

Exxon Valdez Oil Spill
Restoration Project Final Report

Using Otolith Chemical Analysis to Determine Larval Drift of
Prince William Sound Pacific Herring (*Clupea pallasii*)

Restoration Project 060782
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Study History: A Detailed Project Description (DPD), Using Otolith Chemical Analysis to Determine Larval Drift of Prince William Sound Pacific Herring (*Clupea pallasii*) was submitted to the *Exxon Valdez* Oil Spill Trustee Council (EVOS-TC) in 2005. Following review by the Trustee Council, Project 060782 was approved in 2006. Otoliths were removed from fish collected in an earlier project (SEA project) and used for otolith chemical analysis to identify larval drift of Pacific herring in PWS. Laser chemical analysis was performed at the University of Alaska Advanced Instrumentation Laboratory. Annual report was turned in September 2006.

Abstract: Pacific herring (*Clupea pallasii*) is an ecologically, culturally and economically important species in Prince William Sound, Alaska. Following the *Exxon Valdez* oil spill in 1989, this stock of Pacific herring has not recovered to pre-oil spill abundance. To understand the habitat use of larval and juvenile Pacific herring in Prince William Sound, the isotopic signatures of otolith cores and edges, representing natal and nursery areas, were compared to determine the relative contributions of recruits from different spawning regions to juvenile rearing bays. There were two significant regional isotopic groups of otolith signatures identified between the inner to the outer nursery bays and this pattern of isotopic signatures persisted over the three year sampling period. The contributions of spawning regions to nursery bays were not equivalent. Further analysis of the 1996 Pacific herring signatures indicated that a third isotopically unique spawning region of Prince William Sound contributes much of the larvae to nursery bays. Identifying and protecting critical fish habitat at vulnerable life stages is vital to the recovery plans of fisheries; continued research into the natural tags of Pacific coastal fisheries could aid in developing effective fisheries management policies.

Key Words: Pacific herring, Prince William Sound, Otolith Chemistry, Larval Drift

Project Data: *Description of data:* Data collected during the course of study included Pacific herring age, otolith microchemistry profiles from spawning site chemical signature, and nursery chemical signature. *Format* - All electronic data (e.g., age and otolith microchemistry profiles) are maintained in Excel spreadsheets and Word text documents. *Custodian:* Nate Bickford, University of Alaska, Fairbanks, Alaska 99705, email: Nate@sfos.uaf.edu.

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Introduction

Pacific herring (*Clupea pallasii*) (Valenceinnes 1847) is an important species ecologically, culturally and economically. This forage fish is preyed upon by marine mammal, birds, invertebrates, and fish (Rooper et al. 1999) through out their lifecycle. They have been an important subsistence resource to native users (Brown et al. 2002) and fished commercially through out their range, from the Bering Sea to California (Mecklenburg et al. 2002). The commercial herring fishery in Prince William Sound (PWS) Alaska (Figure 1) began in the early 1900's (Brown et al. 2002) and had an average value of \$5.9 million dollars from 1978 through 1988 (Ashe et al. 2005). The toxic effects of the 1989 *Exxon Valdez* oil spill on Pacific herring caused the fishery to be closed in 1989. Following the closure of the fishery the abundance of herring continued to grow to a peak of 90,000 mt in 1992 (Ashe et al. 2005). The stock again collapsed in 1993 due to viral hemorrhagic septicemia virus (VHSV); the fishery was closed from 1994-1996 (Marty et al. 2003). There has been no herring fishery in PWS since 1998. The herring stock was expected to recover in 1999 (Morstad 1999) but instead it crashed once again.

The recovery of this commercial and subsistence fishery is a goal of the 1994 Restoration Plan. The Exxon Valdez Oil Spill Trustee Council (EVOS) has identified Pacific herring as not recovered to a healthy and productive state and the Council stated herring does not exist at pre-spill abundance. As communities are dependent on the herring fishery, the Trustee Council has made herring a priority for recovery. The mechanisms impeding herring recovery in PWS are not well understood. The Sound Ecosystem Assessment (SEA) study focused on the ecological factors affecting the recovery of Pacific herring (Cooney et al. 2001). The survival of herring through age-1 is influenced most significantly during the larval drift period (Norcross et. al 2007) as newly hatched herring larvae are advected from spawning grounds to nursery bays. The validation of a tool describing the potential transport of herring larvae from spawning sites to nursery areas would aid researchers in understanding the recovery status of herring and achieve the goals of the 1994 Restoration Plan.

The SEA project initiated an integrated multi-investigator ecosystem approach to study PWS from 1995-1998 (Cooney 1999; Cooney et al. 2001). SEA had a component

identifying impediments to recruitment during the early life history stages of Pacific herring (Norcross et al. 2001). A three dimensional numerical ocean current model for PWS (3D-PWS model) was developed as a tool to study the potential transport of herring larvae from spawning sites to nursery areas (Norcross et al. 2001). Movement pathways due to advection and larval drift can be determined by retrospectively examining the chronology of otolith chemistry. To date there has been no way to validate correspondence between spawning and nursery locations for herring in PWS. The 3D-PWS model has yet to be validated by sampling larvae in the field. Validation of this model demands that herring larvae be physically tracked from the natal origins to fjords and compared to the 3D-PWS model. Traditional mark-recapture techniques (e.g. mark recapture) are ill-suited to this task, while otolith elemental signature analysis offers a researcher a means of identifying the temporal and spatial movements of larval and juvenile herring. The identification of elemental signatures from natal spawning areas could validate a 3D-PWS model. Survival of herring in the individual rearing bays of PWS is not equal (Norcross et al. 2001); therefore, validation of the 3D-PWS model can help researchers understand the mechanisms impeding herring recovery. Through otolith chemical analysis, the spatial and temporal description of where herring spend their early life history can be described.

The lifecycle of Pacific herring in PWS is conducive to otolith chemical analysis. Herring are demersal spawners that substrate in subtidal waters to fertilize and deposit their eggs (Norcross et al. 1996). Four-year-old adult herring migrate in late March to spawn on 23 – 168 km of coastline in PWS (Norcross et al. 2001). Spawning in mid-April, much of the herring eggs are lost to predation, wave-action, and exposure (Rooper et al. 1999). The surviving herring eggs incubate in these spawning areas for about 24 days before hatching as larvae in May (Brown et al. 1996). After herring larvae are advected from spawning areas, the larvae drift through PWS pushed by surface currents, density changes, and meteorological forces (Brown et al. 1996). Metamorphosis of the larval herring begins to occur in June of that same year (Stokesbury et al., 2002). The herring become nektonic and swim to favorable habitats and are no longer at the mercy of the currents. In August, the young herring begin to form schools and aggregate at the heads of bays far from coastal waters (Brown et al 2002; Stokesbury et al. 2000). These

populations stay isolated in their respective nursery bays until June of their second year (Stokesbury et al. 2000). At that time this cohort of herring leaves the bays and joins adult schools (Stokesbury et al. 2000).

PWS has numerous spawning and nursery areas. Located in the northern most portion of the Gulf of Alaska, PWS is a small inland sea (Muench & Heggie 1978) measuring 60 km wide and 90km long and depths can exceed 700 m. Elevation rises from sea-level to 4000 m within 60 km of the shore. Orographic interactions between the Aleutian low and the coastal mountains causes high levels of precipitation (Weingartner 2007), up to 5 m per year from 1995-1998 (Gay & Vaughan 2001). The numerous fjords, islands and mountains support tidewater and alpine glaciers which are seasonal sources of freshwater in PWS. The sound is connected to the Gulf of Alaska via Hinchinbrook Entrance (HE) and Montague Strait (MS). The Alaska Coastal Current (ACC) and Ekman transport supply GOA water into PWS in the fall, winter, and spring seasons. The flow in PWS is counterclockwise entering through HE and exiting through MS. Coastal downwelling occurs seasonally. As the winds from the Aleutian low subside in the summer, Ekman transport is weakened and there is deep water inflow from the outer shelf. The inflow of deep water into the sound is also a source of oceanic animals and nutrients into the sound. The counterclockwise surface circulation can reverse in the summer as surface water enters through MS and exit through HE. The seasonal precipitation, weakened downwelling favorable winds, and increased sunlight cause stratification and highly productive conditions.

Throughout the life of a herring, as it migrates among PWS fjords and bays, the trace element content of the water is recorded in the otolith. This creates a permanent record of habitat use by an individual fish. Otolith bands are accrued during the fish's time of residence in the spawning areas, thus recording the unique spatial chemical signatures. Otoliths are single cellular crystalline deposits of CaCO_3 , in the form of aragonite, within an otolin-1 protein matrix. There are three calcified otoliths structures found in teleosts; the sagittae is the largest and most studied (Wright et al. 2002). Otoliths are formed in the latter part of the egg stage. The initial deposition of material becomes the core of the otolith (Wright et al. 2002). As the juvenile herring grows it accretes bands of new material, which surrounds its original core deposit. Daily bands

and yearly bands are accrued as layers. The edge portion of the otolith represents the capture location of the fish. Growth is recorded as assorted bandwidths inside the otolith, much as a tree accumulates annual rings. The daily and annual bands (annuli) have long been used as detectors of age and growth rate in fish (Campana & Therrold 2001; Wright 2002). Otolith tissue is not reabsorbed by the body, as other calcified tissues are; it is this quality that makes otoliths unique in fish (Campana 1999). Otoliths, unlike other calcified tissues such as skeletal calcium, are not readily mobilized for homeostasis during times of stress; consequently otoliths are highly suitable for aging (Wright 2002) and chemical analysis (Campana 1999). Otoliths also continue to accrete after somatic growth has naturally ceased (Mugiya & Tanaka 1992) unlike skeletal tissue. In recent years the chemical compositions of individual bands have been used to identify past habitat use of the fish (Rooker et al. 2003; Campana & Therrold 2001; Thresher 1999). During crystallization, divalent cations of similar ionic radii to calcium (e.g., Mg^{+2} , Sr^{+2} , and Ba^{+2}) can substitute for calcium in the otolith matrix or in the protein in the otolith (Campana et al. 1995). The mechanism of substitution and incorporation of trace metals into the otolith are a function of abiotic (i.e., temperature, salinity) and biotic (i.e., diet, fish growth rate) conditions (Thresher 1999).

The objective of this study was to identify the past habitat use of juvenile herring collected during the SEA study. We identified and classified habitat use during spawning and during nursery residence. These data will then be used to examine the possibilities of validating the 3D-PWS model and describing the movements of larvae in PWS.

Methods

Juvenile herring were collected during the Sound Ecosystem Assessment (SEA) project from 1995-1997 (Norcross et al. 2001; Cooney et al. 2001; Cooney 1999). The samples (n=626) that survived nearly a decade of storage were used in this investigation but are not representative of the total samples collected during the SEA project. The herring samples used in this study were not collected for otolith analysis. These samples represent different collection months and locations vary from year to year for most bays. The surviving samples from these collections represent an unbalanced design in both time and space. Pacific herring were collected from the nursery areas within Prince William

Sound Alaska. Juvenile herring samples from collections in Dangerous Passage, Eaglek Bay, Green Island, Jack Bay, Port Nellie Juan, Paddy Bay, Rocky Bay, Simpson Bay, Whale Bay, and Zaikof Bay (Figure 1) remained in cold storage (-18°C) in the Fisheries Oceanography Laboratory at UAF (Table 1&2). Biological data exist for all samples but physical oceanographic data exists for only the four heavily sampled bays (Eaglek Bay, Simpson Bay, Whale Bay, and Zaikof Bay). These nursery bays vary in physical characteristics such as: basin area, drainage area, depth, salinity, temperature, and trace element composition (Cooney 1999).

Sagittal otoliths were extracted from intact frozen juvenile Pacific herring. The otoliths were extracted from herring in a clean environment using standard techniques (Bickford & Hannigan 2005; Campana 1999; Campana et al. 1995). All juvenile samples were analyzed using identical procedures. All tools used for extraction were made of Teflon and were acid washed prior to use to minimize contamination. Thin sections were cut laterally across the otolith using a Beuhler isomet low speed saw to expose the core and edge portions of the otolith. The otoliths were mounted onto slides with crystal-bond thermal glue and polished. Each otolith's annuli were counted with an optical microscope. A year class was assigned to each sample based on the age of fish and the year the fish was captured. Quality control of age estimates was accomplished by comparing the estimates of three otolith readers. Age estimates were accepted only after 95% agreement was reached among all otolith readers.

Trace element analysis conducted on the Laser Ablation (LA; New Wave UP 213nm Nd:YAG) Inductively Coupled Plasma Mass Spectrometer (ICP-MS; Agilent 7500c) at the Advanced Instrumentation Facility on the UAF campus. Standard laser settings were used for all samples: laser spot size 25µm; 10hz. A line across the core and along the edge of the otolith was ablated and data were recorded. The isotopes ⁸⁶Sr, ⁸⁷Sr, ⁸⁸Sr, ²⁴Mg, ¹³⁷Ba, ¹³⁸Ba, ⁴⁴Ca, and ⁴⁸Ca were assessed for relative abundance per sample and calibrated to a standard of known composition (National Institute of Standards and Technology NIST 610) to limit the error associated with instrument drift over a sampling period of 1-4 hrs using GEO Pro™ v1.00 (CETAC technologies 1999). This software corrected for instrument drift by assuming a linear relationship between the standard of known consistency and the otoliths. A set of three replicate standards was run at the

beginning of each data recording session, and a single standard was run after every ten samples. A gas blank was subtracted from each sample data and peak isotopic counts were integrated through time and calibrated to the abundance of ^{44}Ca in the standard. Calibrated and corrected counts for the isotopes ^{88}Sr , ^{24}Mg , and ^{138}Ba were normalized to ^{48}Ca as a ratio. As a ratio, the isotopes ^{87}Sr and ^{86}Sr were used as an additional explanatory variable (Barnett-Johnston et al. 2005).

The software programs PRIMER v6 (Plymouth Routines in Marine Environmental Research) (Clark 1993) and SAS v9.1™ (Barr & Goodnight 1971) were used to analyze the processed data in order to make inferences about larval drift in PWS. The assumptions of ANOVA, i.e., linearity, normality, homogeneity of variance, and absence of outliers, were examined for each variable. The Kolmogorov-Smirnov (K-S) test for normality was chosen as it was most accurate when dealing with sample sizes greater than 100 (Lilliefors 1967). Isotopic ratio data were tested for normality. No outliers were removed from these data.

A stepwise discriminant analysis (Fisher 1936; Rencher & Larson 1980) was used to identify the best set of isotopic data to classify samples to a region. Principal components analysis (PCA) (Chatfield & Collins 1980) was used to reduce the dimensionality of the data. These two components maximize the variance of these multivariate data in two directions of orthogonal space. The principal components are uncorrelated indices of the regional signatures created from combinations of the isotopic data in such a way that PC1 explains more variation than PC2 (Manley 1994).

Both univariate and multivariate statistical techniques were used to test for the sources of variance in the data. Factors were initially compared using one-way ANOVA ($\alpha=0.05$), because there were too many missing samples to do two-way ANOVA. These data were explored to identify the significant sources of variance among the independent variables: management area, regional groups, capture bay, capture year, and year class. Tukey-Kramer honestly significant differences (HSD) post hoc test was used to identify the significantly different instances within capture bays in regards to management area, regional groups, year class, between capture bays, and among bays with multiple capture years.

PRIMER v6 multivariate techniques were applied based on the similarity coefficients calculated between all pairs of juvenile otolith samples to identify significant similar regional signatures in otolith edge composition (Mantel 1967; Clark & Green 1988; Clark 1993; Gillanders & Kingsford 2000). The classification of clusters and calculation of ordination plots correspond to the relative differences in trace element composition within and between capture regions. A combination of hierarchical cluster analysis (HCA) (Everitt 1980), non-metric multi-dimensional scaling (MDS) plots (Kruskal & Wish 1978), and a similarity profile permutation test (Clark 1993) were used to identify statistically significant regional clusters of similar otolith signatures within PWS. Dendrograms and ordination plots were used to illustrate the statistical similarities of replicate samples within regional clusters. Regional groups were assigned as replicates with similarities within a site that were greater than those of replicates between groups. MDS was used to visually display these data and to confirm the results of the HCA.

Quadratic discriminant function analysis (QDFA) (Cover & Hart 1967) was used to discriminate among nursery ground signatures and to assign group membership to natal signatures. QDFA does not assume equal covariance and was applicable to these data (Manley 1994; Gillanders & Kingsford 2000). A cross-validation test was used to validate the QDFA by quantifying the correct classification of samples of known origin (edge). The core signatures were classified to regions having a similar elemental signature found in the edge. All edge data were pooled by regional group. All core signatures were individually classified to a regional group. The QDFA was restricted to the 1996 capture year herring and the classification was limited to the 1996 year class fish, excluding the 1995 (n=178) and 1997 (n=173). During this sampling year more samples survived enabling the QDFA to classify samples with a higher spatial resolution. The contributions of larvae from spawning areas and the amount retained in each region were calculated for each of the sampled nursery bays.

Results

There was a significant latitudinal gradient in habitat use in PWS. Regional signatures were split into two significant regional groups ($p < 0.001$) for 1995, 1996 and

1997. However, in 1996 three significant groups ($p < 0.05$) were identified along a gradient from the head to the mouth of PWS. As a result, the contributions of spawning regions to juvenile rearing bays could be quantified.

There were significant differences in the edge signatures of juvenile Pacific herring otoliths among capture bays, among capture years, by year class, between regional groups, and among management areas when all of the data was pooled (Table 3). When the analysis was restricted by the capture year, the capture location was highly significant ($p < 0.001$) (Table 4). The 1995 year class ($n=14$) and 1997 year class ($n=27$) from the 1997 Whale Bay sampling event were significantly different for PC2, but over all year-class differences were not different within bays each year (Table 5). The regional groupings were significantly different from each other when the analysis was done by pooling all capture years. The regional signatures of all bays sampled in multiple years varied from year to year (Table 6). Eaglek Bay was significantly different from Simpson Bay, Whale Bay, and Zaikof Bay for PC1 in 1995, 1996, and 1997. PC2 indicated significant differences among the bays sampled only in 1996: Dangerous Passage, Nellie Juan, Paddy Bay, and Rocky Bay. All bays sampled in multiple years, have significant temporal differences in that their elemental signature, but follow the same isotopic patterns between bays each year.

Similar regional patterns were found for all of the sampling years in PWS. Regional separation among the edge signatures of Eaglek Bay and the edge signatures of Simpson Bay, Whale Bay, and Zaikof Bay was found for the years 1995, 1996, and 1997 ($P_i: 0.08$; $p=0.020$; Figure 2). Multi dimensional scaling (MDS) and principal component analysis (PCA) ordination displayed similar spatial and temporal split in the regional signatures in PWS (Figure 3 & 4). The regional elemental signatures were split along a roughly north (Jack Bay, Galena Bay, Eaglek Bay, Nellie Juan, Paddy Bay, Dangerous Passage, Rocky Bay, Green Island) to south (Simpson Bay, Whale Bay, Zaikof Bay) gradient. In 1996, Nellie Juan was intermediate between the two groups in both MDS and PCA space. The same pattern in regional signatures is seen through out the individual capture years despite the number of bays sampled each year.

The regional signatures of the samples of known origins (edge) and the samples of unknown origins (core) were classified with the QDFA model. Of the 275 herring

captured in the northern region, 93% were correctly classed by QDFA and of the 351 herring captured in the southern region 82% were correctly classed. The class assignment of core signatures (n=646) revealed 73% of herring captured in a northerly region had core signature matching the edge signatures. Similarly, 75% of herring caught in the southern region were hatched within that region. The amount of fish capture in the same region as they were hatched was not equal for each bay within a regional group: in the northern region, Dangerous Passage (93%), Galena Bay (90%), Paddy Bay (88%), Rocky Bay, (88%) Eaglek Bay (80%), and Green Island (44%); in the southern region, Whale Bay (97%), Zaikof Bay (99%), Simpson Bay (25%), and Nellie Juan (77%).

Because the 1996 sampling year had greater sample resolution and revealed three distinct groupings, data from all sample years were assigned to these three regional groupings. These groupings were: Group A (Eaglek Bay, Galena Bay, Jack Bay, and Green Island); Group B (Dangerous Passage, Nellie Juan, Paddy Bay, and Rocky Bay); and Group C (Simpson Bay, Whale Bay, and Zaikof Bay). Of the 212 herring in Group A 65% were correctly classified to Group A, 75% of Group B were correctly classified to Group B edge (n=89), and 80% correct classification of Group C (n=325) (Table 8). 15% of fish collected in Group A hatched in the Group A region. Similarly Group B (70%) and Group C (67%) retained larvae but at a higher percentage. The core signatures from Group A were predominantly from other regions in PWS. For each bay outside of Group A, a core signature of indicating a Group A origin was found in 0%-18%. The regional signatures of other groups were more abundant in the core signatures of the herring samples: Group B (6%-85%) and Group C (6%-58%) (Table 9).

The 275 herring samples captured in 1996 were analyzed individually for edge signature. The regional groups were classed and the success rates were recorded (Table 10). The classification of core signatures for this one sample year also resulted in Eaglek Bay retaining the least amount of larvae from its region and Whale Bay and Zaikof Bay retaining the most. Simpson Bays has 63% of its juvenile core signatures were similar to Dangerous Passage, Nellie Juan, Paddy Bay, and Rocky Bay (Table 11). Group A does not contribute as many core signatures to other regions as it receives from other regions, conversely Whale and Zaikof bays contribute core signatures to other regions but do not receive many from other regions in the PWS. Larval retention was calculated for each

region and for the two grouping schemes for each sampled year 1995-1997: Northerly (73%) Southerly (75%); Group A (15%), Group B (70%), Group C (67%). When the 1996 year class fish were classified to 1996 edge signature grouping the larval retention was quantified: Group A (14%), Group B (N/A), Group C (48%). In 1996 the spawning grounds from the middle and southerly latitudes of PWS contributed the most larvae to other a bays through out the sound.

Discussion

This study shows that otolith chemical techniques can be applied to the past habitat use of herring in PWS. The otolith chemical analysis demonstrated that larvae advected from spawning grounds are not distributed randomly to PWS nursery regions. There is a gradient in the otolith signatures from the mouth to the head of PWS. This study has quantified the contributions of spawning regions to juvenile nursery areas. . Movement of larval from spawning areas to nursery areas can be compared to larval drift models.

Larval retention in a spawning area can affect a species ability to disperse spawn through out a range. Larvae can maintain their locations by migrating vertically in the water column daily and being passively carried by currents (Sinclair & Iles 1985) or larvae can be retained in bay by favorable physical oceanographic conditions such as fronts (Iles & Sinclair 1982). As in similar studies (Swearer et al. 1999; Gillanders & Kingsford 2000, Forrester & Swearer 2002; Gillanders 2002) identifying the nursery signatures of juvenile herring has enabled this study to quantify a spawning regions contribution to the juvenile members of each nursery bay and to quantify larval retention for each bay. The similarity of the core chemistry to the regional edge signature implies that the fish either were spawned at the same location as the edge signature or the water in the two areas was similar (Stransk et al. 2005). Larval retention was defined by the similarities of core signature to an edge signature. This study indicates that spawning grounds outside of the four intensively studied bays, Eaglek Bay, Simpson Bay, Whale Bay, and Zaikof Bay disperse larvae to more nursery bays.

The herring otolith data supported many of the conclusions about larval transport in PWS proposed by McGurk et al. (1990) but these otolith data do not support many of

the conclusions of the 3D-PWS model (Wang et al. 2001). Both McGurk et al. (1990) and these otolith data show that larval drift and retention are not equal for each bay. The conclusion that advection of larvae from northern site was less than from southern sites (McGurk et al. 1990; Brown 2003) is also inferred from these otolith data. These data also support McGurk et al. (1990) conclusion that southern transport of larvae would dominate northern bays.

These otolith data cannot confirm the conclusion of the 3D-PWS model (Wang et al. 2001; Brown 2003) pertaining to larval drift timing and transport into the central gyre 'trap', but the 3D-PWS model's conclusion about larval retention of spawn in PWS may not be as simple as the model predicts. These otolith data show that larvae was retained at the highest rates in the southern portions (Whale Bay, 100%; Zaikof Bay, 89%) of PWS and that northern areas (Eaglek Bay; 14%) retained the least amount of spawn in 1996, which is contrary to the 3D-PWS model prediction that 4.9% retention in the southeast and 40.2-53.8% retention in the northern regions. The otolith data reveals the relative contributions of spawning regions to a juvenile bay not the abundance of larvae advected from a region lost to early life history mortality.

The regional elemental signatures have the highest spatial resolution in the 1996 sampling season due to collection efforts at many different bays. The otolith data show that larvae from Group B, intermediate region between the northern and southern regions, contribute the most juveniles to both Eaglek Bay (70%) and Simpson Bay (63%) and that juveniles originating in southern regions tends to stay in the south (Whale Bay, Zaikof Bay). No otolith data is available to describe the origins of the juveniles captured in the Group B region in 1996, but when all years were pooled 70% of the juveniles from Group B remain were from Group B. The Wang et al. (2001) model was only run for the 1996 year conditions. Future model confirmation studies should include a broad sampling effort to increase spatial resolution to confirm a model's validity over time.

Increase samples from a broad range of bays in PWS would enable higher spatial resolution in the regional signatures in PWS. The spatial resolution is unclear because samples were pooled from more than one nursery bay and have spatial scales that may not be easily quantified. The regional signatures within the bays of PWS may vary, as demonstrated in reef fish where otolith chemistry can varied over spatial scales on the

order of hundreds of meters (Dove et al. 1996; Patterson et al. 2003). It is possible that variation on scales smaller than regions leads to inappropriate interpretations of otolith signatures (Gillanders & Kingsford 2003). Future studies in PWS should look at spatial and temporal variability within the nursery bays (e.g. Miller 2007) as well as between them.

Larger sample sizes are needed because there is physical and chemical variability in the four heavily sampled bays within PWS that can affect the regional signatures of herring otoliths. As part of a subarctic watershed PWS experiences seasonality in its thermal, chemical and physical properties (Weingartner 2007). The two deepest fjords (Whale Bay and Zaikof Bay) did not experience deep mixing in the winter time during this study. Surface waters were cold (1-3° C) and fresh (29-30) while the subsurface waters were warmer (5-7° C) and saltier (31-31.5° C). The shallower bays (Eaglek Bay, and Simpson Bay) experienced deep winter mixing and had warmer temperatures (3.5-5.5° C) and higher salinities (31-31.5) throughout the water column. Temperature, salinity and freshwater input to the seawater can influence the otolith composition of fish on nursery grounds (Gillanders & Kingsford 2000; Martin et al 2004). Natural variation among regional signatures is likely to fluctuate due to salinity and temperature seasonally (Bath et al. 2000; Gillanders 2002; Martin & Thorrold 2005). The concentrations of isotopes within an otolith and the ambient concentrations vary significantly in saltwater (Kalish 1989; Secor 1992; Chesney et al. 1998; Gillanders & Kingsford 2000).

Variation in temporal signatures can distort spatial resolution of otolith chemical analysis (Gillanders 2002), therefore sampling efforts should be modified in future research. Edge isotopic variability can be influenced by interannual variability in the environment or by fish occupying a different habitat within the past few months (Stransk et al. 2005). Although juvenile herring remain isolated in nursery bays for two years (Stokesbury et al. 2002), the horizontal and vertical scales by which the nursery bay habitats vary seasonally are not clear. During the summer months Eaglek, Simpson, Whale and Zaikof Bays experience strong stratification (Gay & Vaughan 2001), which can affect the otolith chemical signature especially because juvenile herring have diel migrations through the water column (Henri et al. 1985; Munk et al. 1989). Changes in

the amount and composition of fresh water run off changes seasonally and can affect the chemical composition of juvenile fish otoliths (Chesney et al. 1998; Gillanders 2002).

Physical, chemical, and thermal characteristics vary through time, future samples should be collected at the same time and in higher numbers to increase resolution and improve the inferences about spawning grounds, larval drift, and larval retention. Future studies in PWS should focus on similar months or seasons to limit the temporal variation in the data. The grouping of nursery bays could possibly differ from year to year due to the physical, chemical and thermal variation in the sound, but with continued sampling efforts one could infer, through statistical relationships, consistent regional signatures (Gillanders 2002). The absence of samples limited our ability to make inferences about the consistency of regional signatures over time, but the same patterns in regional signatures persisted through the sampling years.

The year class differences in otolith signatures can be eliminated from future studies by targeting juvenile herring of the same year class. Some significant differences were found in the regional signatures of otoliths from different year classes. The age-2 herring, from the 1995 year class, had different otolith growth rates than younger fish, age-0, as is typical of teleost fishes (Wright 2002). This ontogenic effect can affect the trace element chemistry of an otolith and may be a source of variation in regional otolith signatures (Fowler et al. 1995; Thresher 1999). Edge areas vary in fishes due to changing growth rates especially in the replacement of Sr for Ca in the otoliths of juvenile fishes (Campana 1999). Sea surface temperature has a direct effect on growth rate and incorporation of trace elements: Sr and Ba (Gillanders 2002; Martin & Thorrold 2005). Samples collected during the SEA study represented juveniles of ages-0, -1 and -2. Regional signatures are not easily obscured by ontogenic effects, but all ontogenic effects on otolith composition can be minimized by analyzing fish of same age (Gillanders & Kingsford 2000; Gillanders 2002; Miller 2007).

Conclusions

Regional differences in isotopic signatures were found for all juvenile herring in PWS. This signature persisted through the sampling years enabling this study to identify spawning regions and quantify both larval contributions and larval retention in PWS.

These data cannot validate any larval drift model. Future otolith sampling efforts in PWS can validate a larval drift model.