Nisqually River Estuary Baseline Fish Ecology Study: 2003-2006

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Chum salmon captured in the Nisqually River estuary.

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Executive Summary

The restoration of the Nisqually River estuary is recognized as the highest priority action item for the recovery of federally listed threatened Nisqually Fall Chinook in both local and regional recovery plans. To date, the Nisqually Indian Tribe has removed dikes and restored tidal influence to over 150 acres on the east side of the Nisqually River and the Nisqually National Wildlife Refuge plans to restore another 700 acres in the near future. Restoration of this magnitude creates a unique opportunity to study the effects of restoration on juvenile Chinook salmon and other fishes. The purpose of our research was to: (1) Establish a baseline of fish ecology in the Nisqually River estuary complex by determining spatial and temporal patterns of distribution and abundance of salmonids and other fishes. (2) Develop specific hypotheses about the influence of estuary restoration on juvenile salmonids and other fishes by assessing the ecological performance of specific estuary restoring and reference habitats.

We completed over 980 beach seine sets spread out over 26 months between 2004-2006 and over 70 fyke trapping events at three blind channel sloughs from 2003-2006. In addition to identifying and enumerating all of the fishes captured, the diets and otoloths of hundreds of Chinook were analyzed and coded wire tags (CWT) were collected from tagged hatchery Chinook in order to determine hatchery of origin. The sampling provides us with a working 'template' of fish ecology in the Nisqually River, estuary, and adjacent nearshore that includes general community composition, temporal and spatial distribution, hatchery and unmarked Chinook co-occurrence, Chinook salmon prey composition, wild Chinook salmon residence time and growth in the estuary derived from otolith microstructure analysis, and non-natal Chinook use of Nisqually estuarine habitat. In addition, by assessing juvenile Chinook use of restored and reference blind channel sloughs using three metrics (opportunity, capacity, and realized function); we can formulate specific hypotheses about the localized functional response of Chinook to structural changes resulting from estuary restoration.

In general, total fish abundance in the Nisqually estuary peaks in May and June. The May peak catch is primarily composed of hatchery Chinook, followed by Pacific sand lance, chum, sculpin, shiner perch, and unmarked Chinook. The June catch is predominantly shiner perch and hatchery Chinook. Shiner perch were the most abundant fish captured in the estuary. Estuary habitat partitioning in space and time is apparent between hatchery Chinook, unmarked Chinook, chum, and shiner perch although considerable overlap does exist. Most chum salmon were caught between April and May, on average earlier than hatchery Chinook, and were most abundant in freshwater, forested, and nearshore zones. Following hatchery Chinook releases in the Nisqually River in May, catch data indicated that the majority of these fish spent little time in the freshwater tidal zones, but that they were caught in high numbers in the saltier zones during May and June, especially in the lower Nisqually River estuarine habitat zones. Unmarked Chinook salmon, which are much less numerous in the system than chum or hatchery Chinook, had a broader distribution in time and were caught prior to, during, and after the period of hatchery Chinook presence most frequently in the zones associated with the Nisqually River. Peak catches of shiner perch occurred in June and July with

high average catches in the more saline McAllister Creek and Red Salmon Slough sub-estuary and mudflat zones.

Microstructure analysis of wild Nisqually Chinook otoliths conducted by the U. S. Geological Survey indicates that those wild Chinook entering the estuary in late May to June may rear in the estuary for over a month, with a conservative average estuary residency of 16 days. Wild Chinook growth rates in the Nisqually River delta averaged 36% higher than in freshwater.

Over 200 hatchery Chinook CWTs were analyzed for hatchery of origin. The majority of Chinook with CWTs were from Nisqually River hatcheries, but over 26% were Chinook tagged at hatcheries outside the Nisqually. The non-natal component of the CWT recoveries points to the regional significance of the Nisqually River estuary for Chinook.

Unmarked and hatchery Chinook responded quickly to the restoration of approximately 40 acres of historic estuary in 2002. Chinook accessed the site less than a year after the dikes were removed. The site produced large numbers of invertebrates, but low overall diversity, and these invertebrates dominated the diet composition of Chinook captured at the restoration site. We used our monitoring data to make testable hypotheses about the functional responses of Chinook and other fishes to the restored processes and structural changes brought about by large scale estuary restoration.

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Acknowledgements

This study was made possible through the shared resources, personnel, and commitment to a restored Nisqually Estuary by the Nisqually National Wildlife Refuge, Nisqually Indian Tribe, and Ducks Unlimited. The following individuals were the backbone of the study, enduring muddy nine month long field seasons in weather ranging from snow to sunburn: Nano Perez, Lucas Meek, Kelly Martin, Meghan Young, and Erin Degenstein. Refuge and Tribe staff critical to the completion of this study were: Jean Takekawa, Jeanette Dorner, Doug Roster, Florian Leischner, Marian Bailey, and Craig Smith. Special thanks go to the staff and volunteers of the Nisqually Reach Nature Center for invertebrate identification and enumeration; Jennifer Cutler for cartography; and Angie Lind-Null, Kim Larson, and Reg Reisenbichler of the U.S. Geological Survey for the Chinook otolith analysis.

Introduction

Background

Restoring Puget Sound river delta habitat is recognized as a priority action for the recovery of Puget Sound Chinook salmon (*Oncorhynchus tshawytscha*) in both regional and local recovery plans (SSDC 2007; NCRT 2001). The approximately 5,000 acre Nisqually River estuary complex represents one of the most restorable river deltas in the region, with most of the land now owned by the U. S. Fish and Wildlife Service Nisqually National Wildlife Refuge (NNWR), the Nisqually Indian Tribe (Tribe), and the Washington Department of Fish and Wildlife (WDFW). The Tribe, the NNWR, and others are aggressively pursuing large scale restoration of the Nisqually River estuary. Using a phased approach, tidal inundation was restored to reclaim approximately 40 acres of diked pasture in 2002 (Phase 1) and an additional 100 acres of pasture in 2006 (Phase 2), both on the east side of the river. The next step will be the restoration of 700 acres of estuarine habitat on the west side of the Nisqually River in the near future (USFWS 2005).

The Nisqually Fall Chinook stock is one of the 27 stocks in the Puget Sound evolutionarily significant unit listed as threatened under the federal Endangered Species Act (NCRT 2001). Chinook salmon rear extensively in estuaries and are thought to be the most estuary-dependent of the Pacific salmonids (Aitkin 1998; Fresh 2006). The estuary is also important to the Nisqually winter chum (*O. keta*), one of the largest wild runs in Washington State (WDFW and WWTIT 2002), which are known to utilize the estuary for feeding and growth (Fresh et al. 1979; Pearce et al. 1982). Puget Sound/Georgia Basin river delta habitat is also important for many non-salmonid fishes and birds like shiner perch (*Cymatogaster aggregata*), starry flounder (*Platichthys stellatus*), great blue heron (*Ardea herodias*), American wigeon (*Anas americana*), and many others (Levy et al. 1979; Simenstad et al. 1991; UFWS 2005; Eissinger 2007). The restoration of the Nisqually River delta ecosystem represents a unique opportunity to study the response of organisms to a recovering system.

The Tribe started conducting limited fish distribution research in the Nisqually River estuary in 2002 and began monitoring the newly restored Phase 1 site in 2003. The fish research effort was expanded in 2004 with a partnership between the Tribe, NNWR, and Ducks Unlimited. This technical report focuses on the research conducted by the partners from 2004-2006, but includes the first year of restoration monitoring by the Tribe in 2003.

Study Purpose and Objectives

The purpose of our Nisqually River estuary fish research was to: (1) Establish a baseline of fish ecology in the Nisqually River estuary complex by determining spatial and temporal patterns of distribution and abundance of salmonids and other fishes. (2) Develop specific hypotheses about the effects of estuary restoration on juvenile salmonids and other fishes by assessing the ecological performance of specific estuary restoring and reference habitats.

The specific objectives for accomplishing purpose 1 were:

1. Monitor changes in the species composition of fishes in the Nisqually River, estuary, and nearshore over time (monthly and annually).

2. Determine the distribution and relative abundance of fishes in the Nisqually River, estuary, and nearshore over time (monthly and annually).

3. Document the estuary residence time, growth, life history diversity, and prey composition of Nisqually River hatchery and unmarked[1] Chinook salmon.

Monitoring specific, small scale, restoring and reference tidal channel habitats informs the design and management of current and planned estuary restoration projects and enables us develop our restoration hypotheses (purpose 2). The ecological performance of estuarine salt marsh habitats were measured at three levels for juvenile salmon: opportunity, capacity, and realized function (Simenstad and Cordell 2000).

1. *Opportunity*- The ability of juvenile salmon to access the habitat and utilize the habitat's capacity. Opportunity was measured by determining the density and timing of salmonid usage of the restoring and reference habitats through fyke trapping.

2. *Capacity*- Habitat attributes that produce conditions favorable to juvenile salmon growth and survival. Capacity was measured by determining the occurrence and abundance of salmonid prey organisms through benthic core sampling, neuston sampling, and insect fallout trapping.

3. *Realized Function*- Measures of juvenile salmon responses resulting from the fish physically occupying the habitat and taking advantage of the sites capacity. The diet compositions of juvenile hatchery and unmarked Chinook were used as a measure to examine the realized function of the restoring and reference habitats.

[1] The term 'unmarked' is used to indicate that these Chinook may be natural origin (a.k.a. wild) fish or hatchery fish that did not receive a distinguishing mark or tag.

Methods and Materials

Study Area

The mouth of the Nisqually River is located in South Puget Sound, approximately 20 miles southwest of Tacoma and 8 miles northeast of Olympia. The study area includes the lower 2 miles of the Nisqually River, the Nisqually River delta complex, and approximately 2.5 miles of adjacent nearshore in both Pierce and Thurston counties including Anderson Island. For a detailed description of the Nisqually Basin please see the Nisqually Chinook Recovery Plan (NCRT 2001) and the Nisqually National Wildlife Refuge Final Comprehensive Conservation Plan and Environmental Impact Statement (USFWS 2005).

Nisqually Habitat Zones and Beach Seine Sites

The Nisqually River delta complex encompasses the estuarine portions of three distinct riverine systems: Red Salmon Slough (RSS), McAllister Creek (MCA), and the Nisqually River (NIS). In order to examine fish use of different estuarine habitats and to compare and contrast regional fish-habitat relationships and Chinook life-history diversity, the Nisqually River delta complex was subdivided into habitat zones based primarily on salinity and vegetation (Table 1), as well as GIS based habitat mapping (Tanner 1999). The habitat zones are: freshwater, forested riverine tidal (FRT), emergent forested transition (EFT), estuarine emergent marsh (EEM), delta mudflats (Flats), and nearshore (adapted from Beamer et al. 2005). Within the nearshore zone one pocket estuary located at Hogum Bay was also sampled. Beach seine sites were distributed throughout the various zones (Figure 1). See Appendix A for a complete list of all beach seine sites.

Table 1. Nisqually habitat zone salinity ranges and general identifying characteristics, with range in number of sites sampled among years.

Habitat Zone	Salinity Range (ppt)	Characteristics	Number of Sites
Freshwater	0.0	Forested slow water habitat on mainstem Nisqually without tidal influence.	1-2
Forested Riverine Tidal (FRT)	0.0 - 0.3	Riparian forest, mud/silt substrate, and tidal influence.	1-2
Emergent Forested Transition (EFT)	0.1 - 2.0	Scrub/shrub and marsh vegetation, mud/silt substrate, and tidal influence.	2
Estuary Emergent Marsh (EEM)	2.8 - 25.0	Low and high salt marsh vegetation, mud substrate, and full tidal influence.	NIS 2 MCA 1-5 RSS 3
Delta Mudflats (Flats)	18.0 - 28.0	Sparse to no vegetation, mud and/or gravel/cobble substrate, and large tidal fluctuations.	4-5
Nearshore	25.0 - 30.5	Areas outside of Nisqually delta complex, vegetation and substrate variable.	3-8
Hogum Bay Pocket Estuary	18.3 - 30.0	Sand spit enclosed estuary with salt marsh vegetation, sand and mud substrate, and forested bluffs.	1

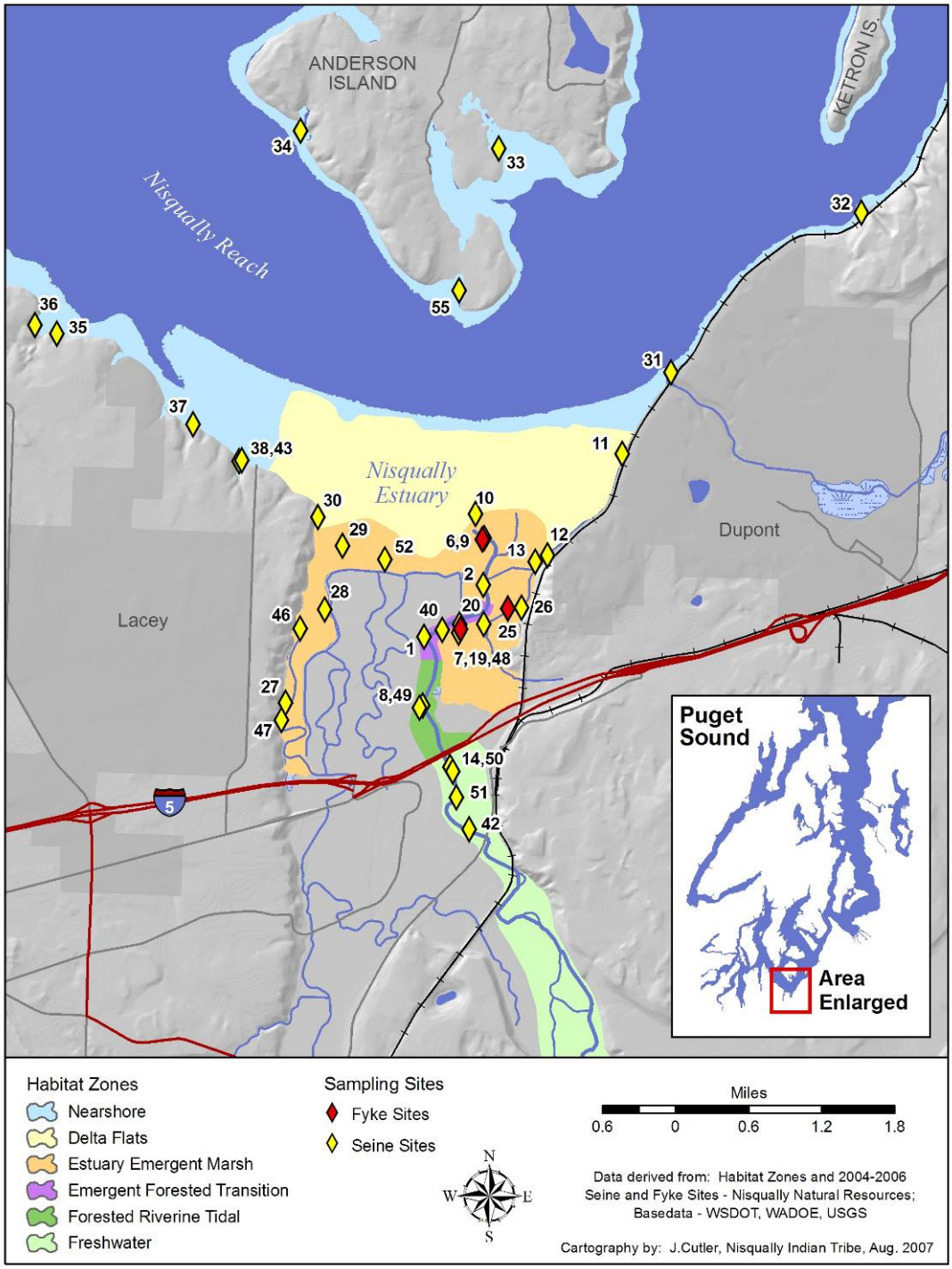

Figure 1. Location of Nisqually river, estuary, and reach habitat zones and fyke trap and beach seine sampling sites.

Fyke Trap Sites

Three fyke trap sites were established on blind channel sloughs in the Nisqually Estuary emergent marsh habitat, two located on tidal channels that drain into Red Salmon Slough and the third on a blind tidal channel that drains directly into the Nisqually River (Figures 1 and 2). The Phase 1 Restoration (Restoration) site was situated at the outlet of a 32 acre (129,499 m^2) area that had tidal inundation restored in late summer 2002 (see Bartlett et al. 2004 for details). The Restoration site wetted surface area was approximately 21,166 m^2 and the volume was 3,971 m^3 at an 11.4 foot (3.5 m) tide, the average height sampled (Table 2). The Red Salmon Slough Control (Control) site was situated at the outlet of a blind channel that drains into Red Salmon Slough about 800 m to the northeast of the Restoration site. The Control channel surface area was approximately 2,542 m^2, with a volume of 1,528 m^3 at tides around 11.2 feet (3.4 m) (Table 2). The Animal Slough (Animal) site is one of the only sloughs that connects estuarine emergent marsh habitat directly to the Nisqually River. The Animal Slough had an approximate surface area of 10,546 m^2 and a volume of 12,745 m^3 at a 10.0 foot (3 m) tide (Table 2). The tidal station at Dupont Wharf, Nisqually Reach (ID 1093), was used for all tide height data.

The surface area and volume at the Control and Animal sites were estimated based on field measurements which included channel cross sections spaced at intervals which equaled twice the width of the channel mouth and at smaller intervals (twice the tributary mouth width) in the tributaries of the main channel.

Due to the shape of the Restoration site, the surface area and volume trapped on a given day was highly variable. The surface area and volume at different tide heights at the site were estimated using a topographic map with 0.5 feet accuracy created by a professional surveyor in September 2003. Cross-sections were drawn every 70 feet on the map and measurements of area length, area widths, and cross-section depths were taken. The area and volume was calculated at six different tide levels ranging from 9.1 to 12.1 feet.

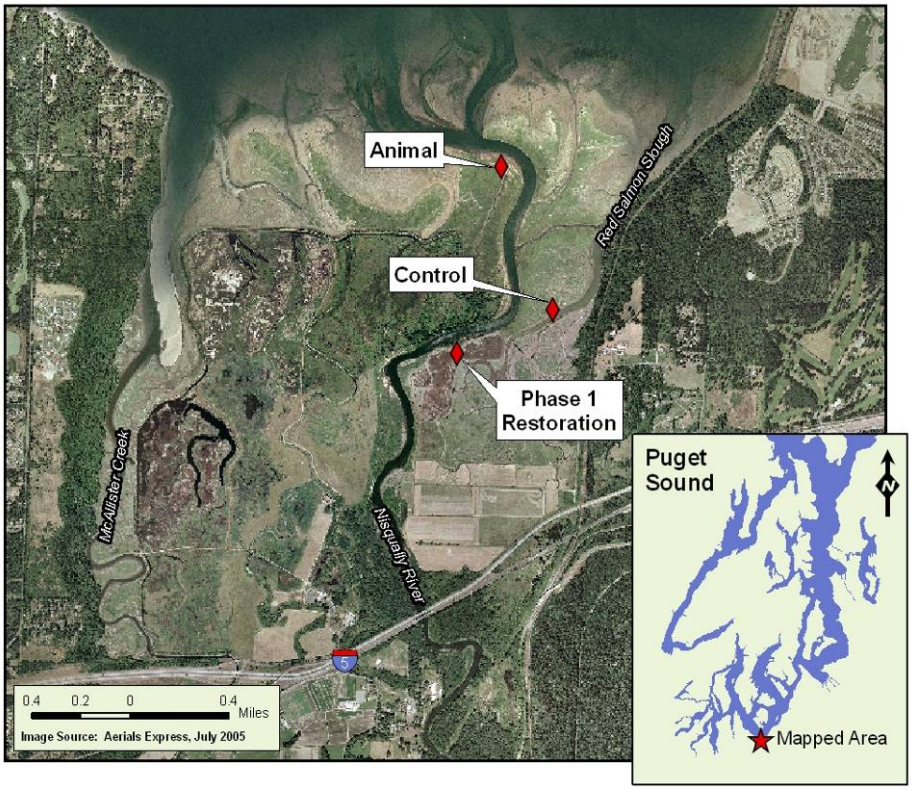

Figure 2. Location of Nisqually River estuary fyke trap sites.

Table 2. Nisqually Estuary fyke trap site descriptions.

Site	Year	Sampling Events per Year	Average High Tide Sampled (ft)	Average Length of Time Sampled (hours:minutes)	Surface Area* (m^2)	Volume* (m^3)	Surface Area/Volume	Mouth Width @ Trap (m)	Average Bottom Temperature (ºC)	Average Bottom Salinity (ppt)	Average Botttom Dissolved Oxygen (mg/L)
Phase 1 Restoration	2003	3	11.4	2:59	21166	3971	5.33	12.2	14.1	22.9	7.6
	2004	9									
	2005	7									
Red Salmon Slough Control	2003	5	11.2	2:53	2542	1528	1.66	16.3	13.1	25.2	8.5
	2004	10									
	2005	7									
Animal Slough	2004	9	10.0	4:44	10546	12745	0.83	13.4	12.9	22.6	9.4
	2005	11									
	2006	10									

*Measured at approximate average high tide sampled.

Sampling Methods

Beach Seining

Field protocols were modeled after similar studies in the Snohomish and Skagit River systems (SRSC Research 2003; Rowse and Fresh 2003) in order to facilitate regional comparisons and compilations. Each zone was sampled at 1 to 5 sites (Figure 1) with fairly consistent effort within zones each year resulting in over 300 sets per year (Figure 3). Our sampling effort did increase in the nearshore zone each year. Sites were representative of areas that could feasibly be sampled. Extremely complex habitats (e.g. logjams) and areas with fast current (e.g. mainstem Nisqually River) could not be sampled and thus were not represented. Each site was generally sampled once every two weeks from March-October in 2004, February-October in 2005, and February-October in 2006. Fish sampling was conducted using a standard 'Puget Sound seine' measuring 37 m x 2 m with a 2.4 m bag of 6 mm delta mesh, set by boat and hauled to shore by hand. Most sites were sampled between mid to high tide, and generally only one set per site was completed on each sampling occasion. Salinity, temperature, conductivity, and dissolved oxygen were measured at each site immediately after sampling using a Yellow Springs Instruments (YSI) Model 85 handheld meter (Appendix A).

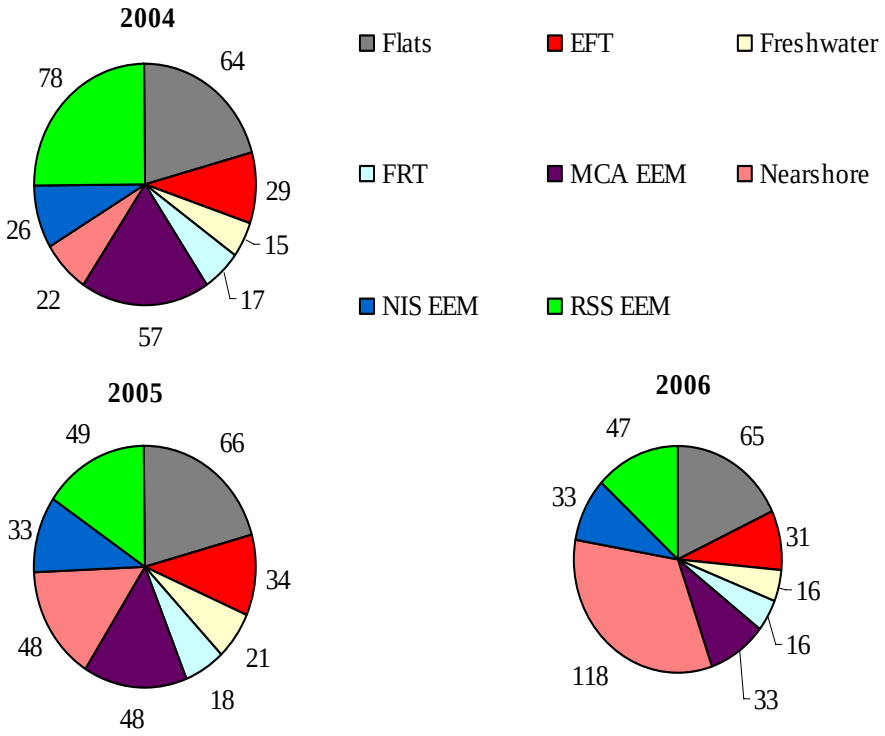

Figure 3. Distribution of beach seine sets among the various Nisqually River delta complex and nearshore habitat zones: 2004-2006.

Fyke Trapping

The Restoration site was trapped three times (May, June, and July) in 2003, 10 times in 2004 (March – August), and 8 times in 2005 (March – July) (Table 2). The Control site was trapped 5 times in 2003 (May – October), 10 times in 2004 (March – August), and 7 times in 2005 (March – July). The Animal site was trapped 9 times in 2004 (April – August), 11 times in 2005 (March – August), and 10 times in 2006 (March – August). The sites were trapped with fyke nets measuring 2.8 m deep with 3.175 mm mesh and a live trap in the center of the net with 2 zippered bays for removing fish while trapping. The Restoration, Control, and Animal site nets were 36.6 m, 30.5 m, and 22.9 m long respectively. Fyke trap nets were set across the channel at high tide (see Table 2 for average tides trapped and duration of trapping) and trapped fish were periodically removed until the traps were pulled several hours later when the site was almost dry. Salinity, temperature, and dissolved oxygen were measured at each site immediately after the trap was set using a YSI Model 85 handheld meter both at the surface (all years) and near the bottom (2004-2006 only).

Fish Processing

All captured fish were enumerated and 10 fish of each species were measured by fork length (nearest mm) at each site. On occasions with extremely large catches or especially muddy conditions, a subsample (by volume) was taken and enumerated and then proportionally expanded by species to estimate the unsampled catch. In a few cases, large catches of certain species such as shiner perch (*Cymatogaster aggregata*) and sculpin (*Cottus and Leptocottus spp*.) made accurate counts of these species unfeasible without causing substantial mortality, so rough estimates were made by eye. All captured coho (*Oncorhynchus kisutch*) and Chinook salmon were examined for clipped adipose fins. All unclipped and the majority of clipped coho and Chinook were "wanded" to detect coded wire tags (CWTs). Lethal Chinook samples were taken for otolith analysis, coded wire tag reading, and stomach content analysis (2004 and 2005 only). Stomachs were cut out of the fish in the field, stored in bags with ethanol, and combined in batches for identification and counting of contents by Robert Wisseman at Aquatic Biology Associates in Corvallis, Oregon.

Chinook stomach sample batches were generally based on location of capture, origin of fish (hatchery or unmarked), and time of capture. Due to limited resources, sample size restraints, and habitat zone labeling inconsistencies early in the project some batches include multiple habitat zones (Table 3).

Table 3. Nisqually unmarked Chinook and hatchery Chinook diet batch names and their corresponding zones.

Diet Batch	Corresponding Zone or Site
Freshwater	Freshwater
Transition	FRT and EFT
Nisqually Estuarine Emergent Marsh	NIS EEM
McAllister/Red Salmon Slough Sub-Estuaries and Inner Flats	RSS EEM, MCA EEM, and Flats
Outer Flats and Nearshore	Flats and Nearshore
Hogum Bay	Pocket Estuary
Restoration	Phase 1 Restoration
Control	Red Salmon Slough Control
Animal	Animal Slough

Fyke Trap Catch Efficiency

Ideally, the fyke trap nets should catch all fish present in the channel above the trap at the time the trap is set, however the actual efficiency of the trap depends on how many fish are able to get past the trap without being captured and how many fish are left above the trap when the trap is pulled. In order to estimate trap efficiency, juvenile salmon (primarily hatchery Chinook) were caught nearby with the beach seine, marked with a small clip of the caudal fin, and released above the trap throughout the channel after the trap had been set. The number of these marked fish that were subsequently recaptured at the trap was then used to estimate the efficiency of the trap.

The efficiency of the Control site fyke trap was estimated on June 18, 2003. 66 Chinook and 10 chum salmon were marked and released above the trap. 68 of these marked fish were recaptured, for a combined chum-Chinook efficiency rate of 89%. The efficiency of the Restoration site fyke trap was estimated on June 7, 2004. Of 118 marked Chinook, 71 were recaptured yielding an efficiency estimate of 60%. The capture rate of the Animal site fyke trap was tested on 05/31/05 with 35 out of 122 marked Chinook recaptured (29%) and on 06/01/06 with 41 out of 108 marked Chinook recaptured (38%). The Animal site does not completely de-water at low tide so fish can reside in pools above the trap site thus the trap efficiency rates are poor.

Catch data were not adjusted by trap efficiency because the intensity of efficiency sampling was not adequate for this purpose. All fyke trap fish density data should be considered an underestimate of the true density, especially the Animal site.

Invertebrate Sampling

Benthic macroinvertebrate sampling was conducted at each of the three fyke trap monitoring sites in March, May, and July 2005. At each site, five replicate sediment core samples were taken at a depth of 0.10 m with a 0.0024 m^2 PVC plastic core described by Cordell et al. (1994; 1998). The samples were sieved in the field with a 0.5 mm mesh

and preserved in 90% ethanol. The replicate samples were batched for a total of one sample per site per event.

The neuston, organisms associated with the air-water interface, was sampled with a 0.4 m x 0.2 m neuston net with a 0.130 mm mesh attached to a 1.835 m pole. The pole was swung in a 120^{o} arc measuring 3.843 m for an estimated volume of 0.33 m^3. Three replicate neuston samples were collected at each fyke trap site on the out-going tide once in March, May, and July 2005. The samples were batched in the field and preserved in 90% ethanol.

Insect fallout traps were placed in the emergent marsh adjacent to the three fyke trapping sites in March, May, and July 2005 in order to measure the density of insects and other marsh associated invertebrates that contribute to a tidal channel salmon prey base. Three replicate fallout samples were collected at each of the three sites. The fallout traps consisted of 0.5 m x 0.4 m plastic storage bins filled with 40 mm of soapy water (Simenstad et al. 2001). The traps were left for approximately 48 hours. At each site, the contents of the three replicate samples were sieved in the field with a 0.106 mm mesh then batched and preserved in 90% ethanol.

The Nisqually Reach Nature Center (NRNC) staff and volunteers identified and enumerated the benthic core, neuston net, and fallout trap samples. See Appendix B for the NRNC invertebrate identification and enumeration methodology.

Data Analysis

A percent similarity index (PSI) was used to examine the similarity between the various batches of Chinook diet samples as well as between the fyke trap Chinook diet batches and the composition of the fallout and benthic core samples (Gray et al. 2002). We used the benthic and fallout data from May and July to bracket the time period from which the diet samples were batched (May 1^{st} – June 30^{th}). The PSI was computed using the following formula (Hurlbert 1978; Yoklavich et al. 1991):

(1) $$PSI = \sum_{i} \min (p_{1i,}\ p_{2i}),$$

where p1i is the percentage of individuals from a taxonomic grouping in sample 1 and p2i is the percentage of individuals from a taxonomic grouping in sample 2.

Most diet and invertebrate samples were identified to the family level. However, if different taxonomic levels were identified in the prey and stomach content analyses they were combined to the most inclusive taxonomic level for analysis. We did not include the neuston samples in the PSI analyses because the identification of the organisms collected with the neuston net were at a more detailed taxanomic level than the stomach content analyses so conducting a PSI analysis would involve an unacceptable loss of detail in the neuston sample.

Length data from 2004-2006 were combined for all beach seine sites and were not adjusted for effort. Unmarked and hatchery Chinook mean lengths were compared with a t-test: two sample assuming unequal variances.

Results

Beach Seine Results

Total Catch for all Species

A large portion of the beach seine catch during the 2004-2006 sampling effort consisted of fishes other than salmonids (Table 4). Shiner perch were the top contributor to the total catch, despite not being captured in the Freshwater and FRT zones, with over 7,000 individuals captured in 2004 and 2006 and over 4,000 individuals captured in 2005. Over 10,000 sculpin were captured in the beach seine during the study with 5869, 1707, and 2612 individuals captured in 2004, 2005, and 2006 respectively. Pacific sand lance (*Ammodytes hexapterus*) were not captured consistently but were found in very high densities on occasion with over 7,000 seined over the course of the study. Starry flounder (*Platichthys stellatus*) catches were also quite variable with 1581, 425, and 4,188 individuals captured in 2004, 2005, and 2006 respectively. Threespine stickleback (*Gasterosteus aculeatus*) and Pacific herring (*Clupea harengus pallasi*) were also abundant non-salmonids in the 2004-2006 total catch with 3,633 and 1,226 individuals captured respectively.

The total salmonid catch was dominated by chum and hatchery Chinook salmon, with 10,180 and 9,686 captured respectively (Table 4). Chum salmon counts were 1,790 captured in 2004, 2,450 captured in 2005, and 5,940 captured in 2006. The 2006 increase in chum salmon counts is due to an increase in the number of sets performed in the Nearshore habitat zone. Hatchery Chinook counts were fairly consistent from year to year with 3,612, 2,634, 3,440 individuals captured in 2004, 2005, and 2006 respectively. Unmarked Chinook catch varied substantially from year to year with 625, 1,551, and 346 individuals captured in 2004, 2005, and 2006 respectively. Pink salmon (*O. gorbuscha*) juveniles were captured in variable densities during even years, with 2,218 counted in 2004 and 204 observed in 2006. Over 1,700 hatchery coho were captured during the study with nearly 1,500 of them counted in 2004. Unmarked coho, steelhead (*O. mykiss*), and coastal cutthroat trout (*O. clarki clarki*) were captured sporadically during the study in low numbers.

Table 4. Nisqually beach seine catch totals by habitat zone for all fish sampled from 2004-2006.

Year	Zone	Number of Sets	Chinook	Chinook (Hatchery)	Chum	Coho	Coho (Hatchery)	Coastal Cutthroat	Pink	Steelhead	Steelhead (Hatchery)	Sockeye	Trout (Unknown)	Salmon (Unknown)
2004	Flats	64	50	355	206	21	1313	15	84			1		
	EFT	29	62	1524	229	9	1		34	9				
	Freshwater	15	209	57	274	15	3	1	1	5			16	4
	FRT	17	164	114	218	2	1	1	2	1				
	MCA EEM	57	8	84	105		11	1	1					
	Nearshore	22	5	23	400	1	1		2091		1			
	NIS EEM	26	85	1153	15	10	125	2						
	RSS EEM	78	41	302	343	1	40		5					
2004 Total		**308**	**625**	**3612**	**1790**	**59**	**1495**	**20**	**2218**	**15**	**1**	**1**	**16**	**4**
2005	Flats	66	119	899	268	9	60	3						
	EFT	34	235	263	95	6				1				
	Freshwater	21	633	1	191	17	1	7		11			8	
	FRT	18	203	25	130	8								
	MCA EEM	48	37	568	58	6	15							
	Nearshore	48	28	122	1402	11	7	4						
	NIS EEM	33	187	195	29	9	3	7		1				
	RSS EEM	49	109	561	277	5	26							
2005 Total		**317**	**1551**	**2634**	**2450**	**70**	**112**	**21**	**0**	**13**	**0**	**0**	**8**	**0**
2006	Flats	65	32	297	301	11	142	3		1				
	EFT	31	32	201	8	2			1					
	Freshwater	16	79	308	308	2		2	1					
	FRT	16	15	62	395	1								
	MCA EEM	33	3	8	227									
	Nearshore	118	145	2136	4558	3	4	8	202					19
	NIS EEM	33	31	299	22	2								
	RSS EEM	47	9	129	122		3							
2006 Total		**359**	**346**	**3440**	**5940**	**21**	**149**	**13**	**204**	**1**	**0**	**0**	**0**	**19**
Grand Total		***984***	***2521***	***9686***	***10180***	***150***	***1756***	***54***	***2422***	***29***	***1***	***1***	***24***	***23***

Table 4 (continued).

Year	Zone	Number of Sets	Arrow Goby	American Shad	Bay Pipefish	Crescent Gunnel	English Sole	Flounder (Unknown)	Goby (Unknown)	Largemouth Bass	Largescale Sucker	Mountain Whitefish	Northern Anchovy	Pacific Herring
2004	Flats	64			4									14
	EFT	29		1										
	Freshwater	15								1	292	2054		
	FRT	17									3	3		
	MCA EEM	57			1									624
	Nearshore	22			3			2						
	NIS EEM	26												
	RSS EEM	78												67
2004 Total		**308**	**0**	**1**	**8**	**0**	**0**	**2**	**0**	**1**	**295**	**2057**	**0**	**705**
2005	Flats	66			3								1	1
	EFT	34												
	Freshwater	21									463	141		
	FRT	18									1	77		
	MCA EEM	48			1		5							30
	Nearshore	48												1
	NIS EEM	33											1	
	RSS EEM	49											58	
2005 Total		**317**	**0**	**0**	**4**	**0**	**5**	**0**	**0**	**0**	**464**	**218**	**60**	**32**
2006	Flats	65			5									34
	EFT	31		2										
	Freshwater	16										243		
	FRT	16									2	1		
	MCA EEM	33												81
	Nearshore	118	10		1	3			4					241
	NIS EEM	33												
	RSS EEM	47												133
2006 Total		**359**	**10**	**2**	**6**	**3**	**0**	**0**	**4**	**0**	**2**	**244**	**0**	**489**
Grand Total		***984***	***10***	***3***	***18***	***3***	***5***	***2***	***4***	***1***	***761***	***2519***	***60***	***1226***

Table 4 (continued).

Year	Zone	Number of Sets	Pacific Sand Lance	Pacific Snake Prickleback	Penpoint Gunnel	Pile Perch	Rock Sole	Saddleback Gunnel	Sculpin (all spp. combined)	Shiner Perch	Speckled Sandab	Starry Flounder	Sunfish (Unknown)	Surf Smelt	Threespine Stickleback	Yellow Perch
2004	Flats	64	258			4		2	2093	2427	8	153		39	1	
	EFT	29							99			77				
	Freshwater	15							166			106	1		1871	
	FRT	17							38			794			3	
	MCA EEM	57	89					2	1160	1969	1	227		10	9	
	Nearshore	22	1						884	636	1	46			1	
	NIS EEM	26							93	117	1	75				
	RSS EEM	78	1						1336	2631		103			4	
2004 Total		**308**	**349**	**0**	**0**	**4**	**0**	**4**	**5869**	**7780**	**11**	**1581**	**1**	**49**	**1889**	**0**
2005	Flats	66	487					6	557	1294	4	41		2	10	
	EFT	34							41	3		4		7	4	
	Freshwater	21							214			56			1506	2
	FRT	18							17			52				
	MCA EEM	48	1538						449	2472	1	70		30	33	
	Nearshore	48		1	1			6	184	125	4	105			25	
	NIS EEM	33							48	19		33			2	
	RSS EEM	49	278						197	565		64		1	37	
2005 Total		**317**	**2303**	**1**	**1**	**0**	**0**	**12**	**1707**	**4477**	**9**	**425**	**0**	**40**	**1617**	**2**
2006	Flats	65	449					18	402	1593		44		6	6	
	EFT	31							10	13		23		16	13	
	Freshwater	16							51			89			7	
	FRT	16							10			3577			2	
	MCA EEM	33	3440						601	2115		146		70	58	
	Nearshore	118	14	5	9	7	1	7	1364	2679	2	73		70	50	
	NIS EEM	33	2						60	243		180		5	4	
	RSS EEM	47	898						114	903		56		7	7	
2006 Total		**359**	**4803**	**5**	**9**	**7**	**1**	**25**	**2612**	**7546**	**2**	**4188**	**0**	**174**	**147**	**0**
Grand Total		***984***	***7455***	***6***	***10***	***11***	***1***	***41***	***10187***	***19803***	***22***	***6194***	***1***	***263***	***3653***	***2***

Average Timing and Abundance of Primary Species in Estuarine Zones

The average catch timing of unmarked and marked Chinook, chum, Pacific herring, sculpin, shiner perch, and Pacific sand lance captured in the estuarine zones (FRT, EFT, all EEM, and Flats) is presented graphically in Figure 4. Unmarked Chinook had a broad temporal distribution in the estuarine zones, with routine captures extending from February to September. The average peak catch per set for unmarked Chinook occurred from May (7.4 per set) to June (6.9 per set). Hatchery Chinook presence in the estuarine zones was characterized by a low duration, high abundance average peak catch (58 per set) in May following releases made by the Clear Creek and Kalama Creek hatcheries located at river miles 6 and 9, respectively (see Appendix C for Nisqually hatchery release data). Chum were captured as early as February in the beach seine, but were at low abundance until their average peak catch of about 25 per set in May.

Shiner perch were first captured in the estuarine zones in May (7.4 per set) and quickly increased in average abundance to over 55 per set in June following the live birth of young of the year perch. Shiner perch abundance in the catch gradually decreased through August and then displayed a secondary peak in September (average 20 per set), dropping off again in October. Sculpin were present in the estuarine zones throughout the study period, with an average peak catch occurring in April (20 per set). Pacific herring average catches were generally low with most fish captured in July (average 4.2 per set) and September (average 2.7 per set). Pacific sand lance average peak catch was over 39 per set in May with average catches of over 8 per set in June and July.

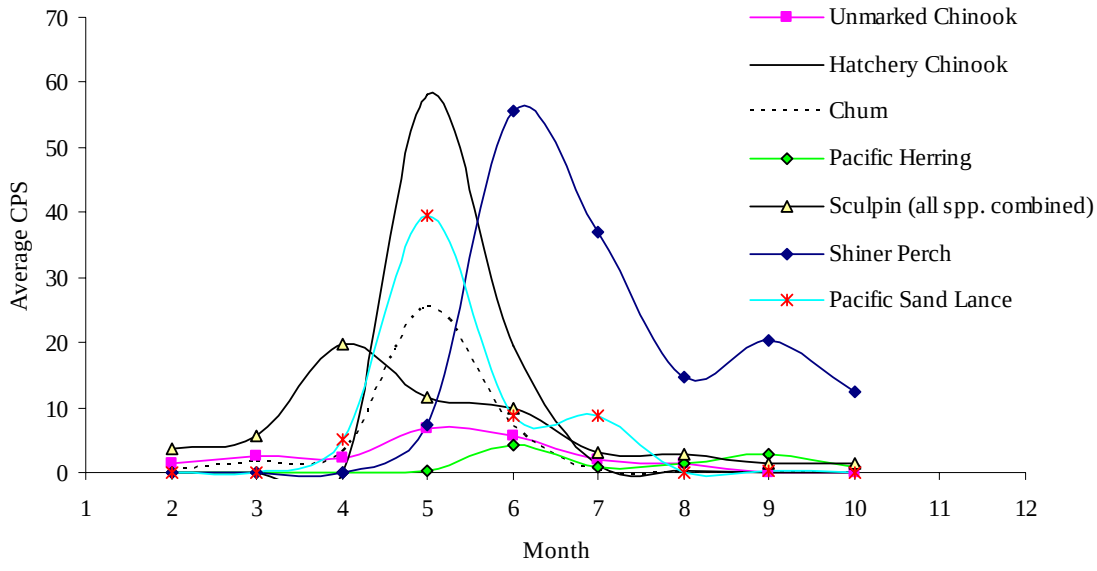

Figure 4. The average catch per set (CPS) per month of the primary fish species from the Nisqually estuarine zones (FRT, EFT, all EEM, and Flats), 2004-2006.

Average Catch per Set of Chinook and Chum per Zone

Unmarked Chinook displayed zone specific average timing and abundance patterns (Figure 5). Unmarked Chinook were captured in the Freshwater habitat zone in February, gradually increasing in average abundance through May to over 50 per set followed by a sharp decline to 10 per set in June, near zero in July and August, and then an average of 3 and 2 per set in September and October respectively. In the FRT zone, unmarked Chinook were present in low numbers in February, increased to an average of 13 per set in March, and maintained a fairly constant abundance ranging from an average of 9.5 to 14 per set from April through June before dropping down to less than 5 per set in July and August and then no captures in September and October. The temporal distribution of unmarked Chinook in the EFT and NIS EEM zones tracked closely together. On average, small numbers of fish were captured in February followed by no to only a few captures in March and April. Peak unmarked Chinook average catch per set for both the EFT and NIS EEM zones occurred in May (approximately 10 per set per zone) proceeded by a gradual decrease in catch from June to August and then no catch in September and October. Average catches of unmarked Chinook in the Flats zone peaked in April (3 fish per set) and again in June (2.5 fish per set). Average unmarked Chinook catches in the RSS EEM, MCA EEM, and nearshore zones were low in all months, with peak average catches in May of approximately 3, 2, and 5 fish per set respectively. When the pocket estuary site (Hogum Bay) is split out of the nearshore zone, two different timing patterns are discernible (Figure 6). The average peak catch of unmarked Chinook at the pocket estuary site peaks at the beginning of the sampling efforts in February at around 6 fish per set and then quickly drops off in March, followed by a secondary peak of almost 2 fish per set in May. With the pocket estuary site excluded, the average peak catch of unmarked Chinook in the nearshore zone is over 6 fish per set in May (Figure 5).

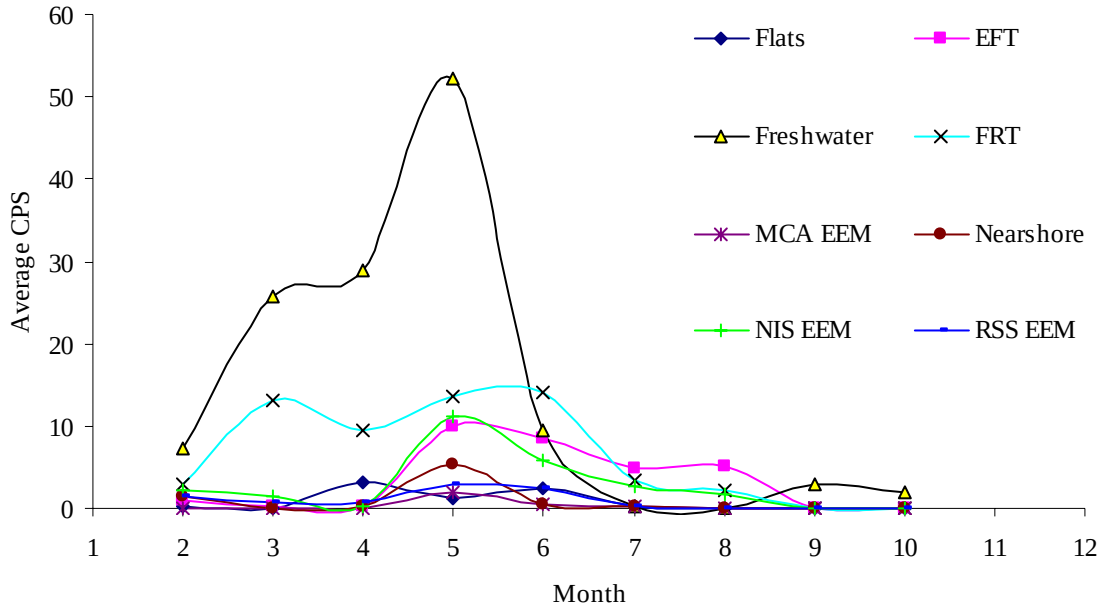

Figure 5. Average catch per set (CPS) per month of unmarked Chinook from all habitat zones sampled, 2004-2006.

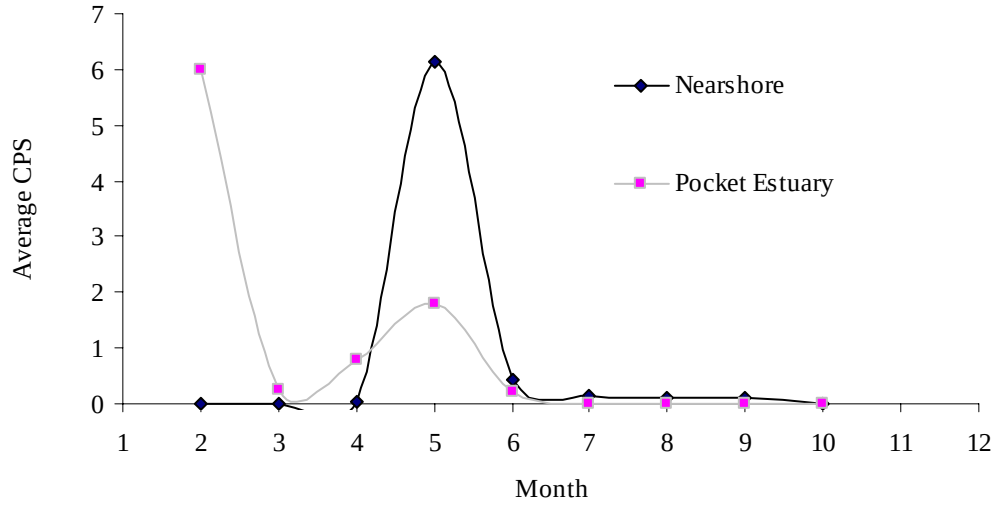

Figure 6. Average catch per set (CPS) per month of unmarked Chinook from the Hogum Bay pocket estuary site and the nearshore, 2004-2006.

The temporal distribution of hatchery Chinook was similar for all zones with a strong peak in May, followed by a rapid decrease in average CPS and then few if any captures from July to October (Figure 7). The FRT zone was the exception to this pattern, with an average peak catch in June rather than May. Average peak CPS for hatchery Chinook was highest at the EFT zone (134 per set), followed by the NIS EEM (96 per set), nearshore (87 per set), Flats (50 per set), MCA EEM (35 per set), RSS EEM (32 per set), Freshwater (29 per set), and FRT (27 per set) zones. Separating out the pocket estuary site from the nearshore zone did not change the observed timing patterns for hatchery Chinook.

Chum salmon were generally present in the study area from March to July, with average peak catches in May for most zones (Figure 8). The nearshore zone had the largest average catches of chum in April (75 per set) and May (141 per set). The April peak of chum occurred primarily in the pocket estuary site, while the May peak occurred at the rest of the nearshore sites (Figure 9). Catches of chum in the FRT zone were characterized by low average abundance in March and April followed by a large average catch in May (89 per set), tapering off quickly to approximately 11 per set in June. The average catch per set of chum also peaked in May (24 per set) in the EFT zone. Chum were captured in the Freshwater zone from March to July, with average peak catches in April (42 per set) and May (42 per set). In the EEM zones, chum had the largest average peak catch in the RSS EEM (15 per zone), followed by the MCA EEM (12 per set), and NIS EEM (2 per set) in May. Chum were captured in the Flats zone as early as March, but did not reach their average peak until June (16 per set).

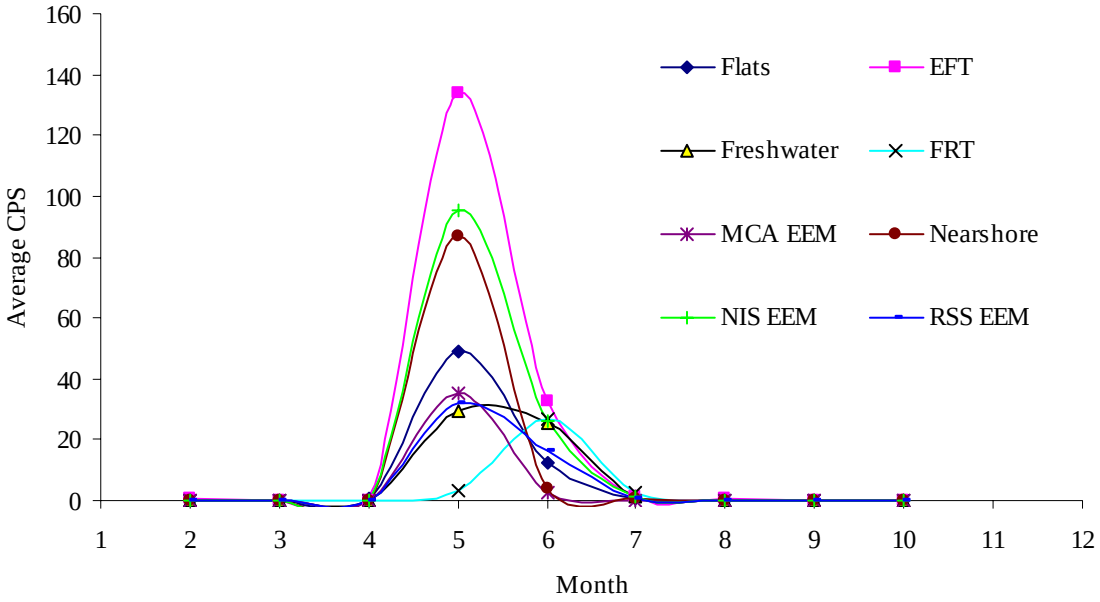

Figure 7. The average catch per set (CPS) per month of hatchery Chinook from all habitat zones sampled, 2004-2006.

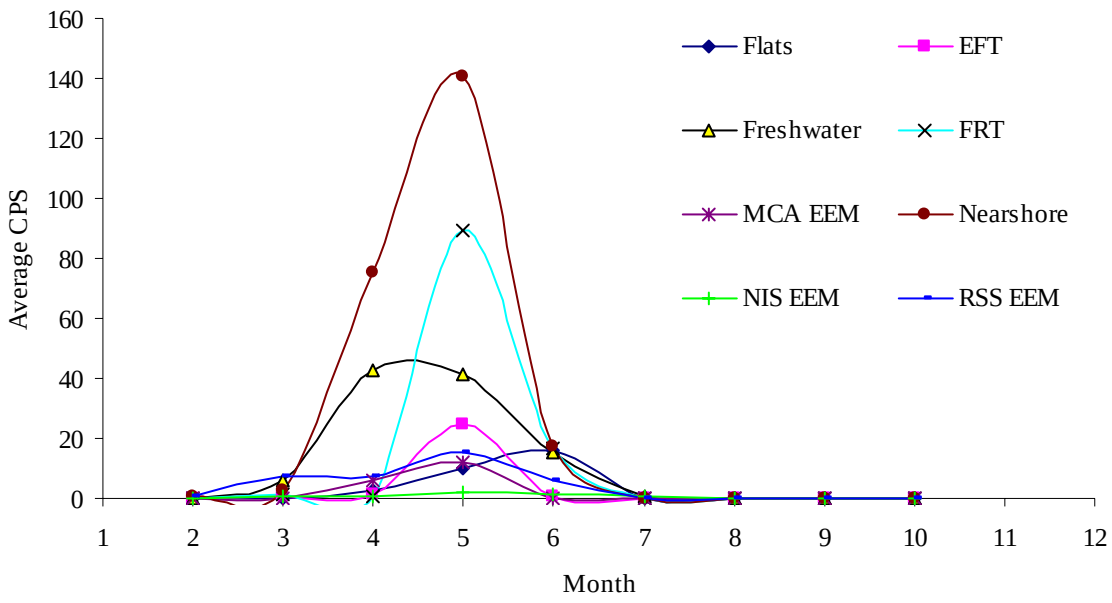

Figure 8. The average catch per set (CPS) per month of chum from all habitat zones sampled, 2004-2006.

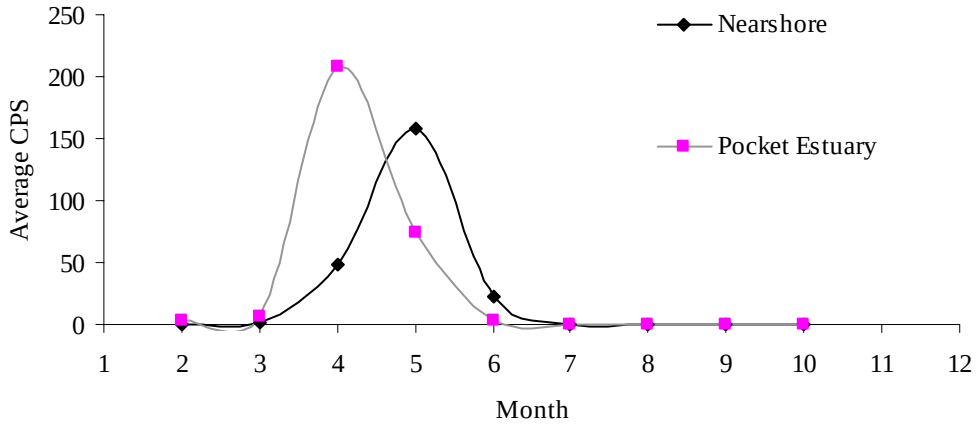

Figure 9. Average catch per set (CPS) per month of chum from the Hogum Bay Pocket Estuary site and the rest of the Nearshore sites (combined), 2004-2006.

Interannual Variability in Catches of Chinook and Chum

Figure 10 shows the interannual variability between average catches of unmarked Chinook, hatchery Chinook, and chum for all the estuarine zones (FRT, EFT, all EEM, and Flats) between years 2004, 2005, and 2006. Unmarked Chinook had the most variability among the primary salmonids in temporal distribution and relative abundance between years. In 2004, average catches of unmarked Chinook remained fairly constant (between 4-6 fish per set) from the start of sampling in March through June followed by a general absence of Chinook from July through October. Unmarked Chinook average catches were highest in 2005, with fish present in low numbers (approximately 2-3 fish per set) from February to April followed by a sharp rise to 17 fish per set in May and were captured in moderate numbers (approximately 4-10 fish per set) from June through August. In 2006, unmarked Chinook catches were very low, with an average peak of only 3 fish per set in May.

Hatchery Chinook catches showed little variability in temporal distribution between 2004 and 2005; however the average peak catch in 2004 was 100 fish per set in May while the average peak catch in 2005 was just over 50 fish per set also in May (Figure 10). In 2006, the average peak catch was reduced from 2005 and 2006, but lasted from May to (14 fish per set) June (21 fish per set).

Chum salmon average peak catches were all in May and ranged from 38 fish per set in 2006, 25 fish per set in 2004, and 14 fish per set in 2005 (Figure 10). The temporal distribution was similar for all years.

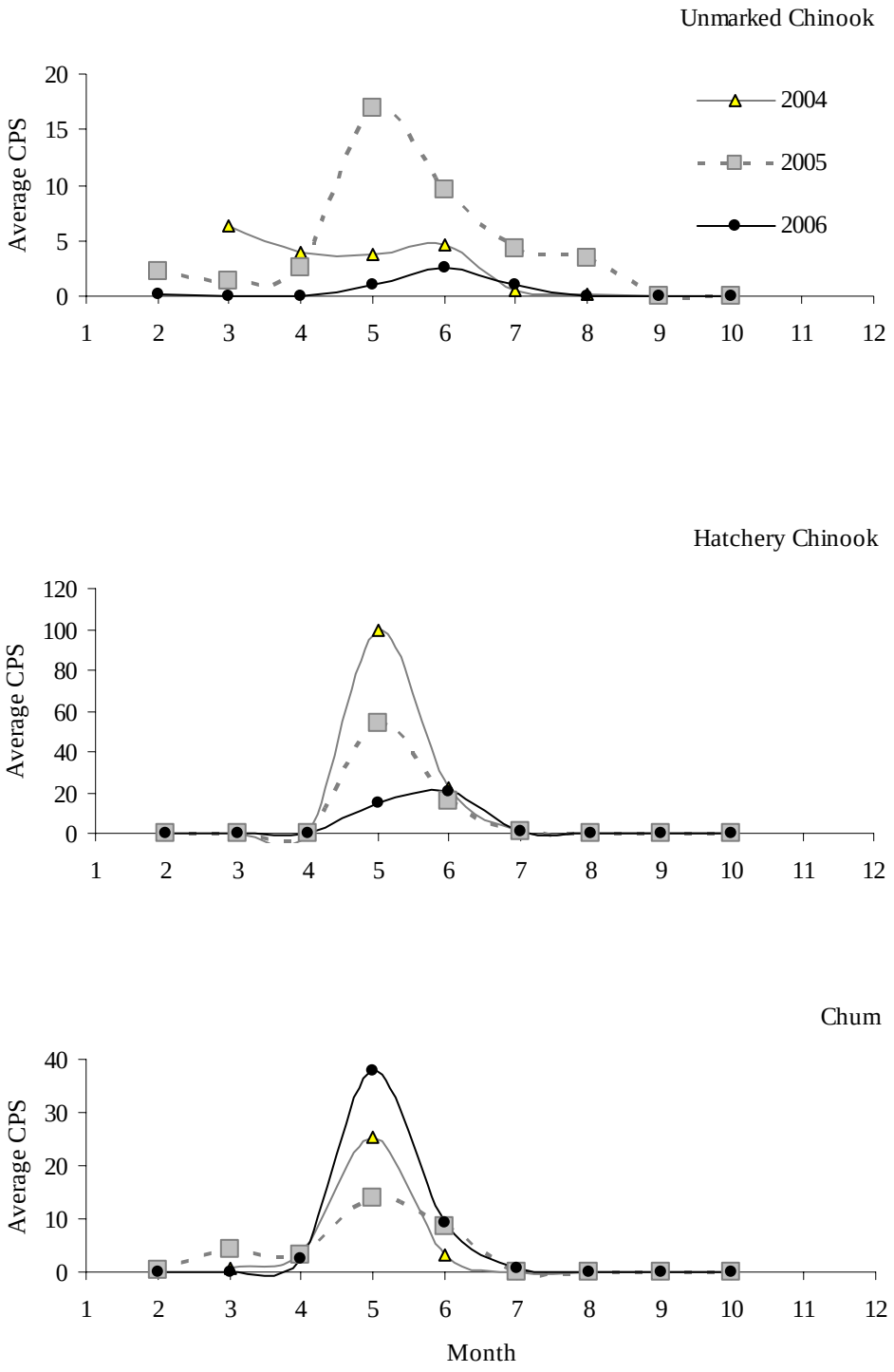

Figure 10. Average catch per set (CPS) per month for unmarked Chinook, hatchery Chinook, and chum captured from all Nisqually estuarine zones (FRT, EFT, all EEM, and Flats) in 2004, 2005, and 2006.

Lengths of Chinook and Chum

The average length of over 1,000 unmarked Chinook measured during this study was 71 mm (Figure 11). Unmarked Chinook length-frequency histogram shows a bimodal distribution, with a group of fish captured in February and March averaging 42 and 47 mm, respectively, and the main group of fish captured after April ranging in average size from 60 to over 95 mm in length. Unmarked Chinook size generally increased over time (Figure 12).

Over 1,200 hatchery Chinook were measured during the study with an average length of 90 mm (Figure 11) and ranging in average size from 80 to 86 mm upon first release in April and May to over 100 mm after July (Figure 12).

Hatchery Chinook were significantly larger than unmarked Chinook ($p < 0.0001$). Hatchery Chinook averaged 11.2 mm longer during the months that they co-occurred with unmarked Chinook in the study area (Table 5). The difference in size between hatchery and unmarked Chinook was especially apparent at the beginning and end of their co-occurrence in the study area. The size difference between hatchery and unmarked Chinook should be considered conservative since an unknown number of unmarked Chinook are unclipped hatchery fish.

The average length of over 1,400 chum that were measured was 51 mm (Figure 11), ranging on average between 38 mm for those captured in February to over 77 mm for those captured after July (Figure 12).

Table 5. The difference between the average hatchery Chinook fork length and the average unmarked Chinook fork length for each month they co-occur in the Nisqually study area.

Month	Average Hatchery Chinook Length (mm)	Number of Hatchery Chinook Measured	Average Unmarked Chinook Length (mm)	Number of Unmarked Chinook Measured	Difference
4	80.8	13	59.8	149	21.1
5	86.6	546	75.4	272	11.1
6	90.1	541	82.6	271	7.6
7	84.4	94	91.9	77	-7.6
8	111.1	15	96.5	36	14.6
9	118.0	1	97.6	10	20.4
			Average Difference		11.2

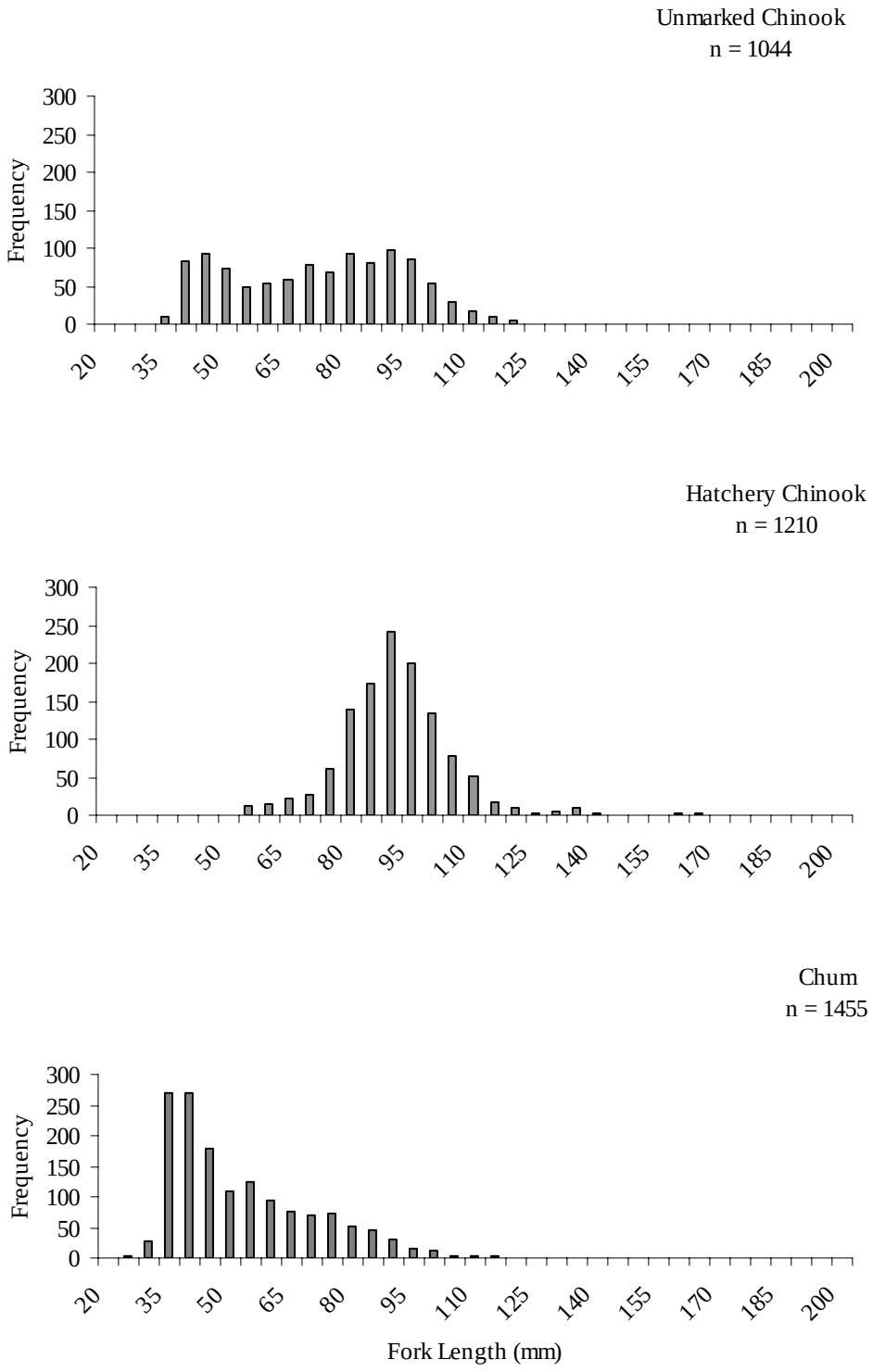

Figure 11. Frequencies of fork lengths measured for unmarked Chinook, hatchery Chinook, and chum from 2004-2006. Data from all zones and years were combined.

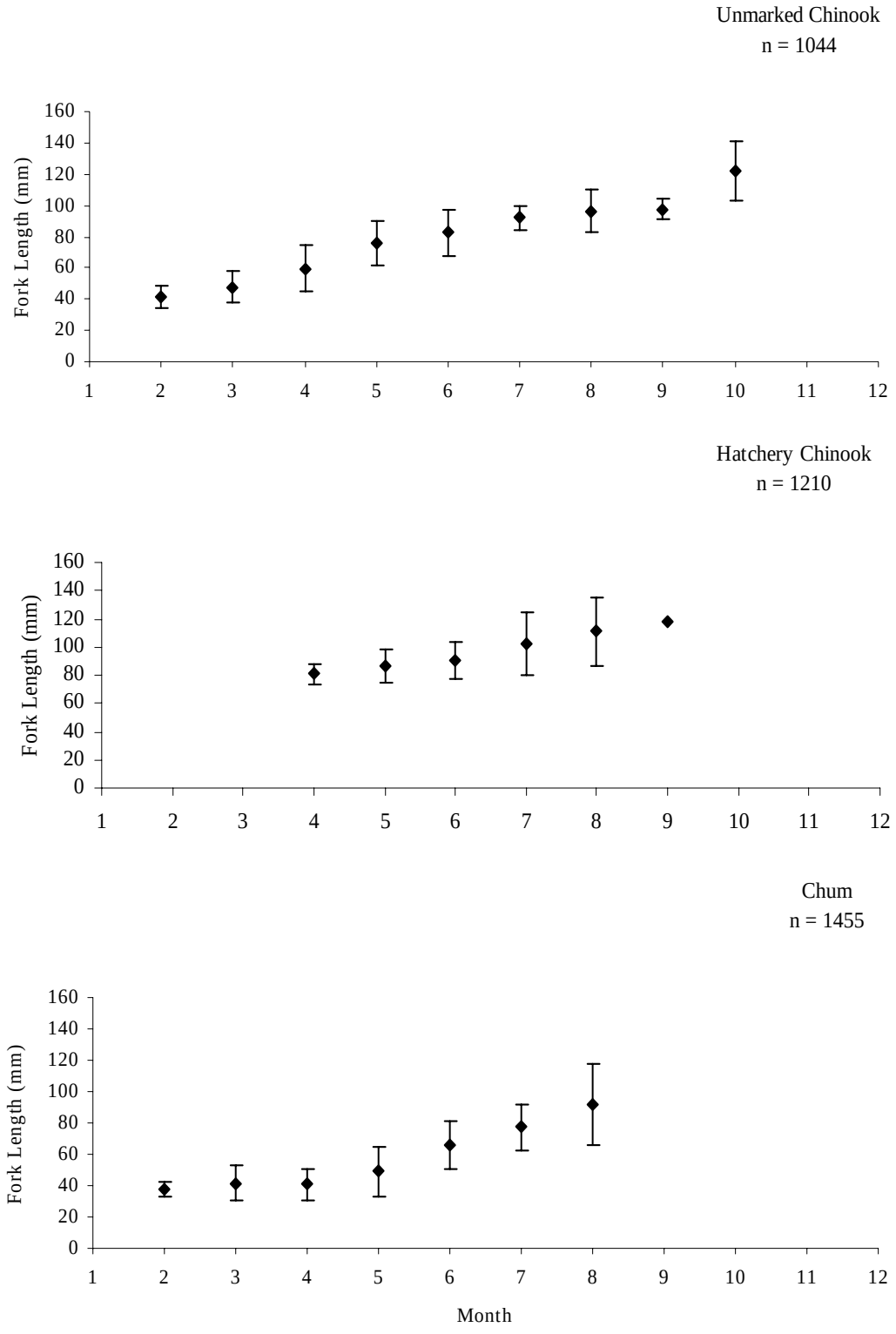

Figure 12. Average fork length (mm) of unmarked Chinook, hatchery Chinook, and chum per month. Error bars represent +/- 1 standard deviation.

Diet Composition of Unmarked and Hatchery Chinook

Freshwater

Unmarked Chinook captured in Freshwater during time 1 (Table 6) consumed primarily Chironomidae in both 2004 and 2005 (Figure 11), making up over 80% and 70% of their total diet composition, respectively. Chironomidae composed over 50% of the diet composition of time 2 Freshwater unmarked Chinook followed by Ephemeroptera (>20%) in 2004. 2005 time 2 Freshwater unmarked Chinook had a diet composed primarily of Brachycera (27%), Chironomidae (23%), Lepidoptera (18%), and Ephemeroptera (11%). The limited number of diet samples collected from 2005 time 3 unmarked Chinook was composed of primarily Hemiptera (54%), Trichoptera (16%), Chironomidae (12%), Salmon Eggs (8%), and Hymenoptera (8%). Hatchery Chinook diet from time 2 in 2004 was comprised primarily of Ephemeroptera (37%) and Chironomidae (28%).

Table 6. Nisqually unmarked Chinook and hatchery Chinook diet batch codes.

Code	Code Definition
U	Unmarked Chinook
H	Hatchery Chinook
1	Captured between February to April
2	Captured between May to June
3	Captured between July to October

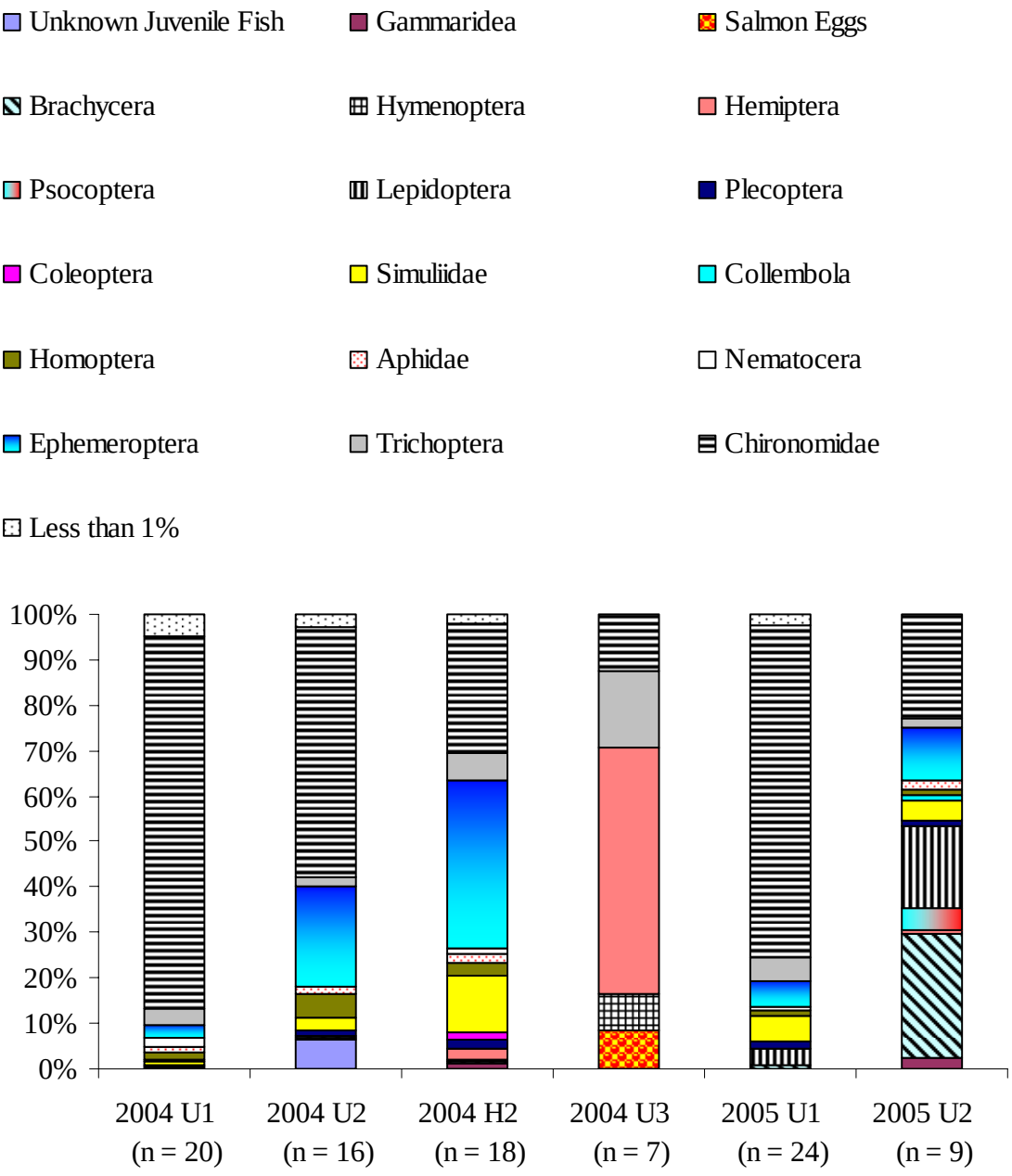

Figure 13. Percent contribution by number of primary prey items to the total diet composition of unmarked and hatchery Chinook captured in the Freshwater habitat zone. See Table 6 for sample code definitions. Diet items that contributed to less than 1% of the diet composition of all batches were combined into the "Less than 1%" item.

Transition

The pooled diet samples of time 1 unmarked Chinook from the freshwater to estuarine transitional habitat zones (FRT and EFT; see Table 3) in 2004 and 2005 were dominated by Chironomidae (91% and 84% respectively) (Figure 14). Time 2 unmarked Chinook in 2004 consumed primarily Mysidacea (58%) and to a lesser extent Chironomidae (13%) and Gammaridea (12%) while 2005 time 2 unmarked Chinook diet was composed of 70% Chironomidae. Time 3 2005 unmarked Chinook fed heavily on Gammaridea (61%) in the Transition zones as well as Chironomidae (11%) and Mysidacea (10%). Hatchery Chinook in 2004 time 2 preyed substantially on Gammaridea (23%), Mysidacea (22%), Aphidae (18%), Chironomidae (13%), and Brachycera (11%) while time 2 hatchery Chinook in 2005 preyed on primarily Chironomidae (46%) and Gammaridea (33%). Time 3 hatchery Chinook from the transition zones had a pooled diet composition of over 80% Gammaridea.

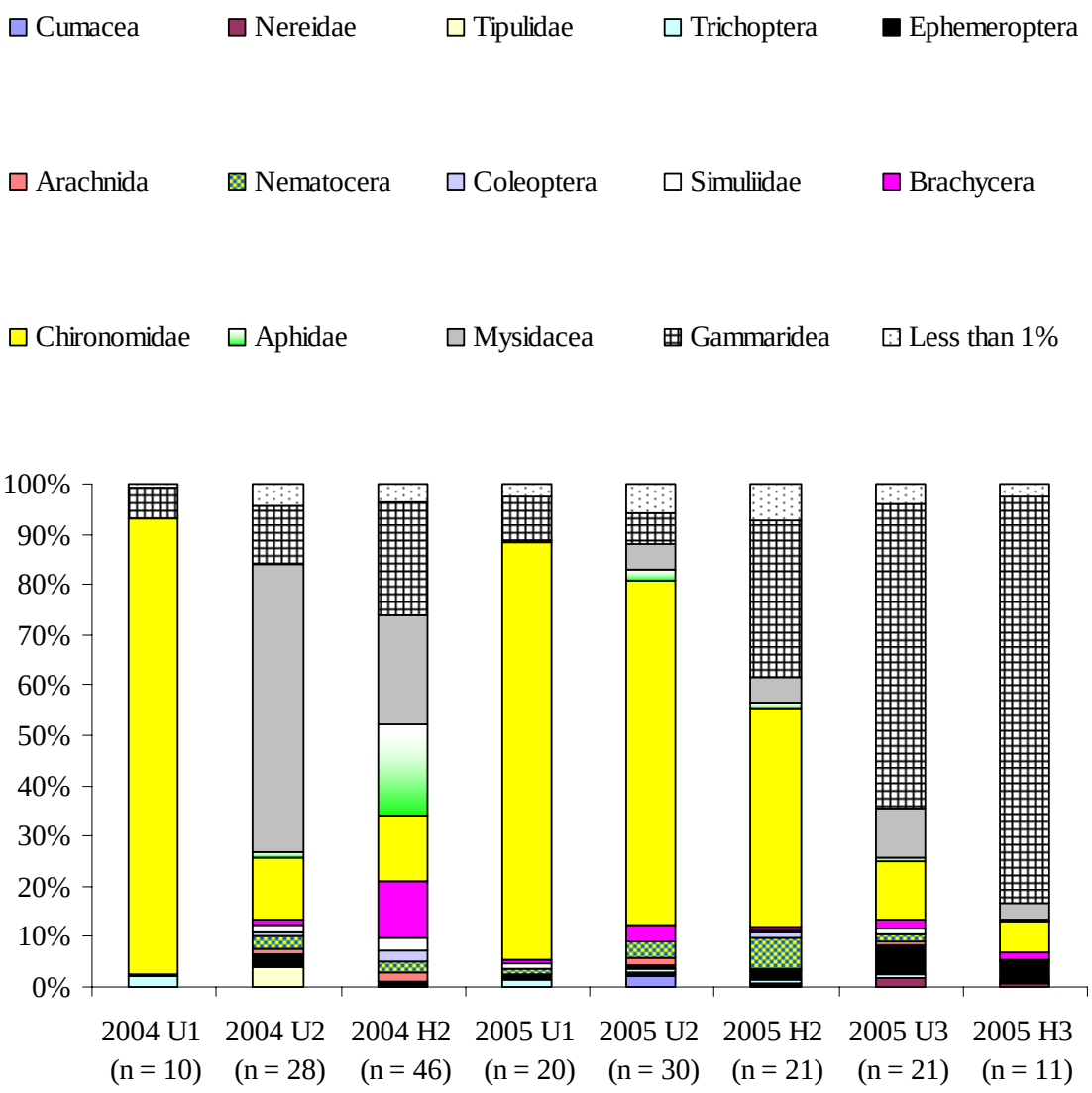

Figure 14. Percent contribution by number of primary prey items to the total diet composition of unmarked and hatchery Chinook captured in the transitional habitat zones. See Table 3 for zone composition of batches and Table 6 for code definitions. Diet items that contributed to less than 1% of the diet composition of all batches were combined into the "Less than 1%" item.

Nisqually Estuarine Emergent Marsh

Nisqually EEM time 1 unmarked Chinook from 2005 consumed primarily Chironomidae (40%), Nematocera (32%), and Gammaridea (23%). Chironomidae, Mysidacea, and Gammaridea were important for time 2 unmarked Chinook in 2005, composing approximately 45%, 27%, and 13% of the total pooled diet composition, respectively (Figure 15). In 2004, time 2 unmarked Chinook diets included large numbers of Gammaridea (23%), Nematocera (19%), Aphidae (14%), and Crustacea Nauplius (13%). Time 2 hatchery Chinook diets were primarily crustaceans, with Crustacea Nauplius (38%), Gammaridea (17%), and Mysidacea (11%) comprising the majority of 2004 time 2 hatchery Chinook diets while Mysidacea (51%) and Gammaridea (35%) dominated the 2005 time 2 hatchery Chinook diets. Time 3 2004 unmarked Chinook diets were composed of primarily insects, namely Brachycera (15%), Nematocera (12%), and Aphidae (10%) among others. Time 3 unmarked Chinook diets were quite different in 2005, with Gammaridea (62%) and Mysidacea (29%) comprising the bulk of the pooled diet sample. Nematocera (40%) and Gammaridea (21%) were the primary items in the time 3 2004 hatchery Chinook diet sample.

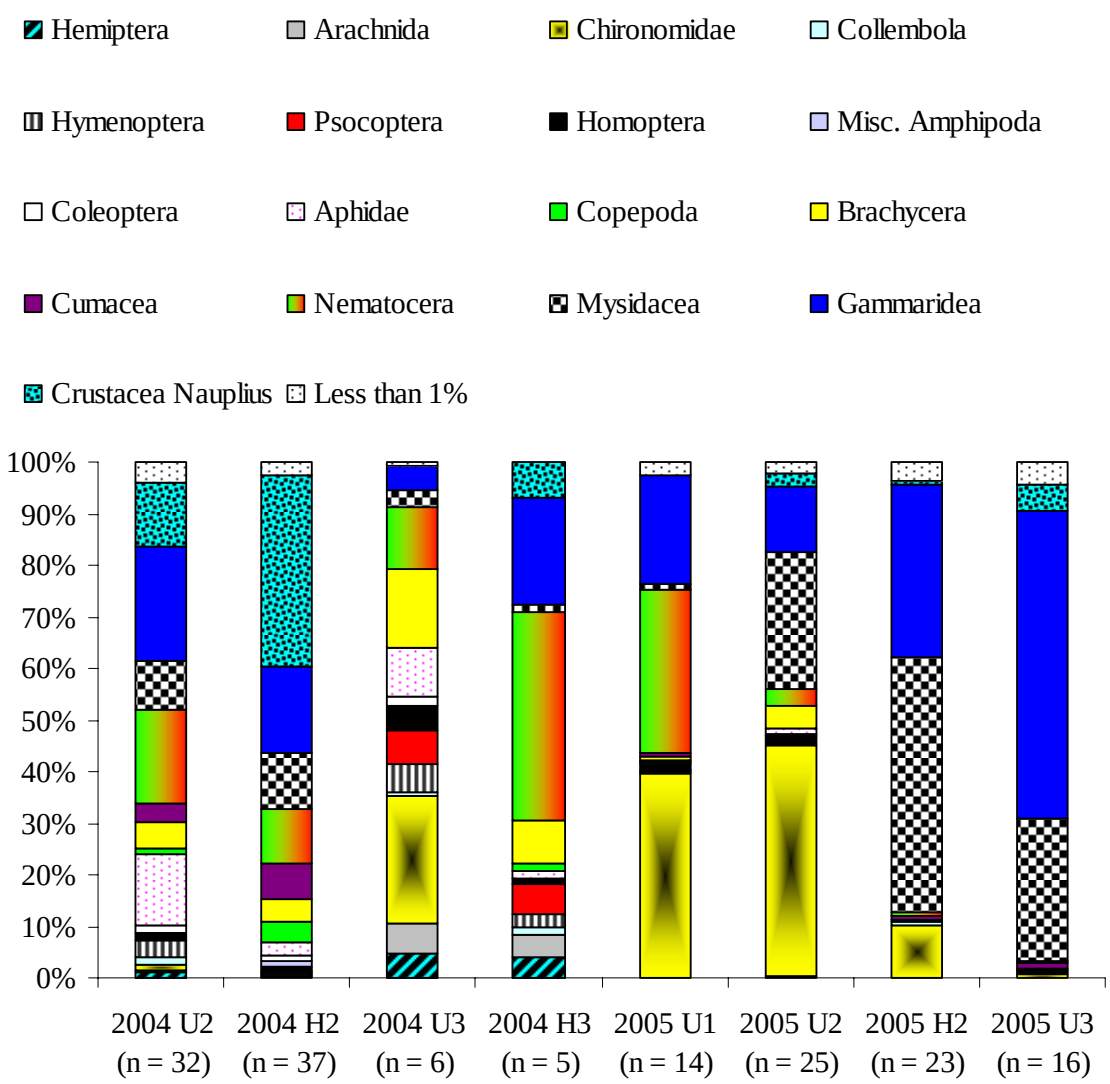

Figure 15. Percent contribution by number of primary prey items to the total diet composition of unmarked and hatchery Chinook captured in the Nisqually Estuarine Emergent Marsh (EEM) habitat zone. See Table 6 for sample code definitions. Diet items that contributed to less than 1% of the diet composition of all batches were combined into the "Less than 1%" item.

Unmarked and hatchery Chinook diets collected from the sub-estuaries and inner flats habitat zones (Table 3) generally preyed on similar insects and crustaceans as fish from the Nisqually EEM zone; however some diet components were quite different (Figure 16). Copepoda, Cumacea, and Unknown Juvenile Fish were all important components of hatchery and unmarked Chinook diets at various time periods for this geographical batch.

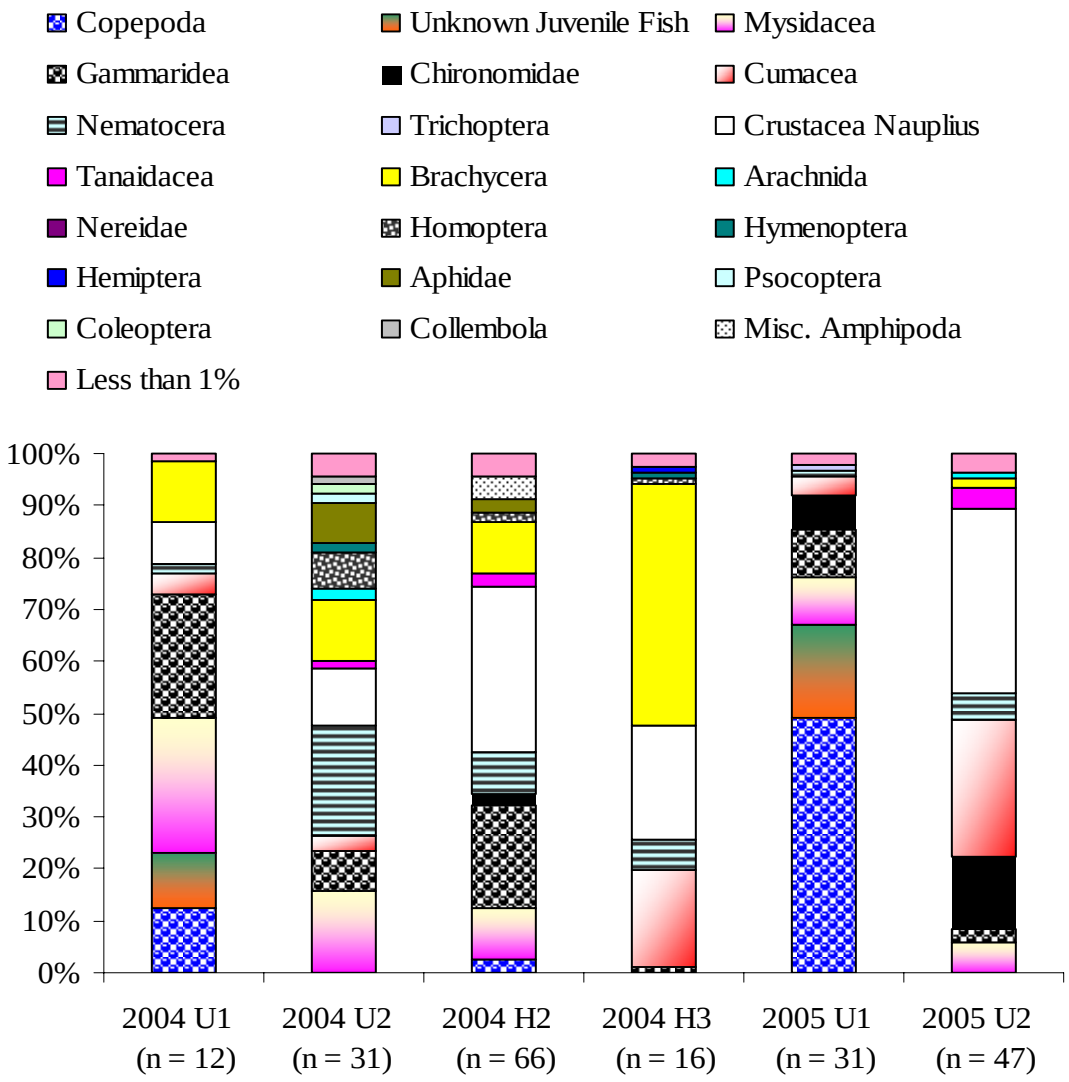

Figure 16. Percent contribution by number of primary prey items to the total diet composition of unmarked and hatchery Chinook captured in the McAllister and Red Salmon Slough sub-estuary zones and the inner flats sites. See Table 3 for zone composition of batches and Table 6 for code definitions. Diet items that contributed to less than 1% of the diet composition of all batches were combined into the "Less than 1%" item.

Outer Flats and Nearshore

Crustacea Nauplius prey comprised a large portion of the total diet composition for time 2 hatchery Chinook in 2004 and time 2 unmarked Chinook in 2005 (Figure 17) from the outer flats and Nearshore batch (Table 3). Nematocera flies were particularly prevalent in the 2004 time 2 unmarked Chinook diet batches. The time 2 hatchery Chinook batch in 2005 was primarily a combination of Cumacea, Crustacea Nauplius, and Tanidacea.

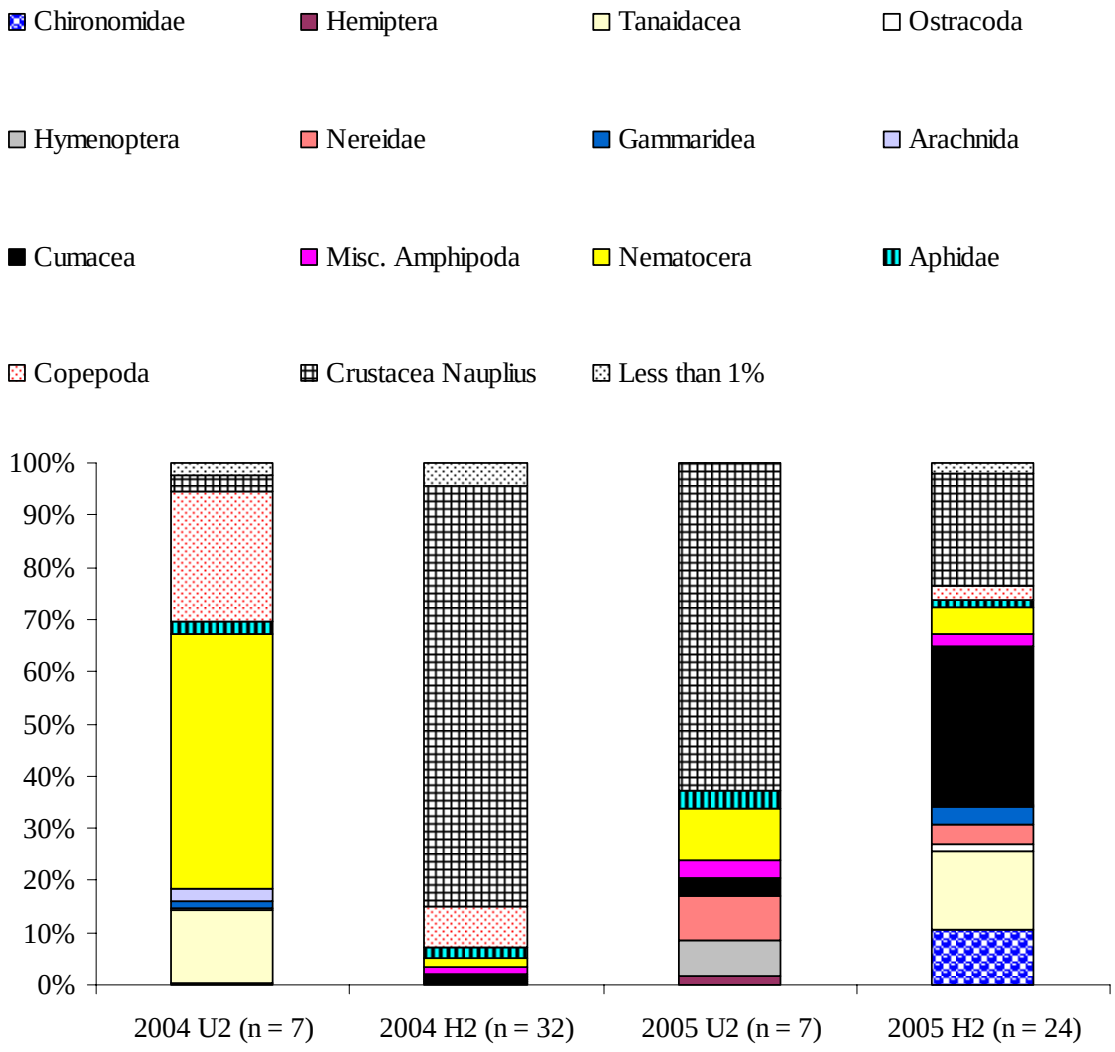

Figure 17. Percent contribution by number of primary prey items to the total diet composition of unmarked and hatchery Chinook captured in the outer flats and nearshore sites. See Table 3 for zone composition of batches and Table 6 for code definitions. Diet items that contributed to less than 1% of the diet composition of all batches were combined into the "Less than 1%" item.

Hogum Bay Pocket Estuary

The Hogum Bay pocket estuary site diet analysis only consists of two batches of Chinook diets from 2005 (Figure 18). The time 1 unmarked Chinook batch of diets was nearly 77% Copepoda and 19% Gammaridea. Time 2 hatchery Chinook diets were primarily composed of Cumacea, Crustacea Nauplius, and Chironomidae.

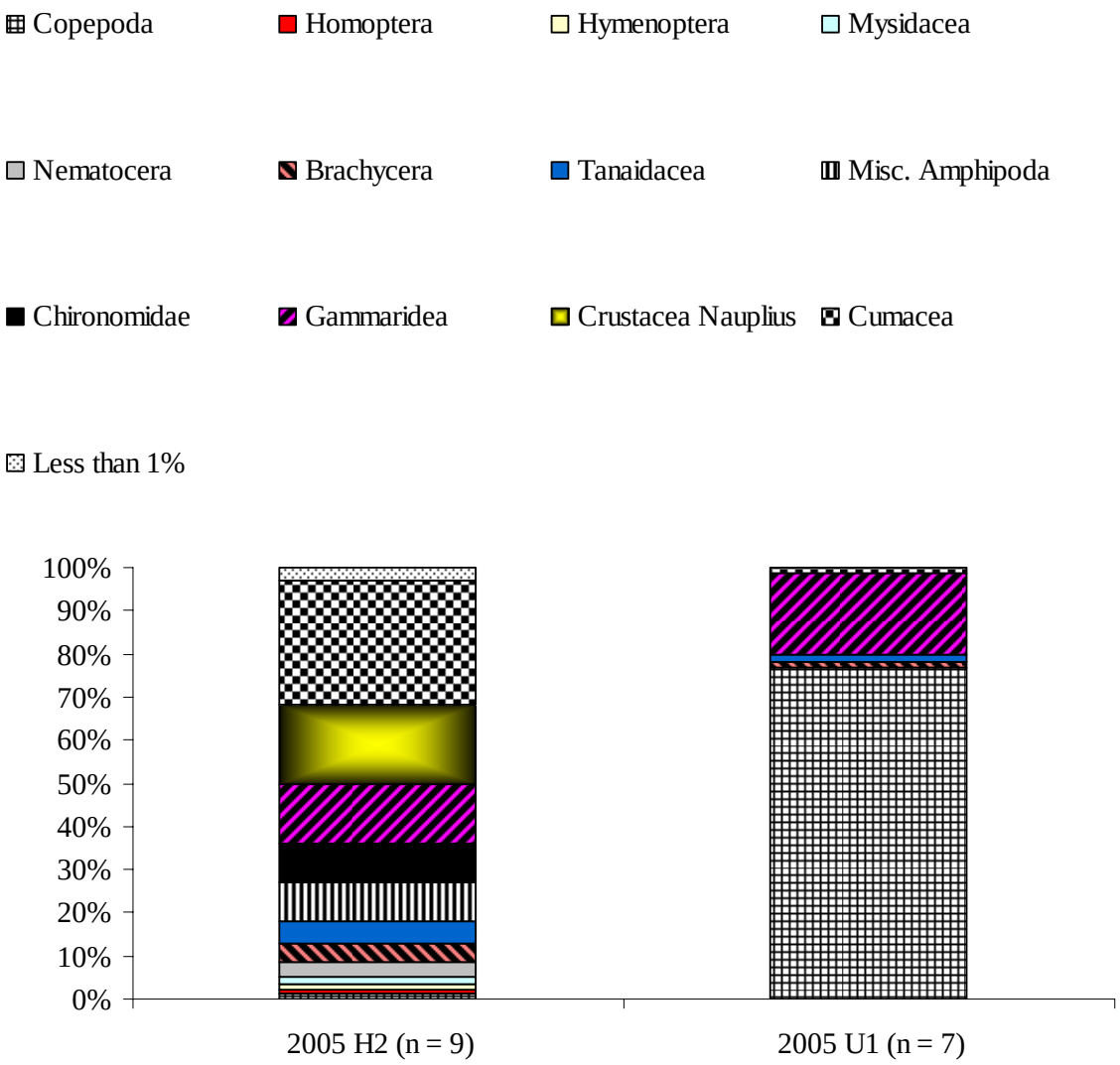

Figure 18. Percent contribution by number of primary prey items to the total diet composition of unmarked and hatchery Chinook captured in the Hogum Bay site. See Table 6 for sample code definitions. Diet items that contributed to less than 1% of the diet composition of all batches were combined into the "Less than 1%" item.

Percent Similarity Index

The percent similarity index (PSI) values (Equation 1) between all of the diet composition batches for 2004 is presented in Table 7 and for 2005 in Table 8. In 2004, the NIS EEM batches were similar in diet composition to batches from the Sub-Estuaries and Inner Flats with diet composition overlap as high as 73% and 80% for time 2 unmarked Chinook and hatchery Chinook, respectively (Table 7). The high PSI values for time 2 Chinook between these two batches are due to large contributions by Crustacea Nauplius, Gammaridea, Mysidacea, and Nematocera to the total diet composition (Figures 15 and 16). Time 1 unmarked Chinook diets from the Freshwater and Transition batches were 86% similar due to the dominance of Chironomidae in the diets of both batches (Figures 13 and 14).

The diet composition of time 2 unmarked Chinook captured in 2005 from the Sub-Estuaries and Inner Flats were very similar (PSI = 80%) to hatchery Chinook from the same time and area (Table 8). Both the hatchery Chinook and the unmarked Chinook batches contained similar proportions of Mysidacea, Gammaridea, Crustacea Nauplius, Nematocera, and Brachycera (Figure 16). The prevalence of Chironomidae in the diet compositions of the time 1 and 2 unmarked and hatchery Chinook Transition batches results in high PSI values ranging from 60% to 81% (Figure 14). The shift in diet to primarily Gammaridea by both hatchery and unmarked Chinook in the time 3 batches results in a 79% PSI (Figure 14).

Table 7. Percent Similarity Index values between diet composition batches from 2004. See Table 3 for zone composition of batches and Table 6 for code definitions. Bold indicates PSI values > 50%.

2004	Sub-Estuary & Inner Flats H2	Sub-Estuary & Inner Flats U2	Sub-Estuary & Inner Flats U1	Sub-Estuary & Inner Flats H3	NIS EEM H2	NIS EEM H3	NIS EEM U2
Sub-Estuary & Inner Flats U2	**58**						
Sub-Estuary & Inner Flats U1	**54**	**50**					
Sub-Estuary & Inner Flats H3	43	38	28				
NIS EEM H2	**80**	**56**	**52**	42			
NIS EEM H3	**52**	**57**	42	26	46		
NIS EEM U2	**65**	**73**	**54**	33	**65**	**63**	
NIS EEM U3	36	**54**	23	26	29	47	45

Table 7 (continued).

2004	Sub-Estuary & Inner Flats H2	Sub-Estuary & Inner Flats U2	Sub-Estuary & Inner Flats U1	Sub-Estuary & Inner Flats H3	NIS EEM H2	NIS EEM H3	NIS EEM U2	NIS EEM U3
Transition H2	**50**	**51**	**59**	17	40	38	**60**	49
Transition U1	9	7	6	1	7	6	8	30
Transition U2	31	32	42	7	30	20	31	28
Fresh H2	12	12	4	6	8	9	11	37
Fresh U1	10	9	4	5	7	7	9	32
Fresh U2	8	10	9	3	4	5	6	33
Fresh U3	4	4	0	2	1	7	5	23
Nearshore & Outer Flats H2	43	20	21	27	**50**	14	21	7
Nearshore & Outer Flats U2	23	34	20	12	23	**52**	29	20

Table 7 (continued).

2004	Transition H2	Transition U1	Transition U2	Fresh H2	Fresh U1	Fresh U2	Fresh U3	Nearshore & Outer Flats H2
Transition U1	20							
Transition U2	**56**	20						
Fresh H2	26	33	24					
Fresh U1	20	**86**	21	42				
Fresh U2	21	**59**	21	**64**	**66**			
Fresh U3	14	15	14	21	16	15		
Nearshore & Outer Flats H2	6	1	5	5	6	4	0	
Nearshore & Outer Flats U2	10	2	7	7	6	4	1	17

Table 8. Percent Similarity Index values between diet composition batches from 2005. See Table 3 for zone composition of batches and Table 6 for code definitions. Bold indicates PSI values > 50%.

2005	Sub-Estuary & Inner Flats H2	Sub-Estuary & Inner Flats U2	Sub-Estuary & Inner Flats U1	Transition H2	Transition H3	Transition U1	Transition U2	Transition U3	Fresh U1
Sub-Estuary & Inner Flats U2	**80**								
Sub-Estuary & Inner Flats U1	27	22							
Transition H2	32	31	24						
Transition H3	17	15	21	48					
Transition U1	19	20	19	**60**	19				
Transition U2	33	32	24	**67**	20	**81**			
Transition U3	32	26	28	**58**	**79**	27	32		
Fresh U1	15	17	11	**52**	13	**79**	**75**	22	
Fresh U2	18	20	12	31	15	31	34	26	43

Table 8 (continued).

2005	Sub-Estuary & Inner Flats H2	Sub-Estuary & Inner Flats U2	Sub-Estuary & Inner Flats U1	Transition H2	Transition H3	Transition U1	Transition U2	Transition U3	Fresh U1	Fresh U2
Hogum H2	**65**	**71**	25	33	25	22	29	29	14	19
Hogum U1	10	8	**60**	20	21	10	9	21	2	4
NIS EEM H2	26	22	28	**50**	45	20	24	**56**	12	13
NIS EEM U1	38	25	21	**72**	31	**52**	**54**	38	44	28
NIS EEM U2	36	32	27	**70**	25	**57**	**66**	40	49	32
NIS EEM U3	22	17	22	42	**68**	12	16	**74**	4	5
Nearshore & Outer Flats H2	**67**	**74**	19	23	12	16	23	19	13	15
Nearshore & Outer Flats U2	44	46	5	9	2	2	9	5	2	3

Table 8 (continued).

2005	2005 Hogum H2	2005 Hogum U1	2005 NIS EEM H2	2005 NIS EEM U1	2005 NIS EEM U2	2005 NIS EEM U3	Nearshore & Outer Flats H2
Hogum U1	19						
NIS EEM H2	27	19					
NIS EEM U1	31	21	35				
NIS EEM U2	36	14	**52**	**60**			
NIS EEM U3	24	20	**66**	26	45		
Nearshore & Outer Flats H2	**75**	10	16	21	23	12	
Nearshore & Outer Flats U2	31	1	2	12	7	7	38

Fyke Trap Results

Total Catch Composition

Shiner perch dominated the catch from the Restoration site trap, accounting for over 66% of the total catch (Table 9). At the Control and Animal sites sculpin were the most abundant fish, constituting over 51% and 56% of the total fish catch respectively. Shiner perch were also extremely abundant at the Control site, comprising over 40% of the total catch. At the Animal fyke trap, over 17% of the total catch consisted of Pacific sand lance and over 17% of the catch was shiner perch. Chum salmon were the most abundant salmonid at the Control and Animal sites, comprising over 7% and nearly 5% of the total catch at the two sites respectively (Table 9). Hatchery Chinook (0.58% of the total catch) were slightly more abundant than chum (0.31%) at the Restoration site. Unmarked Chinook were most abundant at the Animal site (0.65%) followed by the Control (0.23%) and Restoration (0.07%) sites. Several salmonid catches were unique to the Animal site, including 191 steelhead trout, 21 coastal cutthroat trout, and 1 native char (most likely a bull trout, *Salvelinus confluentas*, but a genetic sample was not taken).

Table 9. Fish catch summary for the Phase 1 Restoration, Red Salmon Slough Control, and Animal Slough fyke traps.

		Chum	Hatchery Chinook	Unmarked Chinook	Coho	Hatchery Coho	Pink	Steelhead	Coastal Cutthroat	Bull Trout/Dolly Varden	Mountain Whitefish
Phase 1 Restoration 2003-2005 19 Sampling Events	Total # Captured	235	435	53	1	4					
	% of Total Catch	0.311	0.575	0.070	0.001	0.005					
Red Salmon Slough Control 2003-2005 22 Sampling Events	Total # Captured	3235	143	105	1		16				
	% of Total Catch	7.117	0.315	0.231	0.002		0.035				
Animal Slough 2004-2006 30 Sampling Events	Total # Captured	2305	886	336	68	60	6	191	21	1	1
	% of Total Catch	4.45	1.7	0.650	0.132	0.116	0.012	0.37	0.04	0.002	0.002

		Shiner Perch	Sculpin	Sand Lance	Threespine Stickleback	Starry Flounder	Surf Smelt	Pacific Herring	Saddleback Gunnel	Unknown Cod	Total # Captured
Phase 1 Restoration 2003-2005 19 Sampling Events	Total # Captured	50385	13318	9810	1313	32		14			**75599**
	% of Total Catch	66.648	17.616	12.976	1.737	0.042		0.019			
Red Salmon Slough Control 2003-2005 22 Sampling Events	Total # Captured	18232	23406	2	54	256	2				**45452**
	% of Total Catch	40.112	51.496	0.004	0.119	0.563	0.004				
Animal Slough 2004-2006 30 Sampling Events	Total # Captured	9234	29063	9050	328	76	80	22	1	1	**51730**
	% of Total Catch	17.850	56.181	17.494	0.634	0.147	0.155	0.043	0.002	0.002	

Catch Timing of Chinook and Chum

Hatchery Chinook and unmarked Chinook were captured at the Restoration site on the first trapping event in May 2003, less than a year after dikes at the site were breached (Figure 19), although 2003 densities were very low (<0.0005 fish/m^2). Unmarked and hatchery Chinook catches increased in 2004 with peak catches of 0.001 (June 7th) and 0.012 (June 7th) fish/m^2, respectively. Densities of unmarked and hatchery Chinook were slightly reduced in 2005, with peak catches of 0.0007 (May 12th) and 0.006 (May 12th) fish/m^2, respectively.

Unmarked and hatchery Chinook were captured at the Control site from May through June 2003 with peak densities of 0.0065 unmarked Chinook/m^2 on May 7th and 0.03 hatchery Chinook /m^2 on May 21st (Figure 19). Catches of unmarked and hatchery Chinook declined in 2004 at the Control site to 0.001/m^2 (May 12th) and 0.01/m^2 (May 12th) respectively. The highest densities of unmarked and hatchery Chinook at any site were recorded at the Control site in 2005; unmarked Chinook catch peaked at 0.055/m^2 on April 28th and hatchery Chinook catch reached 0.032/m^2 on May 11th.

Sampling was not conducted at the Animal site in 2003. In 2004, unmarked Chinook were captured at each sampling event from May 25th to July 22nd, peaking on June 9th at 0.001/m^2 (Figure 19). Hatchery Chinook were captured from May 11th to July 22nd and peaked on May 25th at 0.013/m^2. The longest temporal distribution of unmarked Chinook occurred at the Animal site in 2005, where they were captured at each sampling event from March 18th to August 10th. The 2005 peak catch of unmarked Chinook was 0.002/m^2 on March 16th and hatchery Chinook peaked at 0.001/m^2 on March 31st. In 2006 unmarked Chinook were captured at the Animal site from May 19th to the last sampling effort on August 14th, with a peak catch of 0.005/m^2 on June 29th. Animal site catch densities should be considered very conservative, due to very low catch efficiency at the site (see Methods).

Chum salmon were most abundant in the Red Salmon Slough Control site, with a peak density of nearly 2.0/m^2 on May 11th, 2005 (Figure 20). The largest catch of chum at the Animal Slough was in mid-May 2005, similar to the Control site, but at a much lower density (0.04/m^2). The highest density of chum at the Restoration site was nearly 0.008/m^2, observed on May 24th 2004.

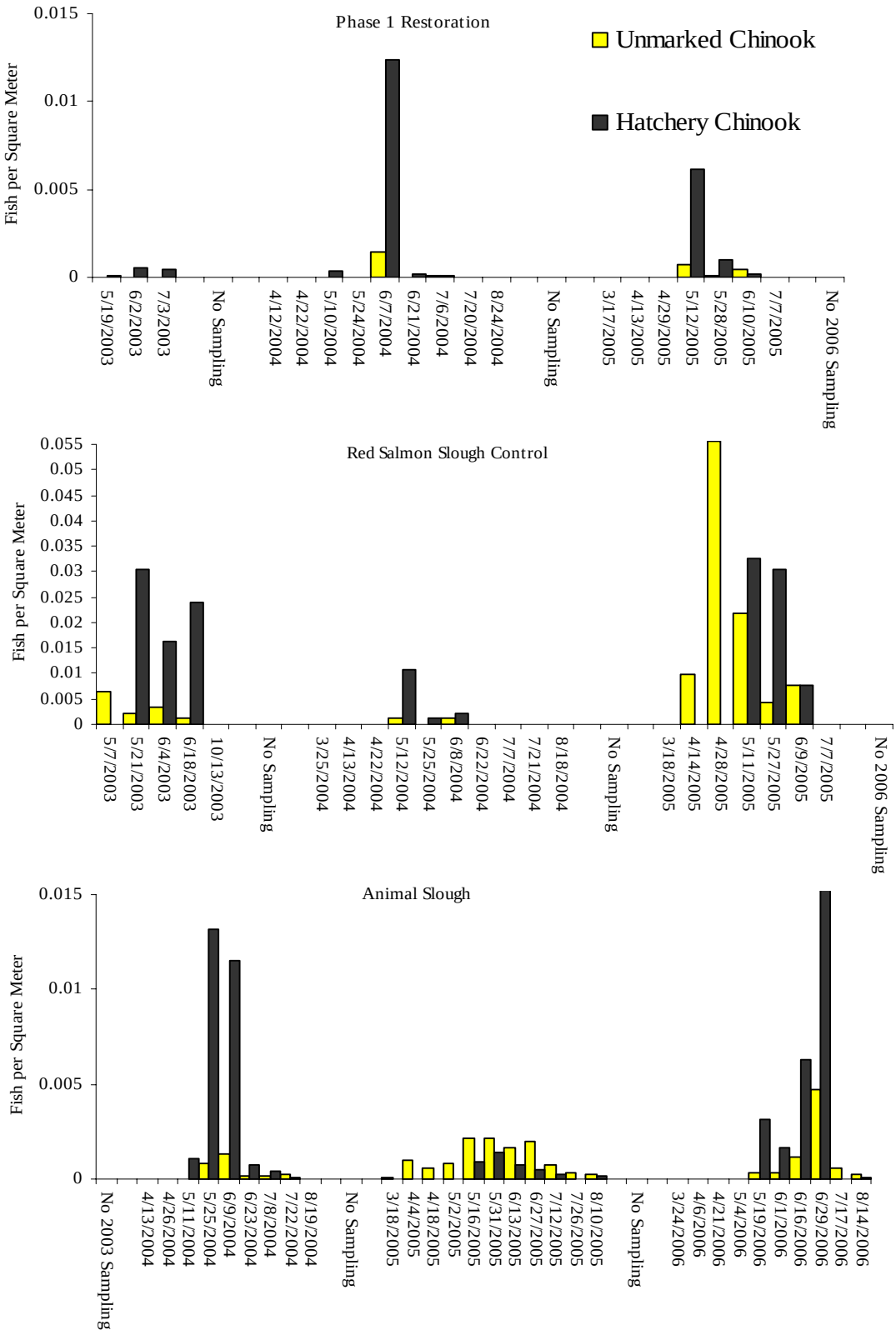

Figure 19. Unmarked and hatchery Chinook catch per square meter from the Phase 1 Restoration, Red Salmon Slough Control, and Animal study sites: 2003-2006.

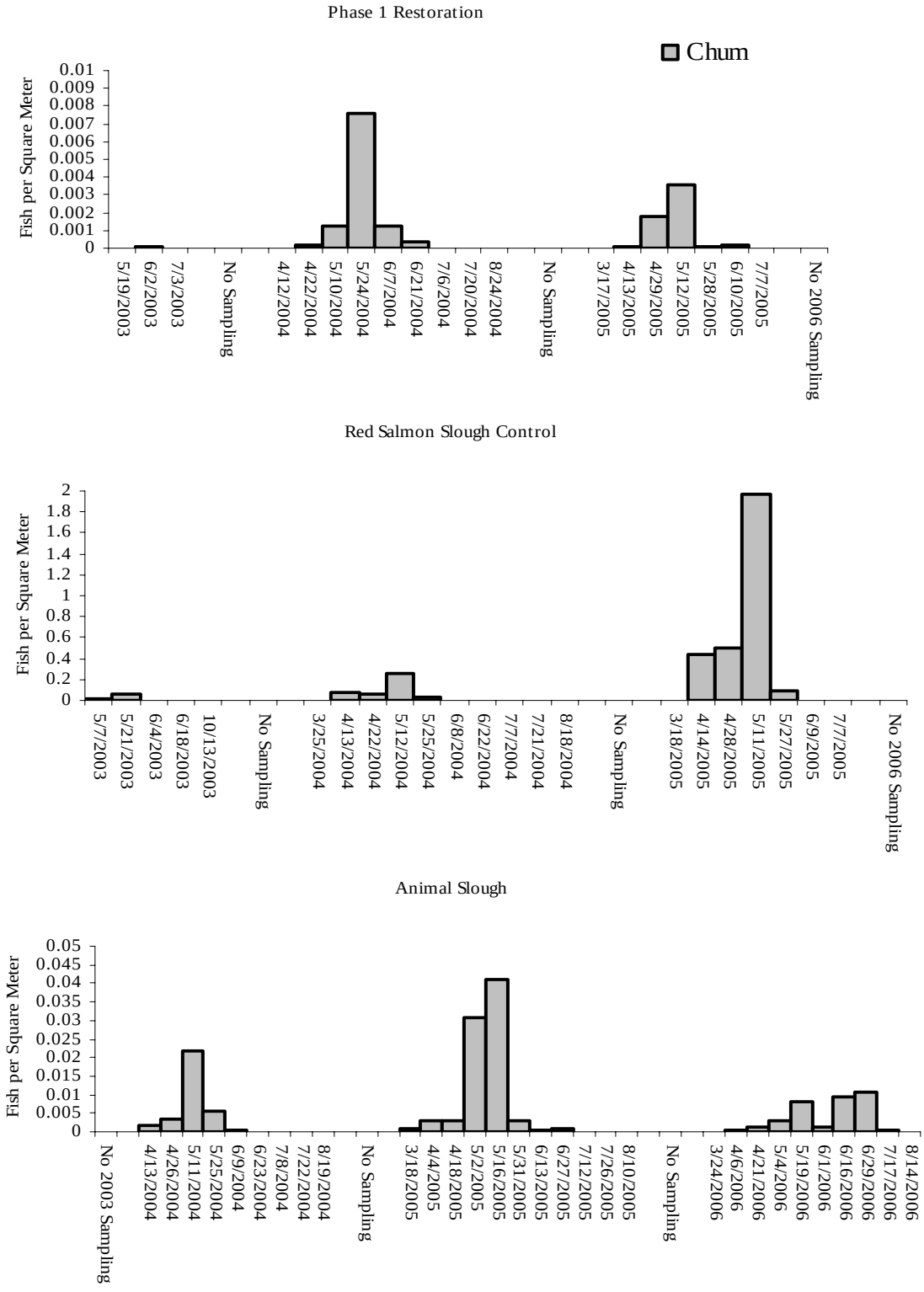

Figure 20. Chum catch per square meter from the Phase 1 Restoration, Red Salmon Slough Control, and Animal study sites: 2003-2006.

Invertebrate Densities

Insect densities in the March fallout trap samples at the Restoration, Control, and Animal sites were 468, 380, and 687 insects/m^2 respectively (Figure 21). The Brachycera suborder of flies, primarily Dolichopodidae, made up nearly half of the insect catch at the Restoration site in March. The Control site was dominated by Chironomidae and the Animal site insect catch was divided nearly equally between Psychodidae, Chironomidae, and Brachycera flies. The Restoration and Control site fallout trap catches increased in May to 1,647 and 1,084 insects/m^2 respectively, while the Animal site catch remained constant at 686 insects/m^2. Brachycera flies accounted for over half of the insect density at the Restoration site in May. Acari and Chironomidae were the primary taxa captured at the Control site while Chironomidae made up nearly half the catch at the Animal site in May. The July insect catch was characterized by dramatic increases in the densities at the Restoration and Animal sites to 5,337 and 4,732 insects/m^2 respectively; the increase at both sites was driven by an explosion in the abundance of Brachycera flies. The July Control site catch actually decreased to 624 insects/m^2 and was composed of primarily Brachycera and Homoptera insects.

The March Restoration benthic core samples had extremely high densities of Annelida with over 1.4 million/m^3. Other organisms with moderate to high densities included Nematoda (314,745/m^3) and Copepoda (81,487/m^3) (Figure 22). The March Control site benthic sample consisted of primarily Annelida (149,733/m^3) and Copepoda (91,673/m^3). The Animal site March benthic sample was dominated by very high densities of Annelida (824,041/m^3) and Gammaridea (374,842/m^3). In May, the Restoration and Control benthic samples were similar in density (415,585 and 489,943 invertebrates/m^3, respectively) and composition, with Annelida dominating the samples. The May Animal benthic sample density was 283,000 invertebrates/m^3 with Gammaridea accounting for 210,848/m^3. Annelida and Nematoda comprised the bulk of the July Restoration benthic sample (624,736 invertebrates/m^3). The July Control benthic sample was primarily Annelida, for a total density of 578,900 invertebrates/m^3. The Animal benthic sample had high densities and diversity of invertebrates in July with Gammaridea (882,779/m^3), Annelida (359,902/m^3), and Isopoda (81,487/m^3) the most abundant taxa.

Harpacticoid copepods were the most abundant organisms in the neuston net collections from all sites and times except for the Animal site in May, which consisted of primarily Ostracods (Figure 23). Harpacticoids were especially dense in the Restoration site in May, with an estimated density of over 6,700 per cubic meter.

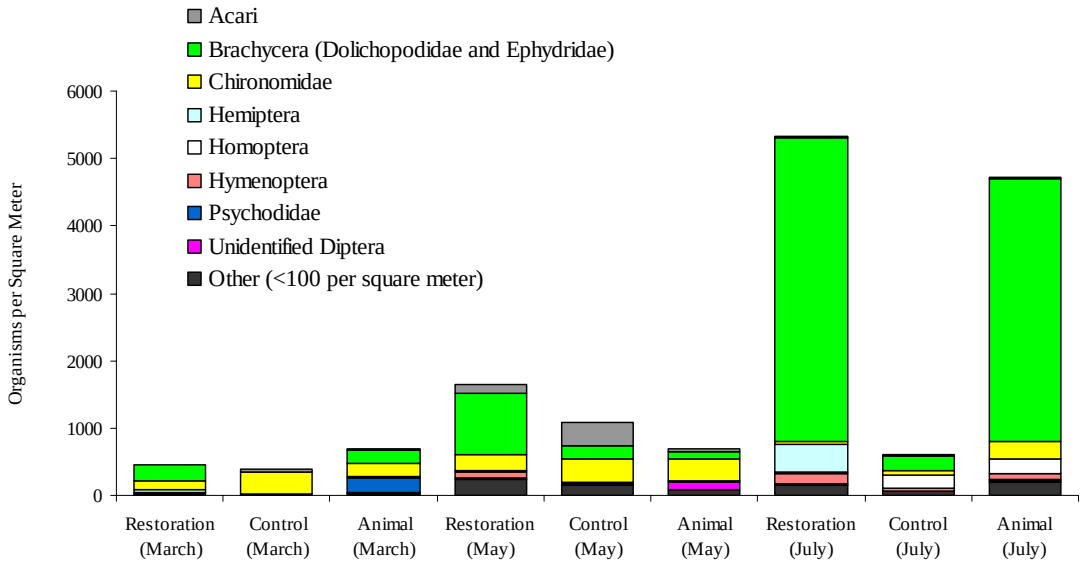

Figure 21. Density of invertebrates sampled with insect fallout traps at the Restoration, Control, and Animal monitoring sites from March, May, and July 2005.

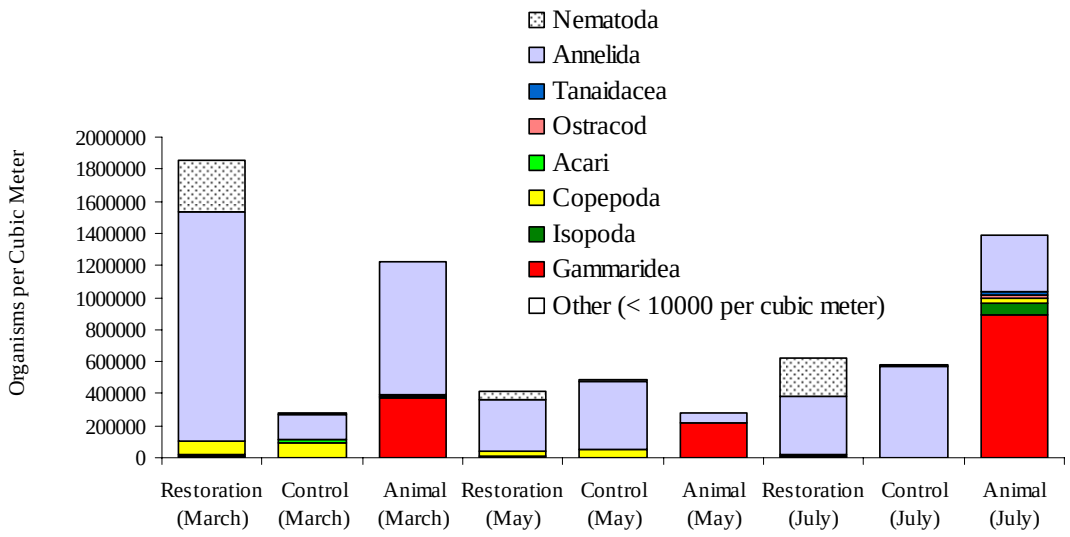

Figure 22. Density of invertebrates sampled with a benthic corer at the Restoration, Control, and Animal monitoring sites from March, May, and July 2005.

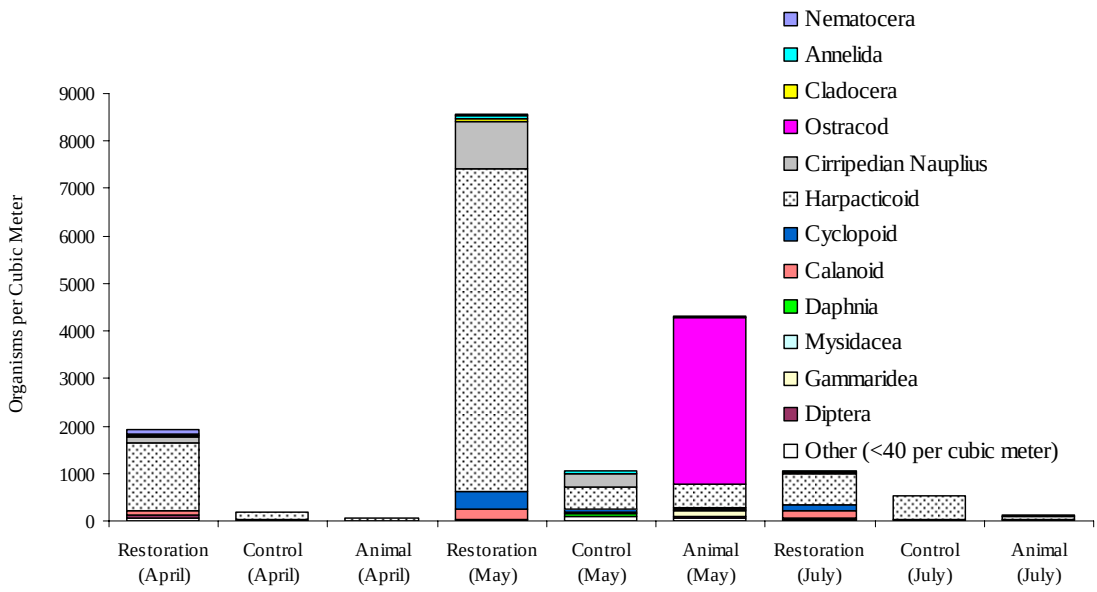

Figure 23. Density of invertebrates sampled with a neuston net at the Restoration, Control, and Animal monitoring sites from March, May, and July 2005.

Diet Composition at Fyke Trap Sites

The percent contribution (by number) of prey items to the total diet of unmarked and hatchery Chinook salmon captured from the Restoration, Control, and Animal sloughs between May 1st and June 30th in 2004 and 2005 are presented in Figure 24. Diets of hatchery and unmarked Chinook captured from the Restoration site were composed of over 80% Brachycera flies in 2004 and nearly 70% in 2005. Unmarked and hatchery Chinook captured at the Control site had a more diverse diet than fish from the Restoration site, with Nematocera and Brachycera flies making up the largest proportion of the diets. Chinook diets from the Animal site differed from both the Control and Restoration sites due to the contribution of crustaceans; Copepoda accounted for 68% of 2004 Animal hatchery Chinook diets, Mysidacea were 28% of the total diet of 2004 unmarked Chinook, Mysidacea and Gammaridea contributed 38% and 36% to the diets of 2005 hatchery Chinook respectively and each accounted for 22.5% of the diets of 2005 unmarked Chinook. Aphidae were also important prey items to unmarked Chinook trapped at the Animal site in 2004 (29%) and Chironomidae were important for unmarked Chinook trapped at the site in 2005 (32%).

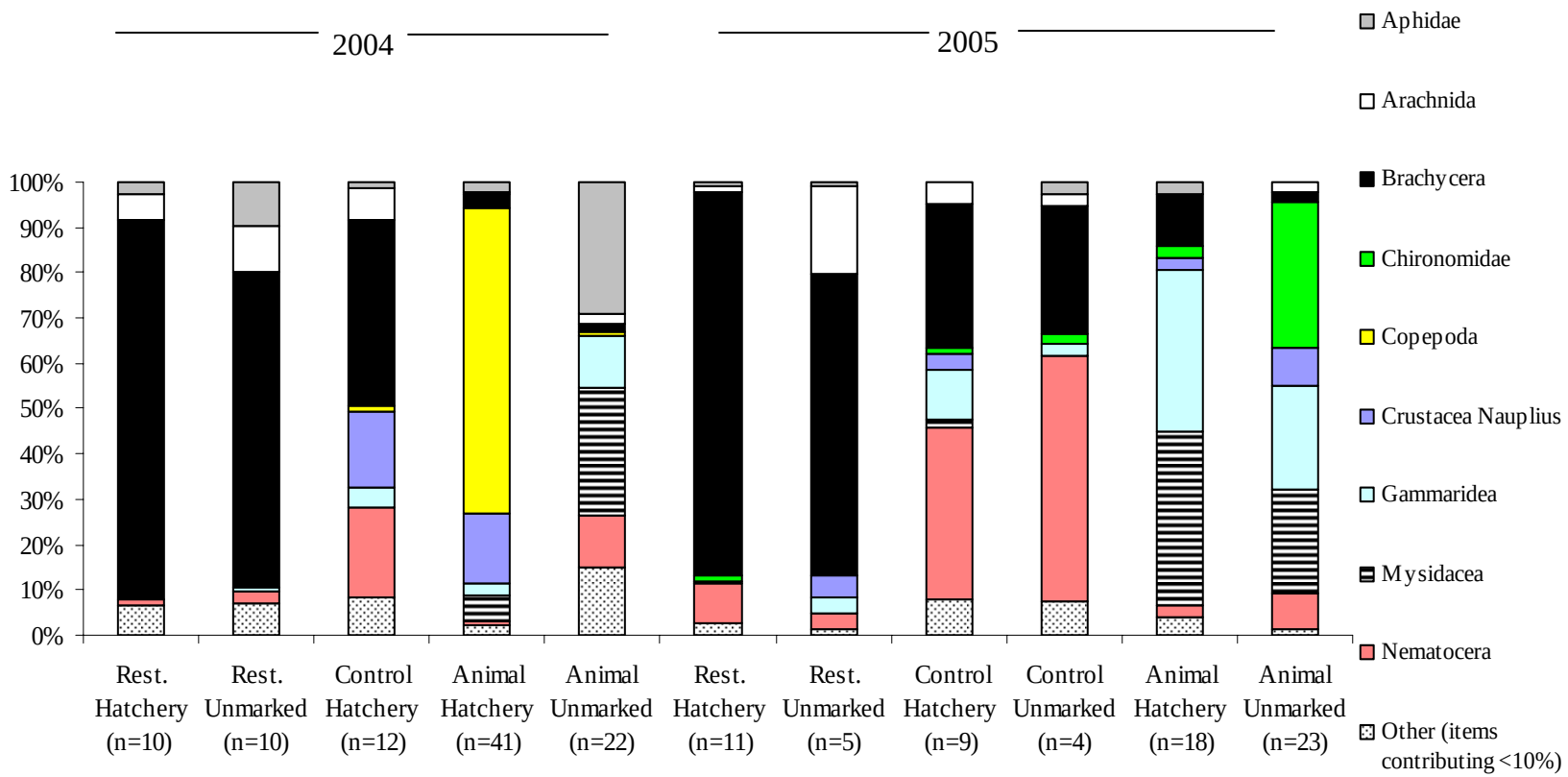

Figure 24. Percent contribution by number of prey items to the total diet composition of unmarked and hatchery Chinook captured at the Restoration (Rest.), Control, and Animal monitoring sites between May 1st and June 30th in 2004 and 2005.

Percent Similarity Index (PSI) values comparing the contribution of organisms to the total stomach contents from Chinook caught in 2005 at each of the three sample sites to the contribution of the same organisms to the total benthic and fallout trap samples from the same sites in 2005 are listed in Table 9. The highest similarity between what was sampled in the environment and what was found in Chinook diets occurred at the Restoration site. The diets of hatchery and unmarked Chinook salmon caught between May 1st and June 30th were 64% and 60% similar to the fallout trap samples from May and 88% and 68% similar to those from July, respectively. At the Control site, hatchery Chinook diets were 43% similar to the July fallout samples and unmarked Chinook diets were 38% similar to the July fallout samples. There was little similarity between benthic samples and Chinook diets (<1.5%) at both the Restoration and Control sites. Chinook diets at the Animal site were moderately similar to both the core and fallout trap samples from May and July.

Table 10. Percent similarity index (PSI) comparing composition of stomach contents from Chinook caught at each of 3 sample sites to invertebrate samples (benthic cores and insect fallout traps) gathered at the same sites. Stomach samples were taken between May 1 and June 30, 2005, and were compared to invertebrate trap samples from both May and July 2005. Bold indicates PSI > 50.

Sample Site	Benthic Cores				Insect Fallout Traps			
	May		July		May		July	
	H	U	H	U	H	U	H	U
Animal	36	23	36	22	17	37	16	10
Restoration	0	1	0	1	**64**	**60**	**88**	**68**
Control	0	0	0	0	23	24	43	38

Discussion

The results of over 980 beach seine sets spread out over 26 months between 2004-2006 and from over 70 fyke trapping events provides us with a working 'template' of fish ecology in the Nisqually River, estuary, and adjacent nearshore from which to predict and measure the effects of large-scale estuary restoration. Our fish ecology assessment includes general community composition, temporal and spatial distribution, hatchery and unmarked Chinook co-occurrence, Chinook salmon prey composition, and unmarked Chinook salmon residence time and growth in the estuary. In addition, by assessing juvenile Chinook use of restoring and reference blind channel sloughs using three metrics (opportunity, capacity, and realized function); we formulated specific hypotheses about the localized functional response of Chinook to structural changes resulting from estuary restoration.

General Fish Ecology Summary

Total fish abundance, as indicated by beach seine catches, in the Nisqually estuarine zones (FRT, EFT, all EEM, and Flats) peaks in May, averaging over 150 fish captured per set, and June, averaging over 110 fish per set (Figure 25). The May peak catch is primarily composed of hatchery Chinook, followed by Pacific sand lance, chum, sculpin, shiner perch, and unmarked Chinook. The June catch is predominantly shiner perch, which enter the estuary to birth fully developed young, and hatchery Chinook.

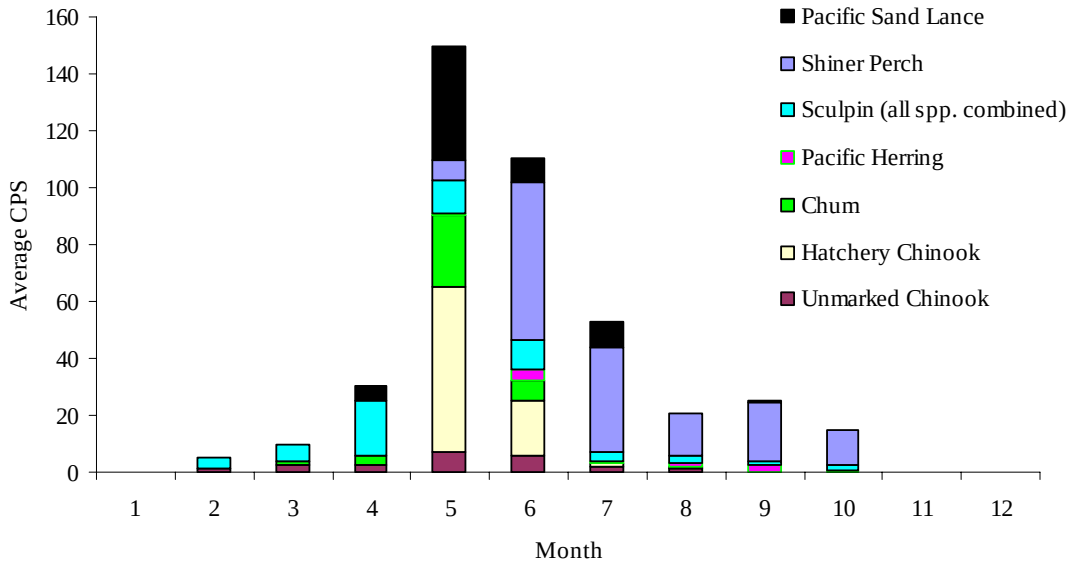

Figure 25. The average catch per set (CPS) per month of the primary fish species captured in the Nisqually estuarine zones (FRT, EFT, all EEM, and Flats), 2004-2006.

Estuary habitat partitioning in space and time is apparent between hatchery Chinook, unmarked Chinook, chum, and shiner perch (the most abundant estuarine fish) although considerable overlap does exist. Most chum salmon were caught between April and May, on average earlier than hatchery Chinook, and were most abundant in freshwater, FRT, and nearshore zones. Following hatchery Chinook releases in the Nisqually River in May, catch data indicated that the majority of these fish spent little time in the freshwater and FRT zones, but that they were caught in high numbers in the saltier zones during May and June, especially in the lower Nisqually River (EFT and NIS EEM zones). Unmarked Chinook salmon, which are much less numerous in the system than chum or hatchery Chinook, had a broader distribution in time and were caught prior to, during, and after the period of hatchery Chinook presence. Unmarked Chinook also appeared to have a broad geographic use of the system; however they were captured most frequently in the freshwater, FRT, EFT, and NIS EEM zones. Peak catches of shiner perch occurred in June and July with high average catches in the RSS EEM, MCA EEM, nearshore, and Flats zones (Figure 26).

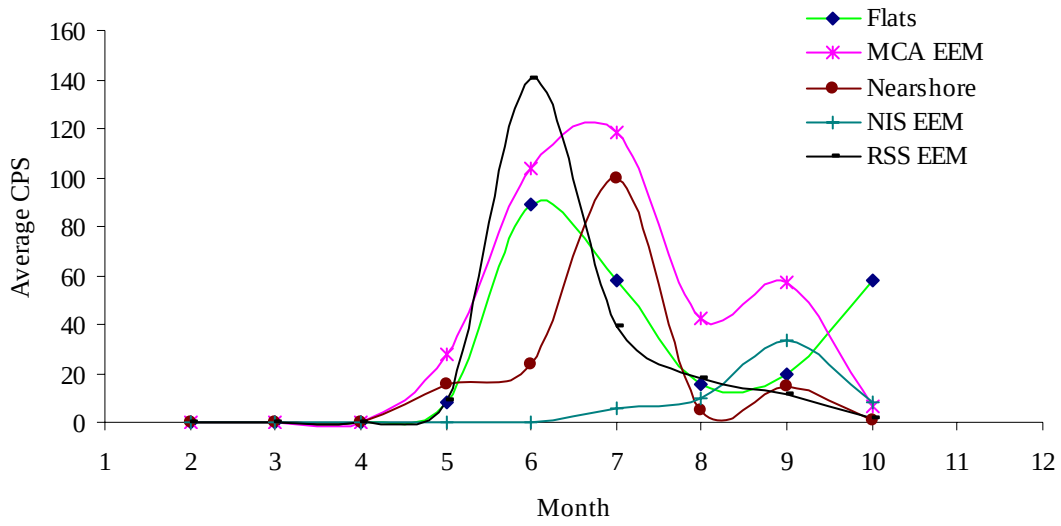

Figure 26. The average catch per month of shiner perch from the primary zones utilized, 2004-2006.

The distribution trends for shiner perch are also apparent in the fyke trap data, with high density shiner perch catches made in late June and July at the Restoration and Control sites which are in the RSS EEM zone, versus relatively low density captures at the NIS EEM zone Animal site. Unmarked Chinook and hatchery Chinook catches overlapped substantially at the three blind channel sloughs which were fyke trapped, especially in the Animal site (Figure 19) indicating that the NIS EEM zone is a heavily utilized zone for Chinook in general. High density catches of chum were made in the Control and in the Animal blind channels even though beach seine catches of chum in the RSS EEM and NIS EEM zones were not correspondingly large, indicating a preference for blind channel slough habitat in the estuarine emergent marsh zones.

Unmarked Chinook and hatchery Chinook preyed on a variety of riparian and marsh insects, epibenthic and planktonic crustaceans, and other organisms. The general trend was for smaller Chinook early in the season to consume primarily insects, especially in the freshwater and transitional zones, and then a mix of insects and miscellaneous crustaceans as they grow larger.

Unmarked and hatchery Chinook diets had variable levels of similarity when they co-occurred in the same geographical area and time period (Table 7). Their diets were most similar in the transition zones in times 2 (May to June) and 3 (July to October), the NIS EEM zone in time 2, and the sub-estuaries/flats zones in time 2. However, the utility of the diet data for assessing resource competition between unmarked Chinook and hatchery Chinook is limited because the diets are batched from multiple sampling events across a time period and, in some cases, from multiple zones. Plus, batching the samples made assessing the consumption rate of individual fish unfeasible. Imperfect mark rates at the Nisqually hatcheries confound hatchery and wild Chinook comparisons even further.

Small Scale Restoration Monitoring

The purpose of this specific component of the project was to assess the ecological performance of restoring and reference estuary habitats for juvenile Chinook. The ecological performance of estuarine salt marsh habitats was measured at three levels for Chinook: opportunity, capacity, and realized function (adapted from: Simenstad and Cordell 2000; Simenstad et al. 2001; Gray et al. 2002).

Opportunity is the ability of juvenile salmon to access the estuarine habitat. We measured opportunity by determining the density and timing of salmonid usage of the restoring and reference habitats through fyke trapping. Capacity is defined as habitat attributes that produce conditions conducive to juvenile salmon growth and survival. Capacity was measured by determining the occurrence and abundance of salmonid prey organisms at the study sites through benthic core sampling, insect fallout trapping, and neuston sampling. The realized function of habitat for juvenile salmon can be assessed using direct measures of physiological and/or behavioral responses resulting from fish occupancy of the habitat. The diet composition of juvenile hatchery and unmarked

Chinook in conjunction with the invertebrate sampling was used to examine the realized function of the restoring and reference estuarine habitats.

Unmarked and hatchery Chinook took advantage of the opportunity to utilize the Phase 1 Restoration site less than a year after the breaching of the dikes, as evidenced by the catch data (Figure 19). Chum salmon, shiner perch, sculpin, Pacific sand lance, and others also took advantage of the newly restored site (Table 9; Figure 20).

The temporal distribution at the Restoration site was similar to the nearby Control site, and both had a much narrower timing distribution than the Animal site. The broader temporal distribution of Chinook at the Animal site may be due to differences in abiotic factors such as temperature and salinity which are moderated by the river, providing a longer period of conditions conducive to estuary rearing.

The overall density of Chinook at the Restoration site was much less than at the Control site but similar to the Animal site. However, the Animal site catch densities are considered to be conservative because efficiency tests at this site have yielded much lower trap efficiency estimates compared to the other two trap sites. The high density at the Control site could be due to its small size because fish density and site area are likely not linearly correlated. Other differences between the sites that make direct comparisons difficult include proximity to refuge at low tide, proximity to the mainstem Nisqually, and level of channel network development.

The Restoration site insect community was dominated by the Brachycera suborder of flies (over 50% of organisms caught in fallout traps in May and almost 90% in July). These flies constituted the bulk of Chinook prey items from the site, as evidenced both in the Chinook diets and in the high percent similarity index (PSI) between the fallout trap catch and the diet composition (Figure 24; Table 10). The diets of Chinook at the Control site had very little to moderate similarity with the insect community and no similarity with the benthic community as sampled at the site. At the Animal site the PSI did reflect moderate to high utilization of both insect (fallout) and crustacean (benthic) prey. The interpretation of the diet data at the Control and Restoration sites is limited by short fyke trapping durations (less than three hours), so there could be carryover in the diet data from feeding that occurred off site. However, the PSI at the Restoration site does provide strong evidence that these fish are feeding at the site and taking advantage of the site's capacity. The PSI should be considered a conservative estimate of the similarity between the composition of the diet and the composition of the invertebrate community because our invertebrate sampling methods may have been biased towards certain taxa or ineffective at capturing important prey items (i.e., Mysidacea).

Percent similarity index values were not computed between Chinook diets and the neuston net samples due to different levels of taxonomic detail between the two data sets. The highest density of neuston organisms, almost 9,000/m^3, were sampled in May at the Restoration site. The vast majority of these organisms were harpacticoid copepods, which are an important prey item for juvenile chum (Pearce and Meyer 1982). The peak

catch of harpacticoids corresponds with the average peak catch of chum at the Restoration site.

Nisqually Wild Chinook Estuary Residency and Growth

A microstructure analysis of wild[2] Nisqually Chinook otoliths conducted by the United States Geological Survey (USGS) indicates that those wild Chinook entering the estuary in late May to June may rear in the estuary for over a month, with a conservative average estuary residency of 16 days (Appendix D). Preliminary otolith results show growth rates in the delta were an average of 36% (0.57 mm/day) higher than in freshwater (0.42 mm/day). Wild Chinook first entering the delta to rear averaged 72.8 mm. The longer residency and higher growth rates of Chinook which rear in the estuary coincide with apparent peak insect production based on our limited invertebrate sampling. The USGS report does indicate that multiple life-history strategies may be expressed by wild Nisqually Chinook, but the data are too limited to accurately define these potential life history types.

The USGS researchers found no difference in wild Chinook otolith microstructure between the freshwater and FRT zones. Our freshwater sites were influenced by tidal elevation changes, but did not have measurable salinity and our FRT sites had very dilute salinity (Table 1). In addition, both zones had similar temporal distribution of unmarked (presumed wild) Chinook (Figure 5) so should be considered one zone. Future research should expand the FRT zone to include the freshwater tidal portion of the lower Nisqually River; this would probably be up to the Mounts Road Bridge. Freshwater sites should be located even further upstream. The FRT and freshwater tidal zones are heavily utilized zones in February through May (Figure 5) and appear to be important for unmarked Chinook pre-estuary entry growth.

Regional Significance of the Nisqually Estuary for Non-Natal Chinook

A total of 237 lethal Chinook samples were taken for coded wire tag (CWT) verification of hatchery origin since juvenile Chinook sampling started in 2002 by the Nisqually Indian Tribe. The majority of the Chinook CWTs sampled were from Nisqually River hatcheries (n = 175), but 26.2% (n = 62) of the tags read came from outside the Nisqually watershed (Figure 27). Some of these Chinook were released from hatcheries in South Puget Sound watersheds, including the Deschutes River (n = 5), Chambers Creek (n = 15), and Minter Creek (n = 4). Some of the CWT recoveries were released from hatcheries in Central and North Puget Sound watersheds including the Puyallup River (n = 33, including 17 spring Chinook from the White River), the Duwamish River (n = 4), and Snohomish River (n = 1). These numbers are raw and are not adjusted for differential mark rates or numbers of fish released at each hatchery. However, even taken conservatively, the CWT data indicates that the Nisqually River estuary is regionally significant for natal and non-natal Chinook.

[2] Otolith analysis confirms natural origin, thus referred to as ‘wild’ and not ‘unmarked’.

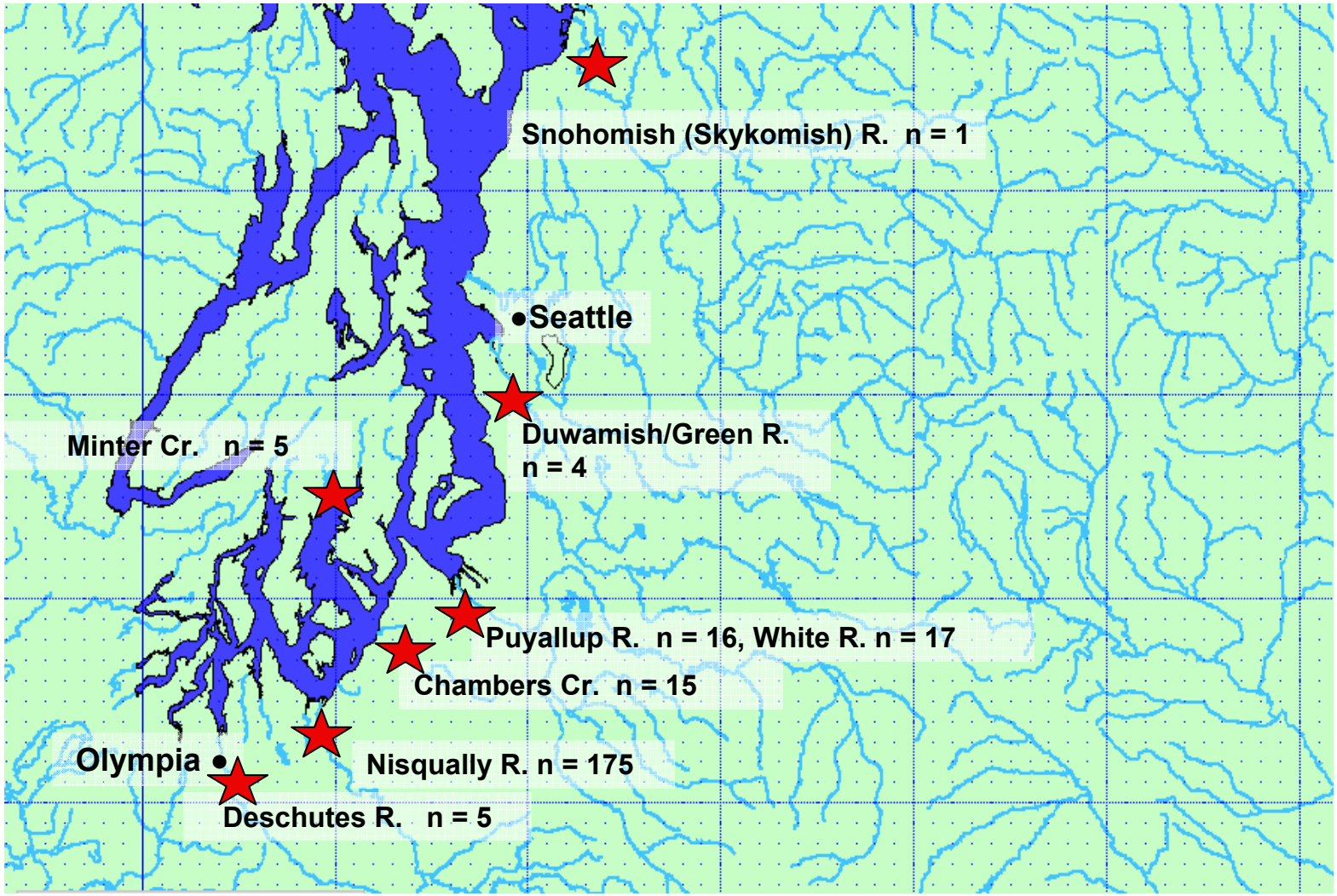

Figure 27. Hatchery of origin for coded wire tagged hatchery Chinook captured in the Nisqually River estuary, 2002-2006.

Nisqually Estuary Restoration Hypotheses

In order to help develop testable hypotheses and to stimulate discussion about the influence of the approximately 700 acre large-scale Nisqually estuary restoration project on juvenile Chinook salmon and other fishes, we propose a rough conceptual model (Figure 28) based on the Level 4 change/action submodel developed by the Puget Sound Nearshore Partnership, Nearshore Science Team (NST) (Simenstad et al. 2006). The NST Level 4 model involves an anthropogenic action (i.e., the estuary restoration project) which affects ecosystem processes, changing habitat structure, resulting in functional responses of target organisms. Each element of the conceptual model is a hypothesized change induced by the restoration project and can be tested. Unlike the NST model, our rough model does not grade degrees of uncertainty; in fact we leave the relationship connections (arrows) blank because they are outside the scope of this study, in need of more discussion, and/or are topics for more analysis. For this particular exercise, we used unmarked (i.e., wild) Chinook as our target organism.

Our conceptual model was populated using the following sources: The modeled restoration action is described in detail in the Nisqually NWR CCP (USFWS 2005); the anticipated restored processes are based on the findings of a Hydrodynamic and Sediment Transport Model (HST) developed to aid the planning and selection of the restoration action (ENSR 1999); our habitat structural change predictions are based on physical and vegetation monitoring of the Tribe's Phase 1 Restoration project (Bartlett et al. 2004) and the findings presented in this study; and we hypothesize functional responses of Chinook based on the findings of this study.

Our specific hypothesized structural and functional outcomes for Chinook (Figure 28; highlighted in light green) are: (1) Chinook will access the new and historic habitat within the project area. (2) The restoring emergent marsh, scrub shrub, and riparian habitat will produce Chinook prey organisms. (3) Chinook will prey on these organisms. (4) Chinook will, on average, reside longer in the estuary than before the project. (5) Chinook will grow more, on average, in the estuary than before the project. Undoubtedly, there will be additional functional outcomes for Chinook (e.g. increased life history diversity) but we do not yet have enough Nisqually specific information to formulate additional hypotheses.

The stated Chinook focused hypotheses are testable using similar methodologies used in this study, including continued otolith analyses with the USGS. Future research should attempt to conduct a more rigorous Chinook diet analysis by keeping the diet samples un-batched, permitting the use of diet metrics that can better quantify the importance of individual taxa, like the index of relative importance (Gray et al. 2002), and allow for site and time-specific diet comparisons. Invertebrate sampling should also continue with increased sampling frequency and over a larger geographic area in order to assess spatial and temporal patterns of invertebrate abundance and diversity in relation to habitat structure.

Monitoring the Tribe's Phase 1 Restoration project has been invaluable for improving our understanding of how organisms (primarily Chinook salmon) respond to localized, small scale estuary restoration, permitting us to develop testable restoration hypotheses. Based on our invertebrate and diet analyses we concluded that the Phase 1 project was indeed providing habitat functions for Chinook, but that those functions were fairly localized. By extrapolating out the functional performance of the 40 acre Phase 1 project, we anticipate that the cumulative effects of the Phase 1, Phase 2 (100 acres completed in 2006, monitoring started 2007), and 700 acre Refuge restoration will result in habitat functions conducive to growth, residency, and ultimately survival of natal and non-natal Chinook and other estuarine organisms, which will permeate throughout the entire Nisqually River delta and possibly into the Nisqually Reach nearshore environment.

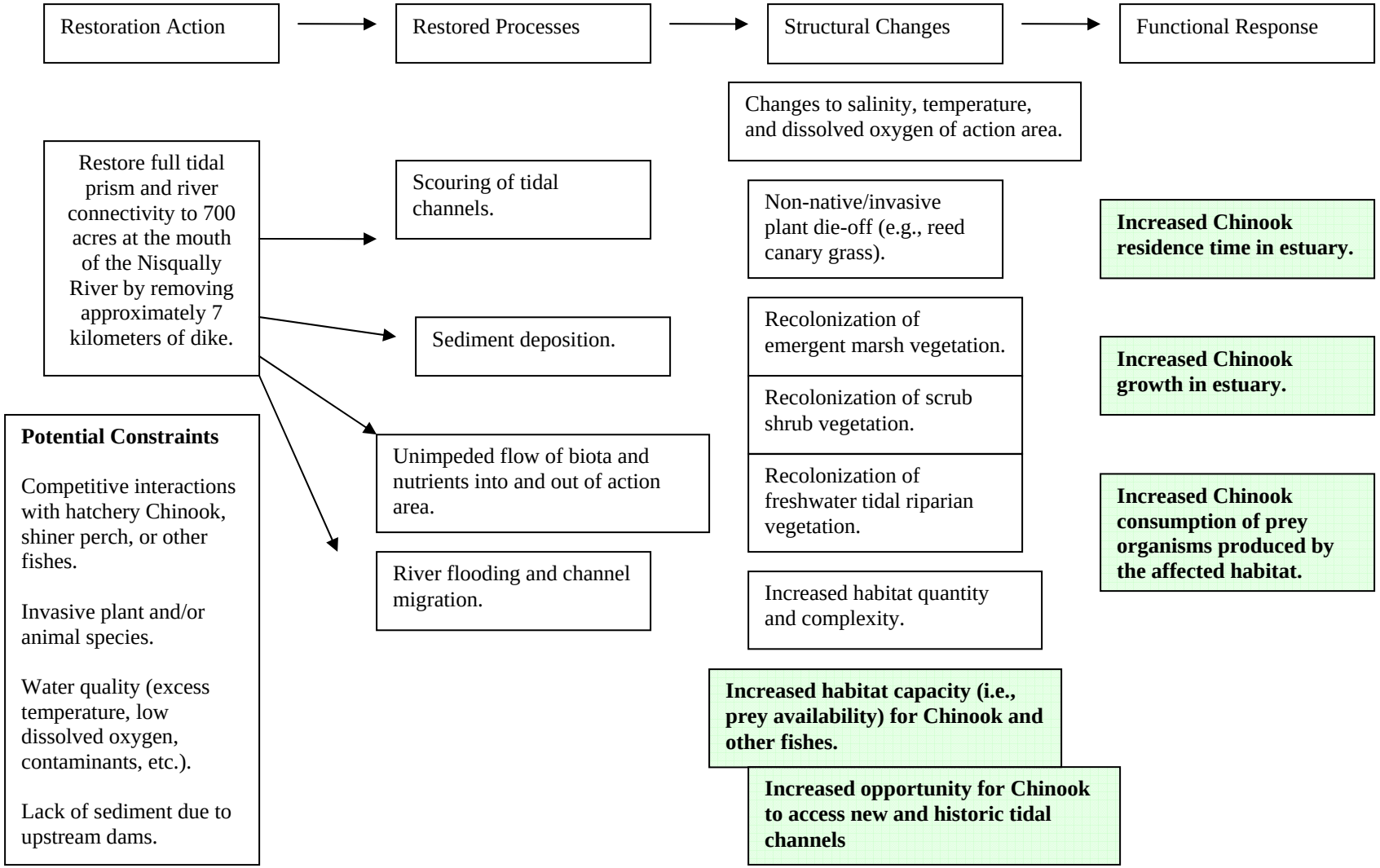

Figure 28. Generalized conceptual model (adapted from Simenstad et al. 2006) used to develop restoration effect hypotheses.

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Appendix A

Nisqually River, Estuary, and Nearshore Beach Seining Sites: 2004-2006

Appendix A. Nisqually fish ecology study beach seine site characteristics: 2004-2006.

Zone	Site	Map Reference Number	Totals Number of Sets in 2004	Totals Number of Sets in 2005	Totals Number of Sets in 2006	Mean Tide Sampled (ft)	Mean Surface Salinity (ppt)	Mean Surface Dissolved Oxygen (mg/l)	Mean Surface Temperature (C)	Mean Near Bottom Salinity (ppt)	Mean Near Bottom Dissolved Oxygen (mg/l)	Mean Near Bottom Temperature (C)
Freshwater	I-5 Alcove	50	0	4	0	N/A	0.1	10.5	9.6	0.1	11.3	9.1
	I-5 RB	14	0	0	10	N/A	0.0	12.2	12.3	0.0	11.4	12.5
	Mounts Alcove	42	15	16	6	N/A	0.0	10.0	12.2	0.0	9.2	12.0
	Wa He Lut	51	0	1	0	N/A	0.1	10.5	6.2	0.1	11.0	6.3
Freshwater Summary			***15***	***21***	***16***	N/A	***0.0***	***10.3***	***12.1***	***0.0***	***9.6***	***11.9***
FRT	Lookout	8	15	16	16	8.9	0.0	10.7	12.8	0.1	10.9	12.7
	LookoutII	49	0	2	0	12.9	0.1	9.9	8.6	0.2	9.5	8.7
FRT Summary			***15***	***18***	***16***	***8.9***	***0.0***	***10.7***	***12.7***	***0.1***	***10.9***	***12.6***
EFT	Kevin's	40	12	0	0	7.9	0.4	10.3	10.8	2.9	10.1	11.2
	Nugie's	1	15	16	15	7.5	0.2	10.9	12.0	0.8	10.4	12.1
	Tidegate	7	0	16	16	10.4	0.9	10.5	13.3	10.8	10.5	13.1
EFT Summary			***27***	***32***	***31***	***8.1***	***0.4***	***10.7***	***12.0***	***3.0***	***10.3***	***12.1***
NIS EEM	NEEM 1	2	11	16	16	8.8	1.7	11.6	12.5	12.4	11.0	12.6
	NEEM 2	9	14	16	16	9.5	5.2	10.5	13.1	14.5	10.6	13.0
NIS EEM Summary			***25***	***32***	***32***	***9.2***	***3.6***	***11.0***	***12.8***	***13.5***	***10.8***	***12.8***
RSS EEM	Mitigation	20	31	16	16	10.9	18.7	9.1	14.9	26.0	9.0	14.4
	RSS LB	13	11	16	16	8.7	16.6	10.1	13.2	27.6	9.8	13.0
	RSS Phase 1	48	0	1	0	13.8	20.9	8.8	7.2	25.0	7.9	7.8
	RSS Point	26	12	16	15	9.0	16.0	10.9	13.8	26.6	9.7	13.1
RSS EEM Summary			***54***	***49***	***47***	***9.5***	***17.1***	***10.0***	***13.9***	***26.8***	***9.5***	***13.5***

Appendix A (continued).

Zone	Site	Map Reference Number	Totals Number of Sets in 2004	Totals Number of Sets in 2005	Totals Number of Sets in 2006	Mean Tide Sampled (ft)	Mean Surface Salinity (ppt)	Mean Surface Dissolved Oxygen (mg/l)	Mean Surface Temperature (C)	Mean Near Bottom Salinity (ppt)	Mean Near Bottom Dissolved Oxygen (mg/l)	Mean Near Bottom Temperature (C)
MCA EEM	Aster	47	10	0	0	10.0	20.8	10.0	16.3	25.6	10.6	15.4
	Eagle Cove	46	3	0	0	11.1	25.6	11.8	15.3	25.2	11.0	14.8
	Hairgrass	27	12	16	16	10.6	13.8	9.3	16.0	23.7	9.5	15.0
	MCA RB	28	15	16	16	8.6	22.3	10.3	14.9	27.1	10.3	13.6
	N1	29	11	16	16	9.7	22.6	10.3	15.5	27.4	38.9	13.7
	N2	52	3	0	0	10.5	24.9	9.3	10.9	26.9	10.7	10.1
MCA EEM Summary			***54***	***48***	***48***	***9.5***	***20.2***	***10.0***	***15.4***	***26.2***	***17.5***	***14.1***
Delta Flats	Breakwater	11	14	16	16	7.9	24.6	10.8	12.8	28.1	10.3	12.4
	Luhr Beach	30	16	16	16	9.2	22.1	10.8	15.0	28.1	10.7	13.4
	RSS RB	12	17	16	17	8.8	16.1	14.0	13.5	27.8	9.9	13.2
	Seal Beach	10	15	16	16	7.9	17.4	10.8	12.8	27.2	10.6	12.8
Delta Flats Summary			***62***	***64***	***65***	***8.5***	***20.1***	***11.7***	***13.5***	***27.8***	***10.3***	***12.9***
Nearshore	Andy	34	0	0	15	9.9	28.2	10.1	13.0	28.3	10.3	12.5
	DeWolf Bight	37	13	16	16	8.5	27.7	10.8	13.2	29.1	11.4	12.9
	East Oro Bay	33	2	0	15	8.5	28.4	11.6	14.4	28.6	11.2	13.4
	Hogum Bay	38	3	16	16	12.9	27.4	10.3	12.5	27.9	9.4	12.6
	Hogum Bay Spit	43	2	0	0	9.0	27.4	9.9	12.5	28.6	10.0	12.4
	Sequalitchew	31	0	16	16	6.7	27.7	10.6	13.0	28.4	10.6	12.6
	Solo Point	32	0	0	15	7.8	28.3	9.2	12.1	28.8	9.1	12.1
	Thompson Cove	55	2	0	0	11.2	28.2	8.4	9.3	28.1	8.7	9.4
	Tolmie Beach	35	0	0	13	11.4	28.5	10.9	11.9	28.5	11.0	11.8
	Tolmie Lagoon	36	0	0	12	11.4	28.0	10.6	13.2	28.7	10.6	13.1
Nearshore Summary			***22***	***48***	***118***	***9.9***	***27.9***	***10.6***	***12.9***	***28.5***	***10.5***	***12.6***

Appendix B

Nisqually Reach Nature Center Invertebrate Identification and Enumeration Methodology

Invertebrate Identification and Enumeration Methodology
Produced by the Nisqually Reach Nature Center

Invertebrate Monitoring Protocol

Invertebrate sampling and identification is important in fully understanding the ecological integrity of the Nisqually estuary. This is a follow up study covering the phase two restoration sites in order to get an assessment of the organisms that are repopulating areas that are being restored to their natural state. The single sampling technique that the center will be looking at in this study is insect fall-out. These samples will be collected in five different sites located in the Nisqually Delta. The results from reference/control sites will be compared with the results from the phase one and phase two restoration site to determine the taxa richness and diversity, assemblage compositions and densities.

The protocol employed in the laboratory identification of these samples needs to be flexible enough to be effective for the different types of samples acquired in the field. The animals will be sorted out into orders and further identified down to family if they are prey to salmon. The following is a list of functional groups of salmonid prey that was compiled based on previous studies regarding estuary habitat assessment:

Class: Order	Common Name	Functional Group
Insecta: Diptera	Midges	Chironomidae (Unid)
Insecta: Diptera	Midges	Chironomidae larvae/pupae
Insecta: Diptera	Shore Flies	Ephydridae (Unid)
Insecta: Diptera	Biting Midges, Punkies, No-see-ums	Heleidae (Unid)
Insecta: Diptera		Diptera (Unid)
Insecta: Diptera	Crane Flies	Tipulidae
Insecta: Lepidoptera	Moths	Microlepidoptera
Insecta: Hemiptera	True Bugs	Hemiptera (Unid)
Insecta: Hymenoptera	Sawflies, Parasitic Wasps, Ants, Wasps, and Bees	Scoloidea (Unid)

The primary invertebrate identifier will sort the samples into orders and then proceed to identify them down to functional group. Quality assurance and quality control will be carried out by a separate individual(s) trained in invertebrate zoology. Their involvement will be based on their time availability. Volunteers will be employed to help sort the sample into different orders after receiving invertebrate identification training. Volunteers will start out by sorting the insect fallout samples into orders using dissecting microscopes and sorting trays. Separate sorting trays will be used for each

sample and labeled accordingly. Volunteers will record order qualification information on Invertebrate Laboratory Bench Sheets located in the Invertebrate Monitoring notebook in the NRNC lab. After volunteers have finished sorting, the Invertebrate Laboratory Bench Sheets should be returned back to the Invertebrate Monitoring notebook containing all pertinent information and the sorting trays should be stored in the lab fire safe cabinet.

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Simenstad, C.A., C.D. Tanner, R. M. Thom, and L.L. Conquest., Prepared for U.S. Environmental Protection Agency, Puget Sound Estuary Program. Estuarine habitat assessment protocol. School of Fisheries, University of Washington; EPA 910/9-91-037

Sample Receiving

Samples were received from the Nisqually Wildlife Refuge (NWR) by the project coordinator and brought to the Nisqually Reach Nature Center (NRNC) for sorting and identification. Samples were received in batches by NRNC; batches typically contained a single sampling date with the three to five different sites and replicates of each site. The samples received were terrestrial. Samples were stored in 95% ethanol in 250 mL nalgene bottles. Received samples were placed under flammable material metal cabinet storage in the NRNC lab. Samples received by the NRNC contained both organisms and debris.

Sample Sorting

The sample storage bottle was removed from the cabinet and the individual removing that sample recorded in the Invertebrate Monitoring Identification Records and Procedure (IMIRP) binder which sample they were removing. Sample material was transferred from storage container to a Petri dish and examined under a dissecting microscope (10x – 40x magnification) or compound microscope (40x – 100x magnification). (The compound microscopes were used mainly for finer identification of individual organisms).

A sorting tray was set up to store smaller vials that contained separate sample material. Trays were labeled with the sample code, sample collection date, date of identification/sorting, and the initials of the sorter. This information was also recorded on a bench sheet, which was to be placed after each use in the “In Use Bench Sheets” section of the IMIRP.

Samples were not split. Any debris that was removed from the sample was inspected by the individual removing it and also checked by an assisting volunteer to ensure that no organisms were removed.

Tools used in the transfer of materials (tweezers and spoons) were rinsed of any sample material with 95% ETOH and inspected to ensure no material was left behind.

All samples were identified and counted in full.

Samples were identified with the help of volunteers who received training from either the project coordinator or key staff with sufficient training in invertebrate zoology. Volunteers with training in invertebrate zoology were asked to concentrate on the type of organisms they were already familiar with.

Organisms were identified (with the use of taxonomic guides (Attachment A) and placed into labeled vials contained in a labeled counting block. Organisms were identified to the lowest possible taxonomic level and according to organism identification goal list (Attachment B). Organisms that were key diet items for salmonids were identified to the lowest possible taxonomic level.

Damage to some organisms made identification to a lower taxonomic level difficult. Some organisms were difficult to pick out form the associated debris and in those cases a rose Bengal solution was used to stain the organisms. A project coordinator or key staff was always present to assist volunteers with identification.

All separate vials were labeled with the sample code, sample collection date, date of identification/sorting, the initials of the identifier, and the type of organism contained in that vial; and filled with 95% ETOH. All organism counts were also recorded on the "In Use" bench sheets and placed into IMIRP. All sorting trays that were still in use were placed in the "In Use" section of the metal cabinet. Once a sample was completed, its bench sheet was placed in the "Completed Samples" section of the IMIRP. The completed sorting trays were placed in the "Completed Samples" section of the metal cabinet for QA/QC.

Quality Assurance/Quality Control

Sorting trays located in the "Completed Samples" area of the metal cabinet were inspected for accuracy by the project coordinator or key staff. Waste removed from samples also underwent QA/QC.

Each individual bench sheet and associated sorting tray underwent QA/QC individually. Organisms were removed from vials and inspected for accuracy of identification and count. Any discrepancies in count were resolved by the involvement of a key staff that was not the original individual working on that sample. Counts were finalized when two separate individuals verified the count. Discrepancies in identification were handled by consulting the appropriate guide and keying out the organism with the aide of the original identifier to resolve any errors made on the original ID.

Final organism counts were transferred to a single "master" bench sheet to be used for the hand off and used to enter the data into the invertebrate database. Samples that were identified by more than one individual were combined into one sorting tray by the project coordinator or key staff and labeled appropriately.

After receiving QA/QC, sample vials were labeled with sample code, the type of organism, and the number of organisms. Vouchers containing the same information found on the outside label were also placed inside the vial. All data contained on finalized bench sheets were entered into the invertebrate data base and brought to the NWR along with the finalized sorting tray for hand off.

Attachment A

Invertebrate Reference Library

Title	Author(s)
How to Know the Insects	Bland, R.G. and H.E. Jaques
The Insect Guide: Orders and Major Families of North American Insects	Swain, R.B.
Aquatic Insect Ecology: 1. Biology and Habitat	Ward, J.V.
Introduction to Insect Biology and Diversity	Daly, H.V., J.T. Doyen, and P.R. Ehrlich
Peterson Field Guides: Insects	Borror, D.J. and R.E. White

Attachment B

Organism Identification Goal List	
Order	***Family***
Diptera	
	Chironomidae
	Ceratopogonidae
	Ephydridae
	Heleidae
	Dolichopodidae
	Phoridae
	Sciaridae
	Tipulidae
Microlepidoptera	
Hemiptera	
Hymenoptera	
	Scoloidea

Hand-over protocol

1. The sample must pass QA/QC

2. All data sheets regarding the sample must be copied and the originals provided with the sample.

3. Data must be either entered into Excel spreadsheet (Note: Entering data is another great way to catch mistakes and to clear up any problems).

4. An electronic copy of the updated spreadsheet must be either e-mailed to Christopher (Christopher_Ellings@fws.gov) or put on a floppy disk and handed over with the sample.

5. Christopher will maintain a sample inventory checklist to track which samples are at the center and which ones are at the refuge.

Appendix C

Nisqually Hatchery Chinook and Coho Releases: 2004-2006
Provided by Dietrich Schmitt, Northwest Indian Fisheries Commission

2004 Release Report for Nisqually Tribe (WA)

NISQUALLY HATCHERY

Fall CHINOOK

Brood Year 2003

Stock	Release Dates First	Release Dates Last	Release Site	Tag Code	Size (#/lb)	Total # Released		Number Tagged/Marked Mark 1	Number Tagged/Marked Mark 2	Final Tag Loss Rate	Mark on Fish Mark 1	Mark on Fish Mark 2
CLEAR CR 11.0013C	05/06/2004	06/04/2004	CLEAR CR 11.0013C	210548	55	103,495	CWT	103,082	310	0.10%	CWT Adipose clip (Ad)	No external marks
Comments ASSOC W/ TAG CODES 210547, 631895 & 631896							NoCWT	103			No-CWT Adipose clip (Ad)	
CLEAR CR 11.0013C	05/06/2004	06/04/2004	CLEAR CR 11.0013C	631896	55	101,957	CWT	100,965		0.98%	CWT No external marks	
Comments ASSOC W/ TAG CODES 631895, 210548 & 210547							NoCWT	992			No-CWT No external marks	
CLEAR CR 11.0013C	05/06/2004	06/04/2004	CLEAR CR 11.0013C	631895	55	769,662	CWT	102,659		0.50%	CWT No external marks	
Comments ASSOC W/ TAG CODES 631896, 210548 & 210547							NoCWT	626,498	40,505		No-CWT Adipose clip (Ad)	No external marks
CLEAR CR 11.0013C	05/06/2004	06/04/2004	CLEAR CR 11.0013C	210547	55	2,564,170	CWT	104,893	955	0.20%	CWT Adipose clip (Ad)	No external marks
Comments ASSOC W/ TAG CODES 631895, 210548 & 631896							NoCWT	2,380,892	77,430		No-CWT Adipose clip (Ad)	No external marks
					Total Fish	3,539,184						

Fall COHO

Brood Year 2002

Stock	Release Dates First	Release Dates Last	Release Site	Tag Code	Size (#/lb)	Total # Released		Number Tagged/Marked Mark 1	Number Tagged/Marked Mark 2	Final Tag Loss Rate	Mark on Fish Mark 1	Mark on Fish Mark 2
MINTER CR 15.0048	04/01/2004	04/21/2004	CLEAR CR 11.0013C	210429	17	546,784	CWT	42,035	1,179	7.39%	CWT Adipose clip (Ad)	No external marks
Comments FISH INFECTED W/ BKD DURING REARING							NoCWT	479,725	24,846		No-CWT Adipose clip (Ad)	No external marks
					Total Fish	546,784						

KALAMA CR HATCHERY

Fall CHINOOK

Brood Year 2003

Stock	Release Dates First	Release Dates Last	Release Site	Tag Code	Size (#/lb)	Total # Released		Number Tagged/Marked Mark 1	Number Tagged/Marked Mark 2	Final Tag Loss Rate	Mark on Fish Mark 1	Mark on Fish Mark 2
KALAMA CR 11.0017	05/14/2004	06/01/2004	KALAMA CR 11.0017	210559	63	627,000	CWT	96,131	342	4.14%	CWT Adipose clip (Ad)	No external marks
Comments							NoCWT	529,737	1,790		No-CWT Adipose clip (Ad)	No external marks
					Total Fish	627,000						

2004 Release Report for Nisqually Tribe (WA)

KALAMA CR HATCHERY

Fall COHO

Brood Year 2002

Stock	Release Dates First	Last	Release Site	Tag Code	Size (#/lb)	Total # Released		Number Tagged/Marked Mark 1	Mark 2	Final Tag Loss Rate	Mark on Fish Mark 1	Mark 2
GREEN R + MINTER	04/01/2004	04/24/2004	KALAMA CR 11.0017	210430	24	299,979	CWT	41,989	1,156	4.90%	CWT Adipose clip (Ad)	No external marks
Comments INCIDENCE OF BKD IN POPULATION							NoCWT	249,145	7,689		No-CWT Adipose clip (Ad)	No external marks
					Total Fish	299,979						

2005 Release Report for Nisqually Tribe (WA)

NISQUALLY HATCHERY

Fall CHINOOK

Brood Year 2004

Stock	Release Dates First	Last	Release Site	Tag Code	Size (#/lb)	Total # Released		Number Tagged/Marked Mark 1	Mark 2	Final Tag Loss Rate	Mark on Fish Mark 1	Mark 2
CLEAR CR 11.0013C	05/02/2005	06/01/2005	CLEAR CR 11.0013C	210599	54	211,123	CWT	209,905		0.59%	CWT No external marks	
Comments DIT ASSOC W/ TAG CODE 632783							NoCWT	1,218			No-CWT No external marks	
CLEAR CR 11.0013C	05/02/2005	06/01/2005	CLEAR CR 11.0013C	632783	54	2,731,291	CWT	208,724	1,202	0.38%	CWT Adipose clip (Ad)	No external marks
Comments DIT ASSOC. W/ TAG CODE 210599							NoCWT	2,354,207	167,158		No-CWT Adipose clip (Ad)	No external marks
					Total Fish	2,942,414						

Fall COHO

Brood Year 2003

Stock	Release Dates First	Last	Release Site	Tag Code	Size (#/lb)	Total # Released		Number Tagged/Marked Mark 1	Mark 2	Final Tag Loss Rate	Mark on Fish Mark 1	Mark 2
MINTER CR 15.0048	04/01/2005	04/24/2005	CLEAR CR 11.0013C	210550	18	572,637	CWT	47,733	721	1.14%	CWT Adipose clip (Ad)	No external marks
Comments							NoCWT	508,475	15,708		No-CWT Adipose clip (Ad)	No external marks
					Total Fish	572,637						

2005 Release Report for Nisqually Tribe (WA)

KALAMA CR HATCHERY

Fall CHINOOK

Brood Year 2004

Stock	Release Dates First	Last	Release Site	Tag Code	Size (#/lb)	Total # Released		Number Tagged/Marked Mark 1	Mark 2	Final Tag Loss Rate	Mark on Fish Mark 1	Mark 2
KALAMA CR 11.0017	05/15/2005	06/09/2005	KALAMA CR 11.0017	210598	55	501,460	CWT	56,177	2,859	9.54%	CWT Adipose clip (Ad)	No external marks
Comments							NoCWT	423,498	19,926		No-CWT Adipose clip (Ad)	No external marks
						Total Fish	501,460					

Fall COHO

Brood Year 2003

Stock	Release Dates First	Last	Release Site	Tag Code	Size (#/lb)	Total # Released		Number Tagged/Marked Mark 1	Mark 2	Final Tag Loss Rate	Mark on Fish Mark 1	Mark 2
MINTER CR 15.0049	04/15/2005	05/12/2005	KALAMA CR 11.0017	210551	21	220,572	CWT	44,827	677	1.14%	CWT Adipose clip (Ad)	No external marks
Comments							NoCWT	2,566	172,502		No-CWT Adipose clip (Ad)	No external marks
						Total Fish	220,572					

2006 Release Report for Nisqually Tribe (WA)

NISQUALLY HATCHERY

Fall CHINOOK

Brood Year 2005

Stock	Release Dates First	Last	Release Site	Tag Code	Size (#/lb)	Total # Released		Number Tagged/Marked Mark 1	Mark 2	Final Tag Loss Rate	Mark on Fish Mark 1	Mark 2
CLEAR CR 11.0013C	09/15/2006	09/15/2006	CLEAR CR 11.0013C	633296	61	3,476,290	CWT	120,154	7,920	0.75%	CWT Adipose clip (Ad)	No external marks
Comments DIT CLIPPED, ASSOC W/ TAG CODE 210691, EXP LOW SURVIVAL DUE TO TAIL DEFORM & PIN							NoCWT	2,943,909	404,407		No-CWT Adipose clip (Ad)	No external marks
CLEAR CR 11.0013C	05/17/2006	05/28/2006	CLEAR CR 11.0013C	210691	61	129,112	CWT	127,293		1.41%	CWT No external marks	
Comments DIT UNCLIPPED, ASSOC. W/ TAG CODE 633296, LOW SURVIVAL DUE TO TAIL DEFORM & PINH							NoCWT	1,819			No-CWT No external marks	
						Total Fish	3,605,402					

2006 Release Report for Nisqually Tribe (WA)

KALAMA CR HATCHERY

Fall CHINOOK

Brood Year 2005

Stock	Release Dates First	Release Dates Last	Release Site	Tag Code	Size (#/lb)	Total # Released		Number Tagged/Marked Mark 1	Number Tagged/Marked Mark 2	Final Tag Loss Rate	Mark on Fish Mark 1	Mark on Fish Mark 2
KALAMA CR 11.0017	05/18/2006	06/05/2006	KALAMA CR 11.0017	210671	55	270,654	CWT	42,333	4,360	5.61%	CWT Adipose clip (Ad)	No external marks
Comments							NoCWT	215,114	9,847		No-CWT Adipose clip (Ad)	No external marks
					Total Fish	270,654						

Fall COHO

Brood Year 2004

Stock	Release Dates First	Release Dates Last	Release Site	Tag Code	Size (#/lb)	Total # Released		Number Tagged/Marked Mark 1	Number Tagged/Marked Mark 2	Final Tag Loss Rate	Mark on Fish Mark 1	Mark on Fish Mark 2
KALAMA CR 11.0017	04/05/2006	05/15/2006	KALAMA CR 11.0017	210595	22	381,143	CWT	35,003	167	16.50%	CWT Adipose clip (Ad)	No external marks
Comments							NoCWT	339,109	6,864		No-CWT Adipose clip (Ad)	No external marks
					Total Fish	381,143						

Appendix D

Pre-Restoration Habitat Use by Chinook Salmon in the Nisqually Estuary using Otolith Analysis. U. S. Geological Survey

Pre-Restoration Habitat Use by Chinook Salmon in the Nisqually Estuary using Otolith Analysis

Final Report presented to the Nisqually Wildlife Refuge
by Angie Lind-Null, Kim Larsen and Reg Reisenbichler
U.S. Geological Survey, Western Fisheries Research Center
July 2007

Introduction

The Nisqually Fall Chinook population is one of 27 stocks in the Puget Sound evolutionarily significant unit listed as threatened under the federal Endangered Species Act. The preservation of the Nisqually delta ecosystem coupled with extensive restoration of approximately 1000 acres of diked estuarine habitat is identified as the highest priority action for the recovery of naturally spawning Nisqually River Fall Chinook salmon (*Oncorhynchus tshawytscha*) in the Nisqually Chinook Recovery Plan.

In order to evaluate the response of Chinook salmon to restoration, a pre-restoration baseline of life history diversity and estuary utilization must be established. Otolith analysis has been proposed as a means to measure Chinook salmon life history diversity, growth, and residence in the Nisqually estuary. Over time, the information from the otolith analyses will be used to: 1) determine if estuary restoration actions cause changes to the population structure (i.e. frequency of the different life history trajectories) for Nisqually River Chinook, 2) compare pre and post restoration residence times and growth rates, and 3) suggest whether estuary restoration yields substantial benefits for Chinook salmon.

Otoliths are calcium carbonate structures in the inner ear that grow in proportion to the overall growth of the fish. Daily growth increments can be measured so date and fish size at various habitat transitions can be back-calculated. Careful analysis of otolith microstructure can be used to determine the number of days that a fish resided in the estuary as a juvenile (increment counts), size at entrance to the estuary, size at egress, and the amount that the fish grew while in the estuary. Juvenile Chinook salmon can exhibit a variety of life history trajectories – some enter the sea (or Puget Sound) as fry, some rear in the estuary before entering the sea, and some rear in the river and then move rapidly through the estuary into the sea as smolts.

The purpose of this study is to evaluate and use analysis of otolith microstructure as a tool for characterizing the importance of the estuary to Chinook salmon in the Nisqually River before and after restoration efforts at the Nisqually National Wildlife Refuge (NNWR). This tool is used to quantify changes in habitat use and help assess restoration benefits to the federally threatened Nisqually River Chinook salmon population.

Analysis of otolith microstructure typically is superior to the alternative of traditional mark-recapture methods. The latter are extremely expensive or inadequate in estuary habitats, typically are biased and substantially underestimate use, and do not directly reveal the importance or contribution to adult recruitment (i.e., they do not account for differential survival afterward in

Puget Sound or the ocean). Analysis of otolith microstructure for these purposes, while new, is proving highly successful in a similar study that USGS and partners are conducting in the Skagit River estuary system located in northern Puget Sound. This work has been based on research by Neilson et al. (1985). We expect to use the Skagit River data as a reference for the before/after restoration comparison in the Nisqually River.

Objectives

Objective #1: Develop a Nisqually-specific signature of otolith microstructure growth patterns and checks that allow us to distinguish growth and residence of juvenile salmon in the estuary from growth in the river (upstream) and in Puget Sound (seaward). Evaluate between-year variation in these characters by comparing otoliths collected in 2004 with those collected in 2005.

Objective #2: Determine whether distinct growth patterns on the otoliths of hatchery and wild salmon in the Nisqually River allow us to recognize unmarked hatchery fish and separate them from wild fish.

Objective #3: Analyze the otoliths of returning adults in order to catalog the juvenile life-history trajectories of these "successful" fish and provide a preliminary estimate for the proportions and numbers of wild and hatchery adults that reared in the delta and estuary as juveniles.

Objective #4: Describe the relationship between juvenile salmon size or date of entry to the estuary with the fish's growth rate or residence time in the estuary.

Methods

Unmarked and marked juvenile Chinook salmon were collected by the Nisqually tribe and U.S. Fish and Wildlife Service – NNWR in March through October of 2004 and February through October of 2005 from various sites within the Nisqually River mainstem, tidal delta, nearshore, and associated habitats (Table 1). No Chinook were caught in the nearshore or EEM (Animal fyke trap) catch during February of 2005. The fish were collected by beach seining in the following distinct habitat zones (Cowardin et al. 1979; Figure 1):

1. *Freshwater* (FW) – forested slow water habitat on the mainstem Nisqually River without tidal influence.
2. *Forested Riverine Tidal* (FRT) – riparian forest, mud/silt substrate, and tidal influence.
3. *Emergent Forested Transition* (EFT) – scrub/shrub and marsh vegetation, mud/silt substrate, and tidal influence.
4. *Estuarine Emergent Marsh* (EEM) – low and high salt marsh vegetation, mud substrate, and full tidal influence.
5. *Delta Flats* (DF) – sparse to no vegetation, mud and/or gravel/cobble substrate, and large tidal fluctuations.

6. *Nearshore* (NS) – areas outside of Nisqually tidal delta, vegetation and substrate variable.
7. *Pocket Estuary* (PE) – sand spit enclosed estuary with salt marsh vegetation, sand and mud substrate, and forested bluffs.

A few sites within the EEM habitat were sampled with fyke nets. Each fish was euthanized and measured for length and weight. The fish were preserved in alcohol and sent to USGS where the sagittal otoliths of unmarked fish were extracted, sectioned, and polished according to the Western Fisheries Research Center's (WFRC) standard protocols.

A total of 167 juvenile Chinook salmon were collected in 2004 directly from the various South Sound hatcheries less than two weeks prior to hatchery releases for determination of unique patterns specific to individual hatcheries. An average of 15 fish per hatchery were sacrificed and the otoliths of at least 8 fish from select hatcheries were processed. These particular hatcheries corresponded to "high incidence" hatchery populations composing at least 5% of the CWT catch for this study in 2004 (shown in italics): *Clear Creek 44.6%, Kalama 28.0%, White River 6.5%, Garrison/Chambers Creek 5.8%, Voights Creek 5.8%,* Lost CWT 5.0%, Soos Creek 1.4%, Tumwater 2.2%, and Clark's Creek 0.7%.

In 2004, a total of 274 pairs of otoliths were collected from unmarked fish. All fish otoliths (one from each pair) were processed and sorted as "high incidence" hatchery or wild fish (Tables 2 and 3). Samples identified as "high incidence" were not analyzed. If the sample was not obviously hatchery or wild, the fish was categorized as "unknown origin" and was not analyzed further. Fish from the pocket estuary also were not analyzed due to small sample size (n=3). A total of 97 fish were identified as hatchery, 119 as wild, and 58 as unknown origin. A total of 97 samples were analyzed out of the 119 available wild fish. Some samples were not suitable for analysis because of: (i) presence of vaterite (a morph of the calcium carbonate structure), (ii) poor initial quality, (iii) uneven microstructural growth along the radial axis or (iv) processing error.

In 2005, a total of 333 pairs of otoliths were collected from unmarked fish. The majority of samples from the 2005 collection were not analyzed due to limited funding in the current funding contract. At the request of our cooperators and project officer we processed otoliths from one particular site in the EEM habitat (Animal fyke trap) (Table 2). A total of 48 pairs of otoliths were collected from unmarked fish at the Animal fyke site. One otolith from each pair was processed and sorted as to hatchery or wild. A total of 8 fish were identified as hatchery, 32 as wild, and 8 as unknown origin. Both hatchery and wild fish were analyzed for a total of 37 out of 45 suitable otoliths, however only wild fish were included in the analyses.

The nearshore collection was supplemented with samples collected in 2005 (n=8) and 2006 (n=19) due to small sample size in 2004 (n=2) (Table 1). A total of 16 fish were identified as hatchery, 10 as wild, and 3 as unknown origin. A total of 8 samples were analyzed out of the 10 available wild fish.

Adult samples were collected from the fishery and spawning grounds by the Nisqually tribe in 2005 and 2006. In 2005, a total of 176 samples were collected from the fishery and 125 were collected from the spawning grounds. In 2006, a total of 189 samples were collected from the fishery and 34 were collected from the spawning grounds. No adult samples were processed or analyzed due to limited funding under the current funding contract. Collections are archived for future funding opportunities.

Fish collected from freshwater showed a pattern which was used as a reference pattern on the otoliths. This reference pattern did not have any "checks" beyond the recognizable emergence and first feed checks. Checks are generally referred to as a consistently prominent mark or pattern on the otolith which interrupts the normal sequence of otolith deposition (Campana 1983). Each increment was interpreted as daily growth for the fish. Otoliths from fish collected in all other habitat types were visually analyzed for additional patterns, checks, or increased growth beyond the identifiers observed on the freshwater residence portion of the otoliths.

Daily growth increments and checks in the otolith microstructure were measured with the aid of a digital imaging system, Image-Pro. We selected a standardized radial axis for measurements at 85 ± 5 degrees ventral of the longitudinal axis passing through an identifiable and preferred nucleus. Distances along the radial axis and individual increment widths between checks or increase in growth representing change in habitat, were recorded for each fish.

Growth rates (mm/day) in the tidal delta were calculated from lengths based on the Fraser-Lee method (Murphy and Willis 1996):

$$L_i = \frac{L_c - a}{S_c} S_i + a$$

where L_i is the back-calculated length of the fish at the beginning of the tidal delta check, L_c is the length of the fish at capture, S_c is the radius of the otolith at capture, S_i is the radius of the otolith at the beginning of the tidal delta check, and a is the intercept from the overall regression of capture fork length verses otolith radius (Figure 2). Average growth rate and mean increment widths (MIW) were determined for all habitat types. Residence time and fork lengths upon entry to the tidal delta and delta flats/nearshore habitat zones were also calculated.

Results

Otolith microstructure pattern varied little over the years in 2004 – 2006; however the timing (i.e. month) of check formation did vary. After first feed, the increments on all otoliths became more legible and consistent across the radial axis (Figure 3). An interruption in the microstructure pattern, designated as a tidal delta check (TDCK), was detected on samples collected within tidal delta habitats EFT and EEM, indicating the fish's transition from freshwater to the

estuarine habitats (Figure 4). Increments were consistently narrow across the radial axis until the tidal delta check appeared where consistently wider increments indicated increased growth. No tidal delta check or increased growth was seen on otoliths from fish collected in the freshwater or the upper most tidal delta habitat (FRT).

In 2004, a tidal delta check was not observed on samples collected in March from EFT and EEM habitats. Insufficient sample sizes in April precluded analysis of the tidal delta check. In mid to late May, the tidal delta check appeared on samples from EFT and EEM habitats. In 2005, the tidal delta check first appeared on samples collected in the EFT and EEM in early June, but was barely detectable on some samples in late May (2 out of 8).

In addition to the tidal delta check, an additional interruption was seen on otoliths collected in the nearshore habitat beginning in June and in the delta flats habitat in April. We called this check a delta-flats check (DFCK). It indicated the fish's transition from estuarine habitat to the nearshore habitat (Figure 5). This check looked identical to the nearshore check located on Chinook in the Skagit River system (Beamer et al. 2000). Due to classification of sites, we called this check a delta-flats check instead of a nearshore check. The check was abbreviated in some samples (3 out of 11), possibly due to the fish being caught immediately upon entrance into the habitat. Insufficient samples were available to determine whether a delta-flats check was visible on samples collected in the nearshore in March or April. The number of samples containing a delta-flats check that were collected in the nearshore habitat were considerably low (1 out of 8).

With samples analyzed from multiple years, a one-way ANOVA was run to test for differences between years among tidal delta and freshwater MIW and growth rates. A significant difference occurred for MIW in the freshwater portion of the otolith for Animal fyke samples ($P<.05$). Therefore, the 2005 Animal fyke trap samples were excluded from the freshwater portion of the analysis.

No difference was visually observed in the microstructure pattern between EFT and EEM. To further validate this observation, a one-way ANOVA was run to test for significant differences between EFT and EEM. No significant differences occurred in growth rate or MIW ($P>.05$) and therefore the data were combined and classified as "tidal delta." FRT was not included as part of the tidal delta habitat for analysis because visually the microstructure pattern did not differ from freshwater samples nor was an additional check or increased growth ever observed.

We tested for differences in MIW in freshwater and tidal delta portions of the otoliths (Figure 6). One-way ANOVA showed a significant difference ($P<.05$) across habitats. On average, delta flats habitat had the lowest freshwater and tidal delta MIW. Overall, the MIW of the freshwater portion of all otolith samples was lowest followed by the tidal delta and nearshore habitats, respectively.

The equivalent results for growth rate were that the freshwater growth rates (mean= .42 mm/day) were lower compared to the tidal delta growth rates for fish residing in the tidal delta (mean = .57 mm/day), nearshore (mean = .57 mm/day), and delta flats (mean = .66 mm/day) habitats, with a 36% increase in

growth from freshwater habitat to tidal delta habitat. The delta flats/nearshore growth rate for fish caught in the delta flats was the same as the tidal delta growth rate. No significant difference was found between tidal delta and delta flat/nearshore growth rates (one-way ANOVA, P>.05).

The average fork length upon entry to the tidal delta was 72.8 mm. Fish caught in the tidal delta spent an average of 16 days with a minimum residence time of 10 days and a maximum of 35. These fish samples provided a minimum estimate of residence because the fish were sacrificed prior to entering the Sound. Evaluation of those fish caught in the delta flats and nearshore habitats exhibited an average residence time of 21 days in the tidal delta (n = 10). This value represents a truer estimate of residence time in the tidal delta, however the sample size was quite small. Fish caught in the delta flats were on average 60.2 mm when they entered the tidal delta and 69.5 mm upon exit, whereas fish caught in the nearshore were 73.1 mm upon entrance to the tidal delta. A positive relationship existed between the growth rate and the date the fish entered the tidal delta or nearshore (Figure 7). This could not be explained by a difference in size at entrance into the tidal delta and nearshore habitats.

Discussion

Hatchery Chinook salmon vastly outnumber wild salmon in the Nisqually River; however distinct microstructure patterns unique to each hatchery allowed us to recognize and separate unmarked hatchery fish from wild. The majority of unmarked hatchery fish were seen in natural habitats during May and June subsequent to hatchery release. Few hatchery strays were seen in March and April prior to release.

We characterized a Nisqually-specific signature of otolith microstructure growth patterns and checks for wild fish that allowed us to distinguish entry into the tidal delta and nearshore habitats. However, we were not able to distinguish between all habitat types prior to mid-May (2004) or early-June (2005) when the tidal delta check first appeared. The tidal delta check was not visible on freshwater or FRT samples regardless of when they were caught nor on samples collected in March in the EEM and EFT. We do not know whether a tidal delta check occurs in April because we had an insufficient sample size (n=1). Samples collected in March displayed few (x = 7) increments following the freshwater pattern which indicated that the fish were collected at or very early after entrance to the habitat and may not have had sufficient time to develop a visible check. This could be clarified by substantially increasing early season (March) sample size or by the addition of otolith microchemical analysis of Sr:Ca ratios (Fowler et al. 1995) in the hypothesized freshwater/tidal delta transition zone.

The delta-flats check first appeared in early June in nearshore samples and in April in delta flats samples. It is unclear whether a delta-flats check appeared in nearshore samples in April due to limited sample size (n=1).

We saw no visual difference in the microstructure pattern between otoliths collected in 2004, 2005, and 2006. However, analysis revealed differences in

MIW for freshwater residence and when the tidal delta check first appeared between the 2004 and 2005 Animal fyke samples. The majority of samples from the 2005 collection have not been analyzed to date due to limited funding. We focused our efforts for this reporting period on one sampling year (2004) rather than divide the effort across two years, lowering the sample size further and possibly missing potential characterization of some life history types.

Mean increment widths generally increased as the fish moved from freshwater to the nearshore habitats. The magnitude of the difference in MIW between the tidal delta and nearshore habitats probably is underestimated and may be an artifact of low sample size compounded by the brief time spent in the nearshore habitat for a large proportion of the fish.

Overall, the growth rate increased as the fish migrated from one habitat to another. Fish were growing faster (36%) in the tidal delta compared to freshwater, but this increase is significantly less than that seen in the Skagit River (U.S. Geological Survey, unpublished data). Our analysis revealed that fish grew at the same rate in the nearshore as in the tidal delta. This may be due to small sample size or that the majority of fish were caught soon after arrival (mean number of days residing before capture = 8; 8 out of 10 residing less than 10 days) in the nearshore habitat.

Funds and allocated time were insufficient to accomplish the analysis of adults during the current funding period. As previously mentioned, it is important to establish baseline information of life history trajectories from the juveniles, and then proceed to examining that portion of the adult otolith corresponding to the juvenile stage. Our resources were exhausted in working with the juveniles so we were not able to proceed to the adult samples. Otoliths collected from carcasses in Fall of 2006 were the first adult collections to correspond to the 2004 collection of juvenile outmigrants (i.e. 2003 brood year) in the estuary. Sampling will be attempted through 2010 to exhaust possible adult returns from 2003 – 2006 brood years.

Our results suggest that otolith microstructure analysis can be a valuable tool to establishing a baseline for use of the Nisqually River estuary habitats by juvenile Chinook salmon under existing conditions. However, this study provides limited information due to small samples sizes in some months, and only looks at contributions of wild-origin fish. The sample sizes contributed to uncertainty about whether Nisqually salmon deposit various habitat transition checks on the otoliths in all months. This uncertainty occurs for both tidal delta checks and delta-flats checks. Collection and analysis of additional fish especially in tidal delta and nearshore habitat zones should be addressed. Furthermore, these collections should occur over several years to allow adequate evaluation of inter-annual variation in microstructure growth patterns and checks, and may reveal additional life history types. Analysis of otolith microchemistry in conjunction with microstructure would provide an additional avenue for identifying early entry (March and April) into the tidal delta and perhaps the nearshore. Of course, further work should include analysis of adults because they show the proportions and numbers of adults that reared in the estuary as juveniles.

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Table 1: Number of otoliths from unmarked juvenile Chinook collected in 2004 – 2006 and used for otolith analysis. Additional fish were collected in 2005 and 2006 but not listed here.

		March	April	May	June	July	August	September	October	TOTAL
2004	**FRESHWATER**	10	20	10	11	0	0	6	1	58
	FRT	17	18	3	7	0	1	0	0	46
	EFT	2	1	9	9	0	0	0	0	21
	EEM	7	0	40	44	17	0	0	0	108
	NEARSHORE	0	0	0	2	0	0	0	0	2
	DELTA FLATS	0	13	3	17	3	0	0	0	36
	POCKET ESTUARY	0	3	0	0	0	0	0	0	3
2005	**EEM (Animal Fyke Trap)**	1	10	12	11	10	4	0	0	48
	NEARSHORE	0	0	2	5	1	0	0	0	8
2006	**NEARSHORE**	0	1	13	1	2	2	0	0	19
	TOTAL	37	66	92	107	33	7	6	1	349

Table 2: Number of otoliths analyzed / processed. With the exception of the 2005 Animal fyke trap, the number analyzed does not include unmarked hatchery fish separated from the catch.

		March	April	May	June	July	August	September	October	TOTAL
2004	**FRESHWATER**	4 / 10	12 / 19	4 / 10	2 / 10	0 / 0	0 / 0	0 / 6	0 / 1	22 / 56
	FRT	8 / 17	10 / 17	1 / 3	2 / 7	0 / 0	1 / 1	0 / 0	0 / 0	22 / 45
	EFT	1 / 1	1 / 1	2 / 9	1 / 9	0 / 0	0 / 0	0 / 0	0 / 0	5 / 20
	EEM	5 / 7	0 / 0	11 / 36	10 / 44	9 / 17	0 / 0	0 / 0	0 / 0	35 / 104
	NEARSHORE	0 / 0	0 / 0	0 / 0	1 / 2	0 / 0	0 / 0	0 / 0	0 / 0	1 / 2
	DELTA FLATS	0 / 0	5 / 13	1 / 3	6 / 17	0 / 3	0 / 0	0 / 0	0 / 0	12 / 36
	POCKET ESTUARY	0 / 0	0 / 3	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 3
2005	**EEM (Animal Fyke Trap)**	1 / 1	7 / 10	11 / 11	7 / 9	7 / 10	4 / 4	0 / 0	0 / 0	37 / 45
	NEARSHORE	0 / 0	0 / 0	0 / 2	1 / 5	1 / 1	0 / 0	0 / 0	0 / 0	2 / 8
2006	**NEARSHORE**	0 / 0	0 / 1	4 / 13	1 / 1	0 / 2	0 / 2	0 / 0	0 / 0	5 / 19
	TOTAL	19 / 36	30 / 64	33 / 87	25 / 104	17 / 33	5 / 7	0 / 6	0 / 1	141 / 338

Table 3: Number of unmarked hatchery samples separated from the catch.

		March	April	May	June	July	August	September	October	TOTAL
2004	**FRESHWATER**	0	0	6	7	0	0	5	1	19
	FRT	1	1	1	5	0	0	0	0	8
	EFT	0	0	5	5	0	0	0	0	10
	EEM	1	0	14	24	5	0	0	0	44
	DELTA FLATS	0	5	1	8	2	0	0	0	16
	NEARSHORE	0	0	0	0	0	0	0	0	0
2005	**NEARSHORE**	0	0	2	4	0	0	0	0	6
	EEM (Animal Fyke Trap)	0	0	3	2	3	0	0	0	8
2006	**NEARSHORE**	0	1	7	0	0	2	0	0	10
	TOTAL	2	2	38	47	8	2	5	1	121

Figure 1: Nisqually field sampling sites.

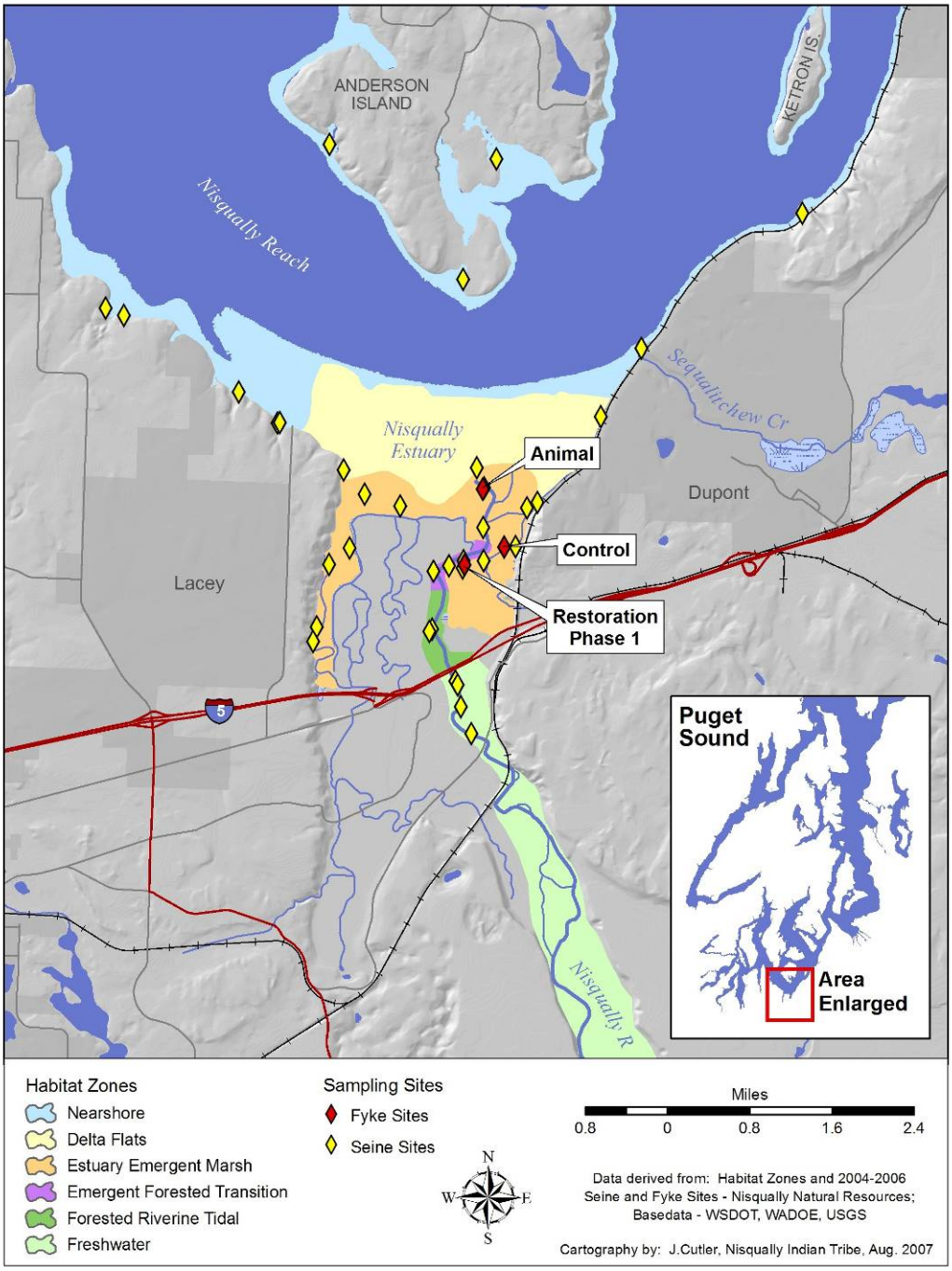


Figure 2: Relationship between fish fork length (mm) and otolith radial distance (mm).

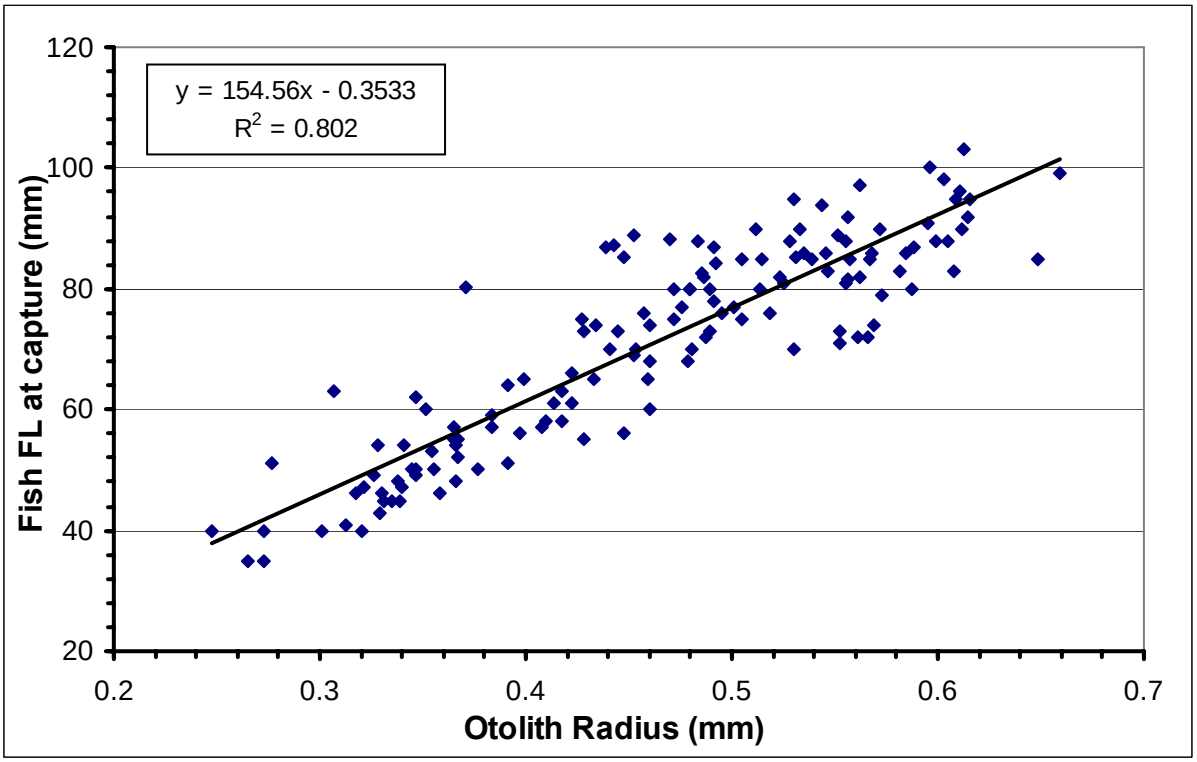
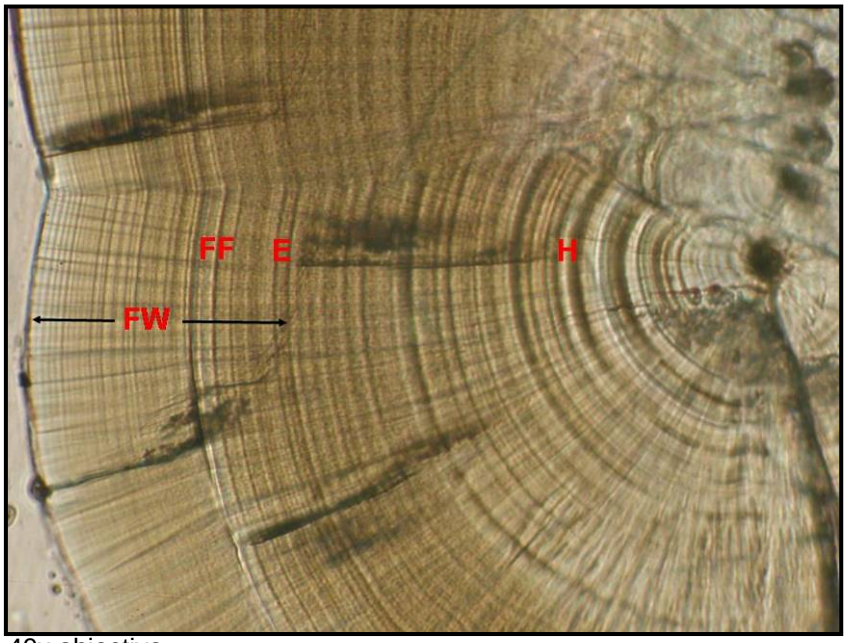


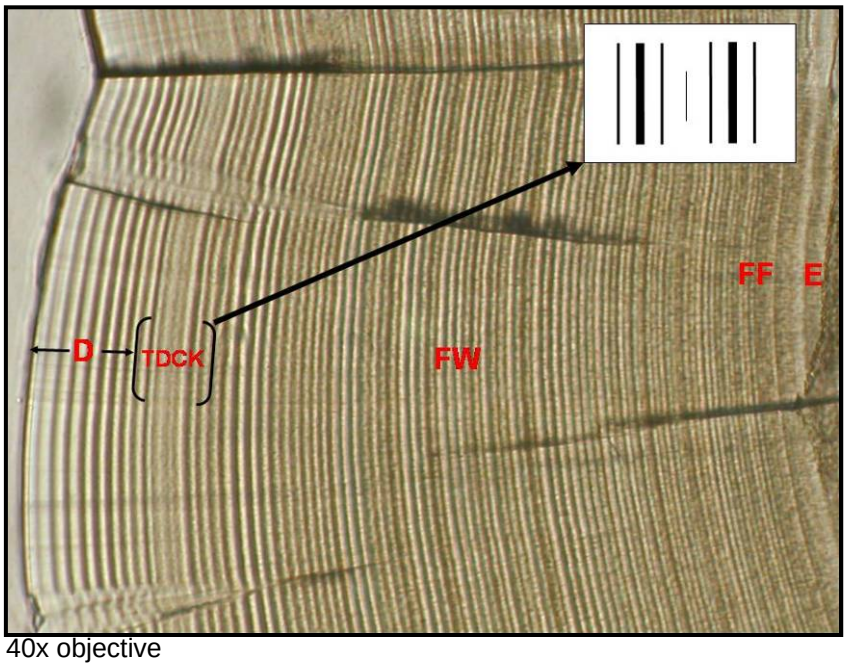
Figure 3: Representative sample of freshwater growth. The letters below represent: H = hatch, E = emergence, FF = first feed, FW = freshwater residence.



40x objective

Figure 4: The tidal delta check (TDCK) was seen on samples collected in the tidal delta in mid to late May (2004) and mid-June (2005). The check was bold and prominent consisting of two thin dark bands encompassing two wide bright bands containing a thick dark band between them. This sequence was then repeated following approximately 1 increment. Beyond the tidal delta check, increments were consistently wider indicating increased growth. The letters below represent: H = hatch, E = emergence, FF = first feed, FW = freshwater residence, TDCK = tidal delta check, and D = tidal delta residence.

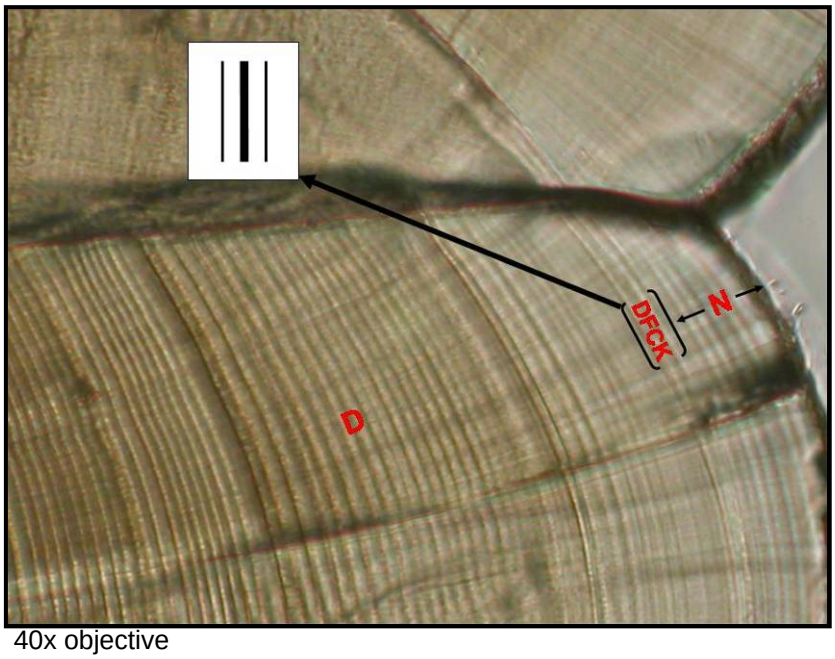
20x objective



40x objective

Figure 5: The delta-flats check (DFCK) was seen on samples collected in the nearshore beginning in mid-June and the delta flats in April. The check was bold and prominent consisting of two thin dark bands encompassing two wide bright bands containing a thick dark band between them. Beyond the delta-flats check, increments were consistently wider indicating increased growth. The letters below represent: H = hatch, E = emergence, FF = first feed, FW = freshwater residence, TDCK = tidal delta check, D = tidal delta residence, DFCK = delta-flats check, and N = delta flats/nearshore residence.

10x objective



40x objective

Figure 6: Mean Increment width (microns) for freshwater, tidal delta, and delta flats/nearshore residence within each habitat. Two samples collected in the delta flats were excluded from the delta flats/nearshore portion of the MIW analysis because residence time was only one day. The number of samples are represented in parentheses. Error bars represent ±1 standard deviation.

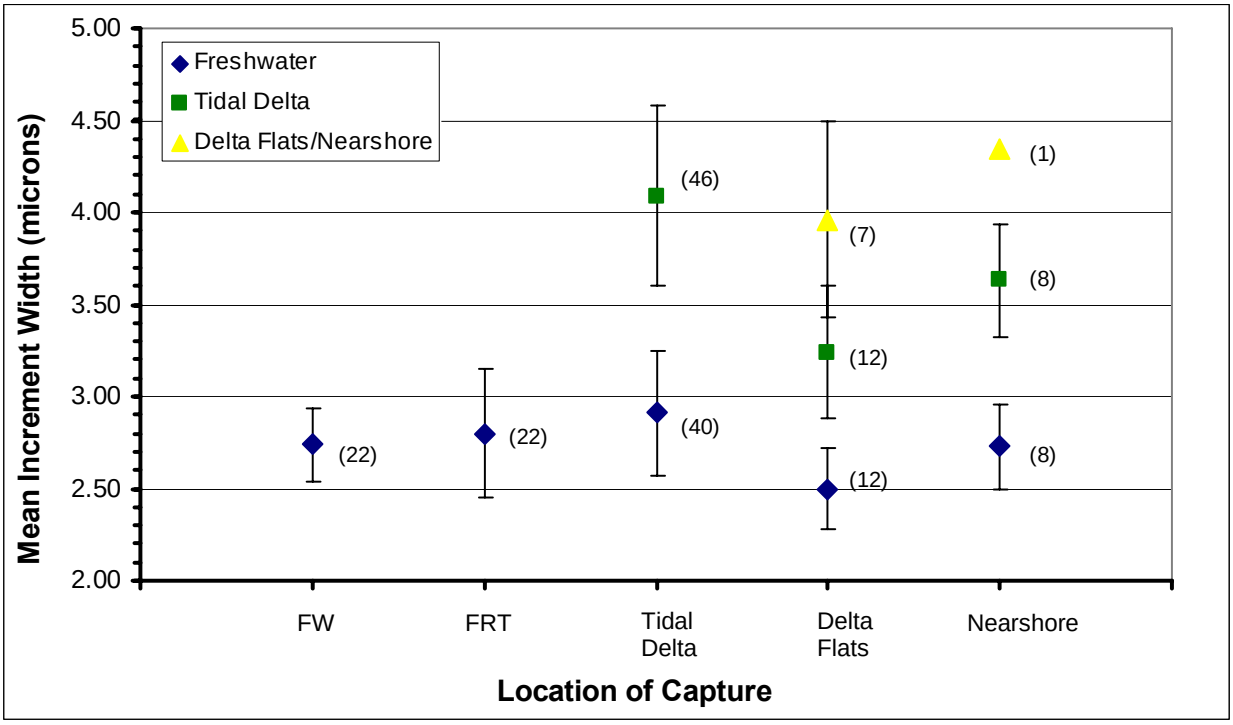


Figure 7: Relationship between the growth rate (mm/day) and the date the fish entered the tidal delta or delta flats/nearshore habitat.