, and larval fish, switching to fish larvae, euphausiids, cladocerans, hyperiid amphipods and calanoid copepods in marine waters. Estuarine chum targeted insects, harpacticoid copepods, gammarid amphipods, and cladocerans, switching to more planktonic crustaceans in the marine phase. These generalizations were derived from a wide variety of sources, methods, and geographical areas along the entire north Pacific range of Pacific salmon. They almost certainly do not represent the diversity of salmon prey; the relative importance of particular prey items to specific stocks of salmon; or the adaptive diversity of foraging strategies within stocks.

The same *caveat* may be applied to extant data on the foraging strategies and diets of juvenile salmon in Puget Sound waters. Simenstad et al (1979) and Miller et al (1980) found mainly epibenthic organisms such as harpacticoid copepods, gammarid amphipods, oniscoidean isopods, and cumaceans in the stomachs of coho, pinks, and to a lesser extent chinook; whilst planktonic organisms such as calanoid copepods and hyperiid amphipods dominated the stomach contents of juvenile chum and were also found in pinks. Decapod larvae and drift insects were frequently found in juvenile Chinook and sockeye. Larval and juvenile fish including herring and sand lance comprised a significant proportion of the diets of juvenile coho and Chinook, and were also frequently seen in sockeye. On the whole, Chinook, chum and sockeye appeared to target more pelagic prey, whereas coho and pink salmon focused more on epibenthic prey (see also Brennan et al. 2004; Fresh

2006). Chinook and coho consumed the most fish, whereas other species relied more on crustaceans. Comparison at the level of species is inadequate for conservation, however, and data do not yet exist for finer scale analyses (*i.e.*, by stock or by nearshore habitat types). In particular, we still lack data on prey use by juveniles congregating in WRIA2, with its extensive beaches and rocky shorelines.

Monthly beach seining in 2005-2006 in a combination of sheltered bays and high-energy exposed beaches of WRIA2 (Barsh and Wyllie-Echeverria 2006) brought several hundred juvenile salmon (Chinook, chum, pink and coho) and thousands of “forage fish” (smelt, herring, sand lance) to hand. Juvenile salmon were most abundant on relatively coarse high-energy beaches. (Compare the negative relationship of salmon fry with fine sediments in fresh water habitats described by Suttle et al. 2004). Sites with the highest juvenile salmon abundance were not vegetated, although sub-tidal eelgrass beds were generally present offshore, and salmon may have sought refuge or prey in those meadows between episodes of feeding closer to shore. Juvenile Chinook, the most piscivorous Pacific salmon, were most abundant from May to August, overlapping the seasonal peak abundance of juvenile smelt and other “forage fish”.

These results are significant because they diverge from observations elsewhere that associate juvenile salmon with eelgrass communities (*e.g.* Simenstad 1994) and “pocket estuaries” (Beamer et al 2003). We may be seeing juvenile salmon from natal areas that do not afford them the protected delta-pocket estuary environments studied by Beamer in Skagit, Island and Snohomish Counties. Or, we may be seeing salmon at a different life-history phase with different habitat preferences. We generally see chum and pink smolts arriving in February at a small size (<50 mm Fork Length), which suggests that they have not spent much time elsewhere, while Chinook in our samples have generally been 80 to 120 mm Fork Length.

There is much more to learn about the kinds of prey and other conditions that attract juvenile Chinook and other Pacific salmon to *particular* high-energy beaches in WRIA2; the extent to which the abundance of particular prey determines juvenile salmon habitat choices (as opposed, *e.g.*, to oceanographic conditions); the extent to which the prey base varies from year to year; and the extent to which prey availability is affected by land-use practices. Answers to these questions will help refine county efforts to identify the most important habitat conditions for Chinook, and to protect and enhance Chinook habitat in ways most likely to increase juvenile Chinook growth and survival during their seasonal residence in WRIA2.

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References:

- Banks, M. A., M. S. Blouin, B. A. Baldwin, V. K. Rashbrook, H. A. Fitzgerald, S.M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in Chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Heredity* 90(2): 281-288.
- Barsh, R.L. 2005. Coast Salish Property Law: An Alternative Paradigm for Environmental Relationships. *Hastings West-Northwest Journal of Environmental Law* 12(1): 1-29.
- Barsh, R.L.; and Wyllie-Echeverria, T. 2006. Fish use of nearshore habitat in San Juan County, 2005. Progress report to SRFB, March 20, 2006.
- Beamer, E., McBride, A., Henderson, R., and Wolf, K. 2003. The importance of nonnatal pocket estuaries in Skagit Bay to wild Chinook salmon: an emerging priority for restoration. Skagit System Cooperative Research Department Technical Report. LaConner, Washington. 9 pp.
- Beamish, R. J., C. Mahnken, and C. M. Neville. 2004. Evidence that reduced early marine growth is associated with lower marine survival of coho salmon. *Transactions of the American Fisheries Society* 133: 26-33.
- Brennan, J. S., K. F. Higgins, J. R. Cordell, and V. A. Stamatiou. 2004. Juvenile salmon composition, timing distribution, and diet in marine nearshore waters of central Puget Sound in 2001-2002. King County, Department of Natural Resources and Parks, Seattle WA. 164 pp.
- Fresh, K. L. 1979. Distribution and abundance of fishes occurring in the nearshore surface waters of northern Puget Sound, Washington. MS thesis. University of Washington, College of Fisheries. Seattle WA. 120 pp.
- Fresh, K.L.; Small, D.J.; Kim, H.; Waldbilling, C; Mizell, M., Carr, M.I.; and Stamatiou, L.2006. Juvenile Salmon *Use of Sinclair Inlet, Washington, in 2001 and 2002*. Washington Department of Fish and Wildlife, Technical Report No. FPT 05-08. Olympia, WA. 180 pp.
- Fukuwaka, M., and Suzuki, T. 1998. Role of a riverine plume as a nursery area for chum salmon *Oncorhynchus keta*. *Marine Ecology Progress Series* 173: 289-297.
- Greene, C. M., and T. J. Beechie. 2004. Consequences of potential density-dependent mechanisms on recovery of ocean-type Chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences* 61: 590-602.

- Greene, C.M., Jensen, D.W., Pess, G.R., Steel, E.A., and Beamer, E. 2005. Effects of environmental conditions during stream, estuary, and ocean residency on Chinook salmon return rates in the Skagit River, Washington. *Transactions of the American Fisheries Society* 124: 1562-1581.
- Higgs, D.A., MacDonald, J.S., Levings, C.D., and Dosanjh, B.S. 1995. Nutrition and feeding habits in relation to life history stage. IN: C. Groot, L. Margolis, and W.C. Clarke eds., *Physiological Ecology of Pacific Salmon* (Vancouver: UBC Press). Pp. 159-315.
- Hutchings, J.A. 2004. Norms of reaction and phenotypic plasticity in salmonid life histories. IN A.P. Hendry and S.C. Stearns eds. *Evolution Illuminated: Salmon and Their Relatives*. New York: Oxford University Press. Pp. 154-174.
- Kim, J. E., R. E. Withler, C. Ritland, and K. M. Cheng. 2004. Genetic variation within and between domesticated Chinook salmon, *Oncorhynchus tshawytscha*, strains and their progenitor populations. *Environmental Biology of Fishes* 69: 371-378.
- Kinnison, M. T., P. Bentzen, M. J. Unwin, and R. P. Quinn. 2002. Reconstructing recent divergence: Evaluating nonequilibrium population structure in New Zealand Chinook salmon. *Molecular Ecology* 11(4): 739-754.
- Miller, B.S., Simenstad, C.A., Moulton, L.L., Fresh, K.L., Funk, F.C., Karp, W.A., and Borton, S.F. 1977. *Puget Sound Baseline Program Nearshore Fish Survey*. Fisheries Research Institute, College of Fisheries, University of Washington, Seattle, Washington.
- Miller, B.S., Simenstad, C.A., Cross, J.N., Fresh, K.L., and Steinfort, S.N. 1980. Nearshore Fish and Macroinvertebrate Assemblages along the Strait of Juan de Fuca Including Food Habits of the Common Nearshore Fish. Fisheries Research Institute, College of Fisheries, University of Washington, Seattle, Washington.
- Moran, Paul et al (2005). Interlaboratory Standardization of Coast-wide Chinook Salmon Genetic Data for International Harvest Management. A draft progress report from the Genetic Analysis of Pacific Salmonids (GAPS) consortium to the Chinook Technical Committee of the Pacific Salmon Commission, FY2004, FY2005.
- Moulton, L. 2004. Personal communication (June 11, 2004).
- Nelson, R. J., and T. D. Beacham. 1999. Isolation and cross species amplification of microsatellite loci useful for study of Pacific salmon. *Animal Genetics* 30: 225-244.

- Nelson, R. J., M. P. Small, T. D. Beacham, and J. K. Supernault. 2001. Population structure of Fraser River Chinook salmon (*Oncorhynchus tsawytscha*): An analysis using microsatellite DNA markers. *Fishery Bulletin* (Seattle) 99(1): 94-107.
- Olsen, J. B., J. K. Wenburg, and P. Bentzen. 1996. Semiautomated multilocus genotyping of Pacific salmon (*Oncorhynchus* spp.) using microsatellite. *Molecular Marine Biology and Biotechnology* 5(4): 259-272.
- Parr, W.H., Jr. 1972. Interactions between sockeye salmon and lake resident fish in the Chignik Lakes, Alaska. M.S. thesis. University of Washington, College of Fisheries. Seattle.
- Quinn, T.P., Vøllestad, L.A., Peterson, J., and Gallucci, V. 2004. Influences of freshwater and marine growth on the egg size—egg number tradeoff in coho and Chinook salmon. *Transactions of the American Fisheries Society* 133: 55-65.
- Rice, C., Greene, C., Beamer, E., Fresh, K., Lomax, D., Henderson, R. and Reisenbichler, R. 2004. Spatial and temporal distribution of marked and unmarked juvenile chinook salmon in nearshore waters of Puget Sound: Preliminary results. Pacific Estuarine Research Society Annual Meeting, May 17-18, 2004, Port Townsend, Washington.
- Simenstad, C. A., K. L. Fresh, and E. O. Salo. 1982. The role of Puget Sound and Washington coastal estuaries in the life history of Pacific salmon: An unappreciated function. In V.S. Kennedy (ed.), *Estuarine Comparisons*, 343–364. Academic Press, New York.
- Simenstad, C.A., Miller, B.S., Nyblade, C.F., Thornburgh, K., and Bledsoe, L.J. 1979. *Food Web Relationships of Northern Puget Sound and the Strait of Juan de Fuca*. U.S. Environmental Protection Agency, Washington, D.C. 335 pp.
- Simenstad, C.A. 1994. Faunal associations and ecological interactions in seagrass communities of the Pacific Northwest Coast. IN: S. Wyllie-Echeverria, A.M. Olson, and J.M. Hershman eds. *Seagrass Science and Policy in the Pacific Northwest: Proceedings of a Seminar Series*. SMA 94-1; EPA 910/R-94-004. Pp. 11-18.
- Sommer, T.R., Nobriga, M.L., Harrell, W.C., Batham, W., and Kimmerer, W.J. 2001. Floodplain rearing of juvenile Chinook salmon: Evidence of enhanced growth and survival. *Canadian Journal of Fisheries and Aquatic Sciences* 58: 325-333.
- Stasko, A.B., R.M. Horrall, and A.D. Hasler. 1976. Coastal movements of adult Fraser River sockeye salmon (*Oncorhynchus nerka*) observed by ultrasonic tracking. *Transactions of the American Fisheries Society* 105 (1): 64-71.

- Stohr, A.J., and J.E. McGowan. 1987. 1985 Results from Micro-tagged Salmon Experimental Groups. Washington Department of Fish & Wildlife Progress Report No. 260. Olympia, Washington.
- Suttle, K.B., Power, M.E., Levine, J.M., and McNeely, C. 2004. How fine sediment in riverbeds impairs growth and survival of juvenile salmonids. *Ecological Applications* 14: 969-974.
- Suttles, W.P. 1974. The Economic Life of the Coast Salish of Haro and Rosario Straits. IN *American Indian Ethnohistory: Coast Salish Indians I*. New York: Garland. Pp 41-570.
- Toonen, Robert J. and Shayne Hughes (2001). Increased Throughput for Fragment Analysis on an ABI Prism ® 377 Automated Sequencer Using a Membrane Comb and STRand Software. *BioTechniques* 31: 1320-1324.
- Willette, T.M. 2001. Foraging behaviour of juvenile pink salmon (*Oncorhynchus gorbuscha*) and size-dependent predation risk. *Fisheries Oceanography* 10 (Suppl. 1): 110-131.

Appendix

INVENTORY OF JUVENILE SALMON DNA SPECIMENS
Fork lengths in mm – Bold Chinook, Italic Probably Coho

<i>Provenience</i>	<i>Date</i>	<i>Gear</i>	<i>Fork Length</i>	<i>Tube</i>
Cowlitz Bay, Waldron	2005 May 15	Beach seine	73	<i>Kw12</i>
			142	Kw13
			117	Kw72
	2005 June 12	Beach seine	69	Kw6
			100	Kw10
			87	<i>Kw15</i>
	2006 July 16	Beach seine	76	Kw2
	2006 Aug 22	Tow net	78	Kw17
			113	<i>Kw18</i>
			95	<i>Kw19</i>
			115	Kw20
			115	Kw21
			107	Kw22
			139	<i>Kw23</i>
			137	Kw24
			123	Kw25
			119	Kw26b
			96	Kw30
			110	Kw31
			95	Kw32
			197	Kw34
			109	<i>Kw39</i>
			110	Kw40
			101	Kw41
			162	Kw42
			100	Kw45
			125	Kw73
			90	Kw54
			113	Kw62
			135	Kw74
115			Kw55	
125			Kw56	
136			Kw57	
107			Kw58	
117	Kw59			
108	Kw60			
118	Kw61			
96	Kw28			

Cowlitz Bay, Waldron	2007 May 26	Tow net	82	Kw69
			85	Kw64
			85	Kw65
			76	Kw66
			95	Kw67
	2007 June 18	Tow net	96	Kw68
Griffin Bay, San Juan	2006 June 18	Beach seine	218	<i>Kw3</i>
			165	<i>Kw5</i>
Hughes Bay, Lopez	2005 July 29	Beach seine	103	Kw16
	2006 Aug 24	Tow net	83	Kw33
			120	Kw35
			103	Kw36
			105	Kw37
			108	Kw38
			92	Kw43
			100	Kw44
			138	Kw46
			65	Kw47
			102	Kw48
			132	Kw49
			111	Kw50
			104	Kw51
			88	Kw52
110	Kw53			
Jones Island	2005 July 27	Beach seine	210	<i>Kw7</i>
			118	Kw14b
Severson's Bay, Waldron	2005 June 12	Beach seine	79	<i>Kw1</i>
Shoal Bay, Lopez	2007 May 24	Tow net	76	Kw63
Spirit Cove, San Juan	2006 June 18	Beach seine	86	Kw4
Watmough Bight, Lopez	2005 July 29	Beach seine	114	Kw9
Zora's Cove, Shaw	2005 July 28	Beach seine	95	Kw8
			118	Kw11

Figure 1

Origins of Juvenile Chinook in San Juan County: Sampling Stations (2005-2007)

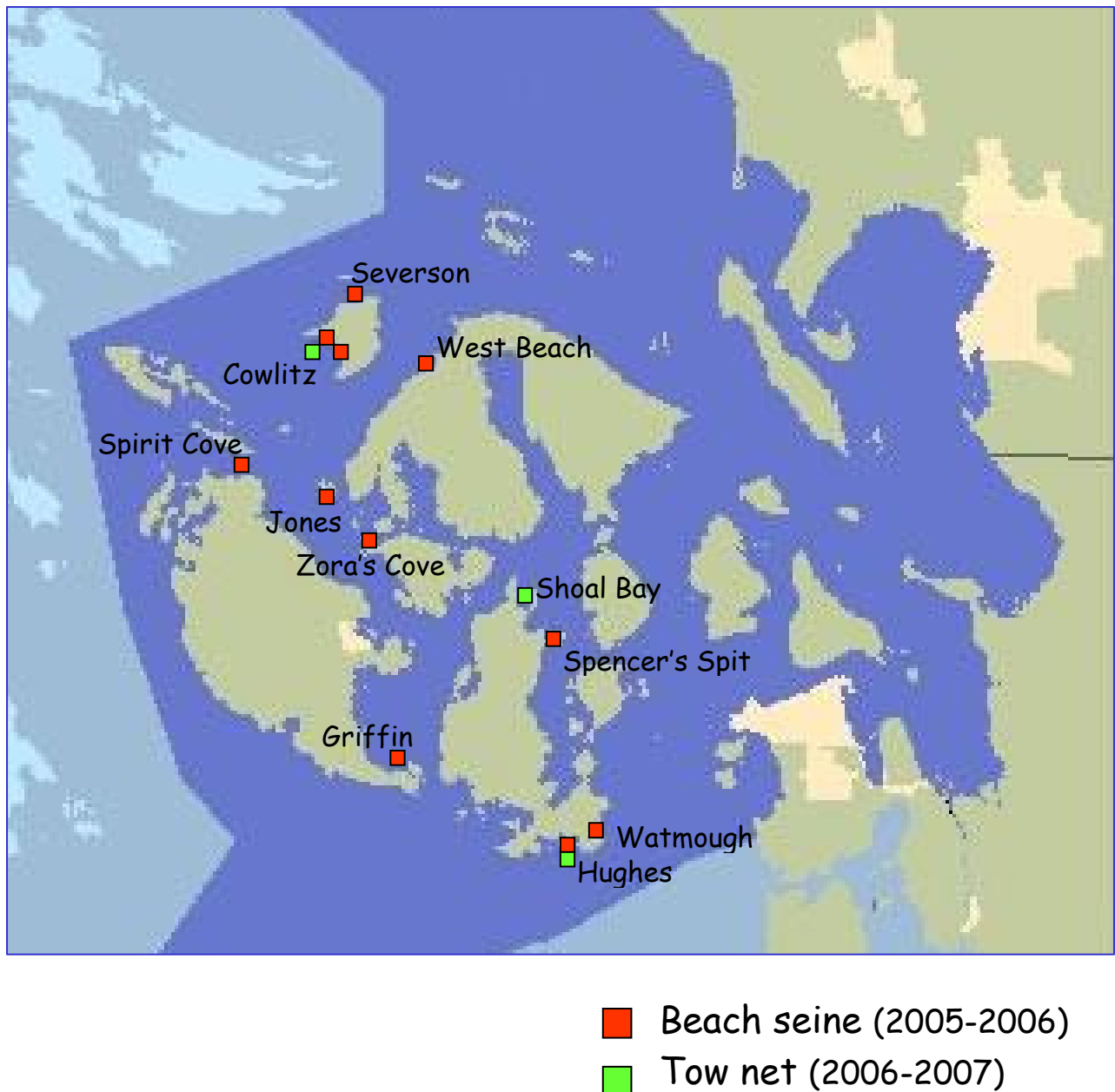


Figure 2

Origins of Juvenile Chinook in San Juan County:
Fork lengths of juvenile salmon in mm
by areas and gears (2005-2007)

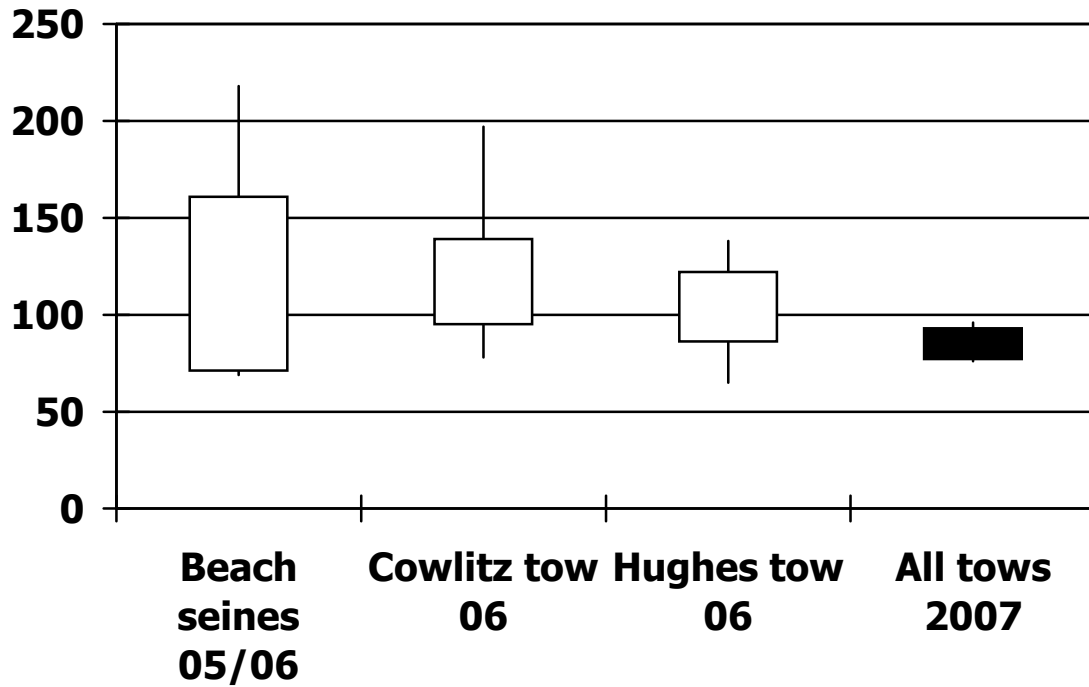


Figure 3

**Origins of Juvenile Chinook in San Juan County:
 Juvenile salmon specimens
 by areas and gears (2005-2007)**

<i>Group</i>	<i>Collection Site</i>	<i>Date</i>	<i>Method</i>	<i>Salmon</i>	<i>Chinook</i>
Northern Aggregation	Cowlitz Bay	May 05	Seine	3	
		June 05	Seine	3	2
		July 06	Seine	1	1
		August 06	Tow	32	1
		May 07	Tow	5	
		June 07	Tow	1	
	Severson's Bay	June 05	Seine	1	
	Spirit Cove SJI	June 06	Seine	1	1
Interior Channels	Jones Island	July 05	Seine	2	
	Zora's Cove, Shaw	July 05	Seine	2	2
	Shoal Bay, Lopez	May 07	Tow	1	
	Griffin Bay, SJI	June 06	Seine	2	
Southern Aggregation	Hughes Bay	July 05	Seine	1	1
		August 06	Tow	15	1
	Watmough Bight	July 05	Seine	1	

Figure 4

**Origins of Juvenile Chinook in San Juan County:
Assignments of Chinook specimens to populations
based on the 13 PSC-CTC microsatellite loci**

<i>Collection site</i>	<i>Date</i>	<i>Method</i>	<i>Region</i>	<i>Population</i>	<i>P</i>
Cowlitz Bay	June 05	Seine	WHIDBEY	NF Stillaguamish	0.9957
	June 05	Seine	FRASER	Lower Thompson	0.9844
	July 06	Seine	FRASER	Nechako	1.0000
	Aug 06	Tow	FRASER	Lower Thompson	0.9064
Spirit Cove SJI	June 06	Seine	WHIDBEY	Skagit-Suiattle	0.9777
Zora's Cove, Shaw	July 05	Seine	FRASER	Chilliwack	0.9998
	July 05	Seine	WHIDBEY	NF Stillaguamish	0.8640
Hughes Bay, Lopez	July 05	Seine	FRASER	Chilliwack	0.9999
	Aug 06	Tow	HOOD CANAL	George Adams	0.9998

Figure 5

Origins of Juvenile Chinook in San Juan County:
Watersheds of origin of juvenile Chinook
Mapped by sampling sites

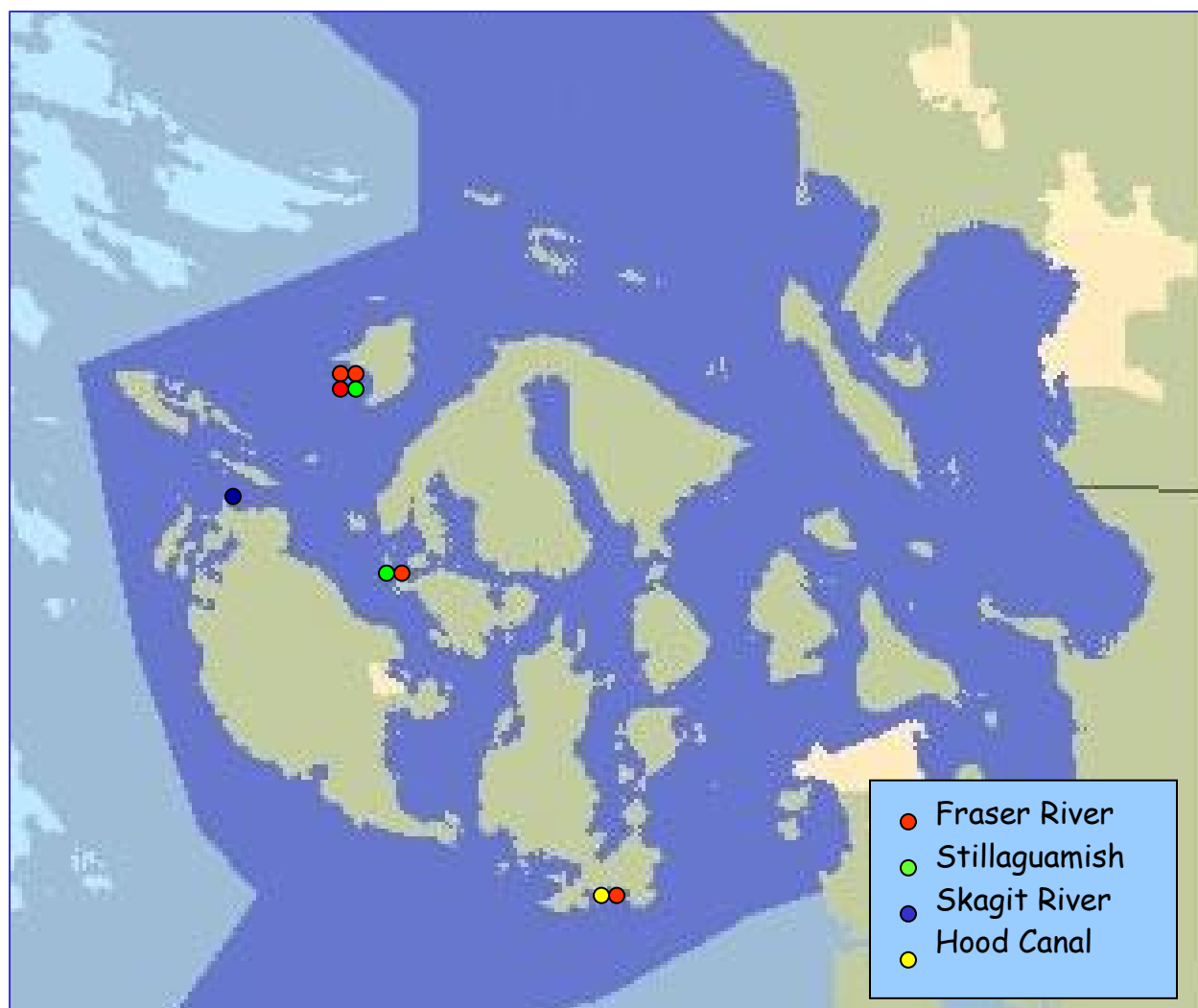


Figure 6

Origins of Juvenile Chinook in San Juan County:
Watersheds of origin of juvenile Chinook
mapped by inferred migration routes

